

ANNI LAMMINSALO

Maternal Diet and Risk of Cow's Milk Allergy, and Cow's Milk Allergy and Risk of Type 1 Diabetes in The Offspring

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ACADEMIC DISSERTATION

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To my family and loved ones

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Helsinki, October 31st, 2025

Anni Lamminsalo

ABSTRACT

One of the earliest manifestations of allergic diseases is cow's milk allergy (CMA), which affects 2-6% of Finnish children under the age of 4 years. In Finland, the incidence and prevalence of type 1 diabetes (T1DM) among children under the age of 15 are among the highest in the world; during 2015–2018, the annual incidence was 52.2 cases per 100,000 children.

Multiple environmental factors, including the maternal diet during pregnancy, are thought to play a role in the development of food allergies in the offspring. For CMA these early environmental factors may have a great role, as it manifests early in life. The focus of interest has included the maternal intake of antioxidant nutrients and fatty acids. It has been suggested that higher maternal intake of antioxidant nutrients, especially vitamin E, and n-3-polyunsaturated fatty acids during pregnancy may protect offspring from allergic diseases, but the evidence is scarce and focused mainly for other allergic diseases than food allergies.

The immunological mechanisms behind allergic diseases and T1DM are different and partly opposite. Thus, it is suggested that these diseases should not appear simultaneously. In addition, the early intake of cow's milk is suggested to be a risk factor for the development of T1DM. The evidence of the association between T1DM and allergic diseases, as well as the cow's milk role as a risk factor for T1DM is scarce.

This study aimed to investigate the association between maternal intake of antioxidant nutrients and fatty acids during pregnancy and development of CMA in the offspring, as well as investigate the association between CMA and development of T1DM.

We investigated the association between maternal intake of nutrients and development of CMA in the offspring in the Type 1 Diabetes Prediction and Prevention cohort. We collected the maternal food consumption data by the validated food frequency questionnaire designed to assess both diet and supplement use over a one-month period during the eighth month of pregnancy. We gathered the information of child's CMA until the age of 3 years from registers and from parents (n=448). Altogether 4921 children had information on CMA and maternal diet during

pregnancy. We used logistic regression in statistical analyses and adjusted the intake of nutrients for energy by residual method.

We investigated the association between CMA and development of T1DM in a register-based case-cohort setting. We collected the data from the Finnish nationwide health registers. We included all children born in Finland between 1.1.1986-31.12.2008 and diagnosed with T1DM before the age of 16 years (n=7 754). As a reference cohort we collected a 10% random sample of each birth year cohort (n=137 798). We defined both T1DM and CMA by the special reimbursement for the costs of drugs/special infant formulas needed in the treatment. We used time-dependent weighted Cox regression in analyses.

The maternal intake of beta-carotene was associated with higher risk of CMA in the offspring, after adjustment for energy and multiple maternal and perinatal factors (OR 1.10 95 % CI 1.02-1.20) for other antioxidants no association was observed. The maternal intake of any single fatty acid during pregnancy was not associated with CMA in the offspring. When we analyzed separately the mothers with and without history of allergic rhinitis or asthma, the maternal intake of Vitamin E was associated with higher risk of CMA only in the offspring of mothers with such a history (OR 1.61 95 % CI 1.14-2.28), whereas the maternal intake of alfa linolenic acid was associated with lower risk of CMA only in the offspring of mothers without such a history (OR 0.72; 95 % CI 0.56-0.93).

CMA was directly associated with the development of T1DM when adjusted for multiple background factors related to the mother, the child and the perinatal period (HR=1.17; 95 % CI 1.02-1.34). In addition, CMA was directly associated with T1DM in children without asthma (HR=1.27; 95 % CI 1.10-1.47), but not in children with asthma (HR=0.80; 95 % CI 0.92-1.27). In all children included in the analysis, CMA was diagnosed prior to the onset of T1DM.

We did not observe an association between maternal intake of fatty acids or the majority of individual antioxidant nutrient during pregnancy and the development of CMA in the offspring.

We observed that children with CMA may have higher risk to develop T1DM. This association has not been reported before, thus more studies are needed.

TIIVISTELMÄ

Yksi varhaisimmista allergisista sairauksista on maitoallergia, jonka esiintyvyys suomalaisilla alle 4-vuotiailla lapsilla on 2-6 %. Suomessa tyypin 1 diabeteksen ilmaantuvuus ja esiintyvyys alle 15-vuotiaiden ikäryhmässä ovat maailman korkeimpia; vuosina 2015–2018 vuosittainen ilmaantuvuus oli 52,2 tapausta 100 000 lasta kohden.

Useiden ympäristötekijöiden, kuten raskauden aikaisen ravinnon, ajatellaan vaikuttavan lapsen riskiin sairastua ruoka-allergiaan. Varhaisilla ympäristötekijöillä saattaa olla merkittävä rooli maitoallergian kehittymiselle, sillä maitoallergia puhkeaa tyypillisesti varhaislapsuudessa. Äidin raskaudenaikaisessa ruokavaliossa kiinnostusta on herättänyt niin antioksidanttien saanti kuin ravinnon rasvahappokoostumuskin. Tutkimuksissa on havaittu, että raskauden aikainen suurempi antioksidanttien, erityisesti E-vitamiinin, sekä n-3-rasvahappojen saanti suojaisi jälkeläisiä allergisilta sairauksilta, mutta tutkimustulokset ovat osittain ristiriitaisia ja keskittyneet suurilta osin muihin allergisiin sairauksiin kuin ruoka-allergioihin.

Tyypin 1 diabeteksen sekä allergisten sairauksien taustalla olevat immunologiset mekanismit ovat erilaiset sekä osittain vastakkaiset ja siksi on ajateltu näiden sairauksien välisen yhteyden olevan käänteinen. Lisäksi varhaista maidonsaantia on esitetty yhdeksi riskitekijäksi tyypin 1 diabetekselle. Tutkimustieto tyypin 1 diabeteksen ja allergisten sairauksien yhteydestä, sekä maidon merkityksestä riskitekijänä tyypin 1 diabetekselle on ristiriitaista.

Tämän tutkimuksen tavoitteena oli selvittää raskauden aikaisen ravinnon antioksidantti- sekä rasvahappokoostumuksen yhteyttä lapsen riskiin sairastua maitoallergiaan, sekä selvittää maitoallergian yhteyttä tyypin 1 diabeteksen kehittymisen riskiin.

Selvitimme raskauden aikaisen ravinnon yhteyttä lapsen riskiin sairastua maitoallergiaan osana tyypin 1 diabeteksen ennustaminen ja ehkäisy (DIPP) -syntymäkohorttitutkimusta. Äidin ruoankäyttötiedot keräsimme validoidulla ruokafrekvenssilomakkeella, joka kartoitti ruokavaliota sekä ravintolisien käyttöä yhden kuukauden ajalta raskauden kahdeksannelta kuukaudelta. Tiedon lapsen maitoallergiasta 3 vuoden ikään mennessä keräsimme sekä rekistereistä että

vanhemmilta (n=448). Yhteensä 4921 lapsella oli tieto sekä äidin raskaudenaikaisesta ruokavaliosta että maitoallergiasta. Tilastolliset analyysit teimme logistisella regressioanalyysillä, jossa ravintoaineiden saanti energiavakioitiin.

Selvitimme maitoallergian yhteyttä tyypin 1 diabeteksen kehittymisen riskiin suomalaisiin kansallisiin terveysrekisteritietoihin pohjautuvassa tapauskohorttiasetelmassa. Tutkimukseen otettiin mukaan kaikki Suomessa 1.1.1986-31.12.2008 syntyneet lapset, joilla diagnosoitiin tyypin 1 diabetes (n=7 754). Vertailujoukoksi valitsimme 10 % satunnaisotoksen jokaista syntymävuosikohorttia kohden (n=137 798). Määritelmämme tyypin 1 diabetekselle sekä maitoallergialle perustui näiden sairauksien hoitoon tarvittavien lääkkeiden/erityiskorvikkeiden erityiskorvattavuudelle. Tilastolliset analyysit teimme aikariippuvaisella, painotetulla Coxin regressioanalyysillä.

Äidin raskaudenaikainen beetakaroteenin saanti oli yhteydessä lapsen suurempaan riskiin sairastua maitoallergiaan, kun huomioitiin useat äitiin, lapsen sekä perinataalikauteen liittyvät tekijät (OR 1.10 95 % CI 1.02-1.20). Äidin raskaudenaikainen rasvahappojen saanti ei ollut yhteydessä lapsen riskiin sairastua maitoallergiaan. Äidin raskaudenaikainen E-vitamiinin saanti oli yhteydessä lapsen suurempaan riskiin sairastua maitoallergiaan niiden äitien lapsilla, joilla oli sairaushistoriassa allerginen nuha tai astma (OR 1.61 95 % CI 1.14-2.28), kun taas äidin raskaudenaikainen alfa-linoleiinihapon saanti oli yhteydessä lapsen pienempään riskiin sairastua maitoallergiaan niiden äitien lapsilla, joilla ei ollut sairaushistoriassa allergista nuhaa tai astmaa (OR 0.72; 95 % CI 0.56-0.93).

Maitoallergia oli suorassa yhteydessä riskiin sairastua tyypin 1 diabetekseen, kun huomioimme useat äitiin, lapseen ja perinataalikauteen liittyvät tekijät (HR=1.17; 95 % CI 1.02-1.34). Lisäksi maitoallergia oli suorassa yhteydessä riskiin sairastua tyypin 1 diabetekseen lapsilla, joilla ei ollut samanaikaista astmaa (HR=1.27; 95 % CI 1.10-1.47), mutta ei lapsilla, joilla oli astma (HR=0.80; 95 % CI 0.92-1.27). Kaikilla tutkimukseen sisällytetyillä lapsilla maitoallergia diagnosoitiin ennen tyypin 1 diabetesta.

Emme havainneet yhteyttä äidin raskaudenaikaisen rasvahappojen tai suurimman osan yksittäisten antioksidanttien saannin ja lapsen maitoallergian välillä.

Maitoallergisilla lapsilla saattaa olla suurentunut riski sairastua tyypin 1 diabetekseen. Tätä yhteyttä ei ole aiemmin raportoitu, joten tulevaisuudessa yhteys on varmistettava lisätutkimuksin.

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ABBREVIATIONS

AA	Arachidonic acid
ALA	Alfa-linolenic acid
CMA	Cow's milk allergy
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFQ	181-item semi quantitative food frequency questionnaire
HLA	Human Leukocyte Antigen
LA	Linoleic acid
MUFA	Monounsaturated fatty acids
N-3 PUFA	Omega 3 polyunsaturated fatty acids
N-6 PUFA	Omega 6 polyunsaturated fatty acids
PUFA	Polyunsaturated fatty acids
PG	Prostaglandins
RCT	Randomized Controlled Trial
SAFA	Saturated fatty acids
SCFA	Short chain fatty acids
SII	Social Insurance Institution
Th1-cell	T helper type 1 cell
Th2-cell	T helper type 2 cell
T1DM	Type 1 diabetes mellitus

ORIGINAL PUBLICATIONS

This thesis is based on the original publications, which are referred in the text by their roman numerals. The publications in the list are not presented chronologically, instead the order is based on the academic reasonableness.

- Publication I Tuokkola J*, Lamminsalo A*, Metsälä J, Takkinen HM, Tapanainen H, Åkerlund M, Niinistö S, Toppari J, Ilonen J, Veijola R, Knip M, Kaila M, Virtanen SM. Maternal antioxidant intake during pregnancy and the development of cows' milk allergy in the offspring. *British Journal of Nutrition*. 2021;125(12):1386-1393.
- Publication II Lamminsalo A, Metsälä J, Takkinen HM, Tapanainen H, Åkerlund M, Niinistö S, Toppari J, Ilonen J, Veijola R, Knip M, Kaila M, Virtanen SM. Maternal energy-adjusted fatty acid intake during pregnancy and the development of cows' milk allergy in the offspring. *Br J Nutr*. 2022 Oct 28;128(8):1607-1614
- Publication III Lamminsalo A, Lundqvist A, Virta LJ, Gissler M, Kaila M, Metsälä J, Virtanen SM. Cow's milk allergy in infancy and later development of type 1 diabetes—nationwide case-cohort study. *Pediatric Diabetes*. 2021;22(3):400-406.

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Anni Lamminsalo's contributions in each publication:

Publication I: responsible of writing the first version of the manuscript together with Jetta Tuokkola, participated in the critical revision of the manuscript and accepted the final version of the manuscript

Publication II: participated in formulating the research questions, was responsible of analysing the data together with Hanna-Mari Takkinen, wrote the first version of the article and participated in the critical revision of the manuscript and accepted the final version of the manuscript

Publication III: responsible of writing the first version of the manuscript, participated in interpretation of the data, participated in the critical revision of the manuscript and accepted the final version of the manuscript

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1 INTRODUCTION

Cow's milk allergy (CMA) is one of the most common food allergies in childhood, and affects 2-6% of Finnish children under the age of 4 years (Pyrhönen et al., 2009, 2011, 2019; Saarinen et al., 1999; Saarinen & Savilahti, 2000). There are no time-trends available on the incidence of CMA, but the overall prevalence of food allergies seems to be increasing in developed countries (Nwaru et al., 2014).

The increase in incidence of allergic diseases was first observed in developed countries and is now seen also in developing countries all over the world. Even though the allergic diseases are strongly hereditary, this increase has been too steep to be solely explained by genetics. Thus, the environmental factors distinct to lifestyle in developed countries, including dietary changes, increased amount of pollutants, changes in microbial exposures, has been suggested as potential risk factors for the development of allergic diseases (Prescott & Saffery, 2011).

As the allergic diseases begin early in life, the early environmental factors may have a significant role, and these effects may begin already in utero (S. L. Prescott, 2013). The environmental factors can affect to the immune environment in utero through maternal circulation and placenta (Mor et al., 2017). The developing immune system of the foetus is vulnerable, and the environmental factors may modify the gene expression by epigenetic changes increasing the susceptibility of allergic diseases in childhood (Prescott & Saffery, 2011). For food allergies, including CMA, these prenatal factors may have even greater role as they typically manifest early in life. One of these environmental factors is maternal diet during pregnancy, including the maternal intake of antioxidant nutrients and fatty acids. Both, antioxidants and fatty acids, have observed to have immunological effects (Allan et al., 2010; Devereux & Seaton, 2005; Miles & Calder, 2017). However, the evidence on their role in the development of food allergy in the offspring is limited, and randomized controlled trials have shown little or no effect on prevention of food allergy by maternal vitamin or fish oil supplementation during pregnancy (De Silva et al., 2020). Further the studies are focused on prevention of food allergies as a group rather than specific food allergies, thus the current knowledge about the role of maternal diet in development of CMA is limited.

The incidence of type 1 diabetes (T1DM) among children and adolescents in Finland is one of the highest in the world (Patterson et al., 2019). T1DM and CMA are connected by at least two background factors, immunology, and cow's milk. The immunological background behind T1DM and CMA are different and partly opposite, as the autoimmunity in T1DM is characterized by T-helper 1 (Th1) immune reactions, and the allergic inflammation in food allergy by Th2-immune reactions. Because of the counter-regulation between Th1- and Th2-cells, the Th1/Th2 paradigm has suggested that these reactions could not appear simultaneously (Sornasse et al., 1996), suggesting an inverse relationship between T1DM and CMA. The early consumption of cow's milk has suggested to be a risk factor for the development of T1DM (Virtanen, 2016). Because of the immunology of CMA and the elimination of cow's milk from the diet, children with CMA could be in a lower risk of developing T1DM later in life.

Identification of potential risk and protective factors behind CMA and T1DM is important for the prevention of these diseases as it helps creating new prevention strategies as well as targeting these to the children with the highest risk.

The aim of our study was to investigate the association between maternal intake of antioxidant nutrients and fatty acids during pregnancy and development of CMA in the offspring as well as investigate the association between CMA and development of T1DM. We hypothesized that maternal higher intake of n-3 PUFA and antioxidant nutrients decreases and higher intake of n-6 PUFA increases the risk of CMA in the offspring, and that children with CMA have a decreased risk for developing T1DM.

2 REVIEW OF THE LITERATURE

2.1 Definition, mechanisms, and occurrence of CMA

Cow's milk allergy (CMA) is one of the most common food allergies among children and the symptoms usually appear early in infancy. The symptoms vary based on the immune response behind the CMA (Figure 1). The immune responses behind CMA are: IgE- and non-IgE, mediated allergy as well as simultaneous appearing of both immune responses. (Flom et al., 2019). In IgE-mediated food allergy the sensitization for the allergen, in case of CMA for the cow's milk protein, happens first. The mucosal-resident dendritic cells capture allergens from skin, bowel mucosa, or airways. The allergen is then presented by dendritic cells to the naïve CD4+ -cells in lymph nodes leading to differentiation to allergen-specific CD4+ T helper type 2 cells (Th2-cells) producing cytokines such as Il-4 and Il-13. These cytokines cause B-cell class switching to the IgE-isotype. The IgE B-cells can mature to plasma cells, which produce allergen specific IgE-antibodies, which bind on surface of basophils and mast cells. After sensitization the individual do not have any clinical symptoms of allergy. The clinical symptoms are caused by the effector phase, where the ingested allergen, for which the individual is sensitized, binds to specific IgE-antibody and causes the activation of the basophils and mast cells, which release the molecules

Mechanisms	Disease	Symptoms
IgE	Atopic food allergy	<ul style="list-style-type: none"> • Urticaria • Angioedema • Erythema • Anaphylaxis
Non-IgE	Food protein induced enterocolitis	Vomiting and diarrhea 1-6 hours after ingestion
	Allergic proctolitis	Bloody stools in 6-72h after ingestion
	Food protein induced enteropathy	Vomiting and diarrhea in 40-72 hours after ingestion

Figure 1. Symptoms of cow's milk allergy (Flom et al., 2019, Connors et al., 2018)

such as histamine causing almost immediately the typical IgE-allergic symptoms (Palomares et al., 2017).

The spectrum of non-IgE mediated food allergies is large including food-protein induced enterocolitis syndrome, allergic proctocolitis, and food-protein induced enteropathy (Connors et al., 2018). In these diseases the inflammation on bowel mucosa is dominated by eosinophils, but otherwise the pathophysiology is poorly understood (Caubet et al., 2017). For food-protein induced enterocolitis syndrome it has been suggested that cow's milk causes local inflammation on bowel epithelia, which increases intestinal permeability and fluid shift, causing the typical symptoms. The T-cells have thought to have a role in the development of this inflammation, but the exact role is still unclear (Caubet & Nowak-Węgrzyn, 2011). For food-protein induced enteropathy, it is suggested that increased production of eosinophils, cow's milk-specific Th2-lymphocytes, as well as IgE on small bowel may have a role in the development of the small bowel injury (Connors et al., 2018).

The overall prevalence of food allergies seems to be increasing in developed countries (Nwaru et al., 2014). In Finland the prevalence of CMA among children has reported to be between 2-6% (Pyrhönen et al., 2009, 2011, 2019; Saarinen et al., 1999; Saarinen & Savilahti, 2000). In studies the reported prevalence of CMA has varied and is dependent on the used definition of CMA. According to the systematic review of the prevalence of food allergies in Europe the prevalence of self-reported CMA was 2.3% (95% CI 2.1-2.5), for food challenge positive 0.6% (95% CI 0.5-0.8), and for food challenge positive or history of diagnosed CMA 1.6% (95%CI 1.2-1.9). The estimates were usually higher in younger age groups and in Northern Europe (Nwaru et al., 2014).

2.2 Diagnosis and treatment of CMA

The golden standard for the diagnosis of CMA is double blinded placebo controlled food challenge, but for young children the open food challenge is considered adequate (Vandenplas et al., 2024). In Finland the common diagnostic practice is to start an elimination diet for symptomatic children and if the symptoms relief, then do an open food challenge in hospital (Kaila et al., 2000, Food allergy (children): current care guidelines, 2025)), which is observed to be as reliable protocol as double blinded placebo controlled food challenge (Isolauri & Turjanmaa, 1996). Because the double blinded placebo controlled food challenge is rarely used, the definition for CMA used in studies varies, including all self-reported, positive skin prick tests, increased IgE

for cow's milk protein, and oral food challenge based definitions, complicating the comparison between the studies (Flom et al., 2019).

CMA is treated by eliminating the cow's milk protein from the diet. For infants in need of infant formula, the typical commercial formula is supplemented by the special formulas including extensively hydrolyzed formulas and amino acid-based formulas. In Finland the use of these special formulas is recommended up to child's age of 1-2 years depending on the child's growth and diversity of diet. After which the cow's milk can be substituted with vegetable milk with attention to adequate intake of calcium, energy, protein, vitamins, and minerals from diet. If the dietary intake is not adequate, the supplements are used (Food allergy (children): current care guidelines, 2025). The level of elimination diet is individual as part of the children with CMA can use baked milk products while others get anaphylactic reactions even from the small amount of cow's milk protein and might need also avoidance of skin and inhalant contacts (Fiocchi et al., 2010).

For non-IgE mediated CMA the prognosis is good and most of the children develop tolerance in childhood (Caubet et al., 2017). For IgE-mediated CMA the tolerance is developed for approximately 50% of the children up to age of 5 years, but the individual prognosis differs. The high IgE-levels, atopic co-morbidity, and severe reactions are associated with worse prognosis (Flom et al., 2019). Overall the prognosis of tolerance development is approximated to be 85-90% (Høst, 2002). Because of the good prognosis of CMA, the regularly performed food challenges are important for the avoidance of the irrelevant food elimination diet (Fiocchi et al., 2010)

2.3 Factors associated with CMA and other food allergies

The incidence of allergic diseases, including food allergy, have increased rapidly during the 20th century. The rapid increase can't be solely explained by genetics, therefore the environmental factors have also thought to have a role in development of allergic diseases, including CMA. (Lack, 2012). Even though CMA is one of the most common food allergies in children, the knowledge about the factors behind the CMA is limited (Abrams & Sicherer, 2021).

2.3.1 Genetical risk factors

As allergic diseases are strongly hereditary, any allergic disease in the child, parents, or siblings increases child's risk for the development of food allergy (Nwaru et al.,

2014). For CMA this increase in risk may be cumulative as in the population based survey the number of parents affected is directly associated with the risk of CMA in the offspring (Pyrhönen et al., 2011). Because the genetic background is complex, it is likely that multiple genes affect the risk of CMA. A systematic review has reported an association of genetic variants at FLG, HLA, and IL13 with food allergy (Suaini et al., 2019).

Sex has thought to have a role in development of food allergy. The studies have reported increased risk of food allergy for both males (Karpa et al., 2012; McGowan et al., 2015) and females (Acker et al., 2017; Mitselou et al., 2018). In case of CMA the Finnish nested case-control study has reported increased risk for males (Metsala et al., 2010) whereas the Swedish population based cohort study has reported increased risk for females (Strinnholm et al., 2014). The methods between studies vary, including the study design, definition of CMA and food allergy, which may explain this discrepancy. Thus, the role of sex as a risk factor is not yet known.

The race and ethnicity are also observed to affect the susceptibility for the development of food allergy. The systematic review from the USA observed that black children might be in higher risk of food allergy than white children. Because of the differences in study methods and the definition of food allergy among the studies, the real role of ethnicity or race as a risk factor remains unknown (Greenhawt et al., 2013). It is notable that race and ethnicity are also associated with the environmental factors, including the socioeconomical factors, health awareness, and feeding practices, which are better known as risk factors for food allergy and these could possibly explain the discrepant results between studies (Sicherer & Sampson, 2018).

The interactions with genes and environment are complex and constant. It is observed that the risk for development of diseases may differ between individuals living in the same environment with different genetic background or between individuals living in the same environment with similar genetic background, suggesting possible gene-environment interactions. (Kauffmann & Demenais, 2012). In case of food allergy, this has been studied in Australia in population-based cohort setting. They observed that in children born in Australia food allergy is more common in children with parents born in Asia compared with parents born in Australia. Thus, suggesting that genetic background affects how the environmental risk factors affect to the development of food allergy (Koplin et al., 2014). Even though the allergic diseases are strongly hereditary it seems that the genetical risk factors cannot independently explain the development of CMA.

2.3.2 Environmental risk factors

Food allergies appear early in life, therefore the environmental factors during early childhood is thought to have a significant role, and their effects may begin already in utero (Prescott, 2013). The increase in prevalence of allergic diseases was first observed in more developed western countries. The same is now seen in developing countries all over the world, where simultaneously the westernization of lifestyle happens. Therefore the environmental factors distinctive for western lifestyle, (including dietary changes, increased amount of pollutants, changes in microbial exposures) are suggested to increase the risk of allergic diseases (Prescott & Saffery, 2011). For CMA these early environmental factors may have even greater role as it is mostly a disease of infancy and early childhood (Vandenplas et al. 2023). For CMA a nested case-control study has reported that assisted birth and high birth weight are associated with higher risk, whereas the lower maternal socioeconomic status, smoking during pregnancy, and number of previous pregnancies are associated with lower risk of CMA (Metsala et al., 2010).

The way these environmental factors alter immunology is suggested to be in their ability to modify the gene expression by epigenetic mechanisms such as DNA-methylation and histone modifications. These changes modify the development of maturing immune system, leading to increased susceptibility to allergic diseases. As the developing immune system is most vulnerable to these changes, the early environmental factors, including the prenatal factors, may have a major role. Further, these changes seem to be permanent why it is possible that they are hereditary, which could explain the rapid increase in prevalence of allergic diseases (Prescott & Saffery, 2011).

The hygiene hypothesis has been a base for the studies of environmental and lifestyle factors affecting the risk of allergic diseases. The hypothesis originated from the observation that family size and the order of siblings was indirectly associated with development of certain allergic diseases, and from this data it was suggested that early exposure for microbial agents could be protective for allergic diseases (Strachan, 1989). Since then, the hygiene hypothesis has been studied in multiple cohorts comparing the prevalence of allergic diseases, typically asthma and hay fever, between the urban and rural areas, and based on these studies the rural living environment seems to be protective for the development of allergic disease. The urban and rural living differentiates in multiple ways, including the amount of microbial exposures through diet and environment, infections, dietary habits, family size, as well as genetical background, therefore the exact protective factors and the mechanisms how these different factors affects to the risk is still undiscovered

(Schröder et al., 2015). For food allergy this phenomenon has been noticed in South African cross-sectional study, which observed that the prevalence of food allergy for black African participants was significantly lower in rural areas compared to overall prevalence and prevalence in black Africans in urban areas (Botha et al., 2019).

Based on this original hygiene hypothesis, a hypothesis “gut microbial deprive hypothesis” has been developed. It suggests, that the increased hygiene standards have altered the intestinal colonization, thus leading to failure in the development and maintenance of oral tolerance, and further leading to unwanted immunological reactions toward harmless antigens, including food proteins (Wold, 1998). Thus, there is growing interest in the effects of lifestyle changes on the development and composition of gut microbiota. The early development of gut microbiota during perinatal period is influenced by multiple environmental factors including mode of delivery, use of antibiotics, early nutrition, and the living environment (Renz et al 2017). The maturation towards the adult like gut microbiota happens during the first 3 years of life and the variation of bacterial communities between individuals is greater in children than adults, suggesting the importance of the early environmental factors in the development of the gut microbiota (Yatsunenکو et al., 2012). There is also growing knowledge on the importance of the normal microbial colonization of the gut in the development of the healthy immune system and the prevention of allergic diseases including food allergy (West et al., 2015). The effects to immune system by microbiota seem to begin already in utero as it has been noticed that maternal microbiome can affect growing fetus by transplacental transfusion (De Agüero et al., 2016).

The mode of birth may alter infant’s gut microbiota as the gut microbiota is observed to differ between children born vaginally and by cesarean section (Rutayisire et al., 2016). Therefore, the mode of delivery could affect to child’s risk of food allergy. The systematic review reported an increased risk for sensitization in children born with caesarean section, but the effect on the clinical food allergy is still unsure because of the lack of studies (Koplin et al., 2008). For CMA, case-control study has observed an increased risk in children born with caesarean section (Metsälä et al., 2010).

Also the use of antibiotics given perinatally have suggested to have both short- and long-term effects on the gut microbiota (Jernberg et al., 2010). Two case-control studies have reported that a child’s early use of antibiotics is associated with the increased risk of CMA (Hirsch et al., 2017; Metsälä et al., 2013). In the study from Finland, this association was also observed between maternal use of antibiotics before and during pregnancy and offspring’s CMA (Metsälä et al., 2013).

It is observed that the gut microbiota differs when compared children with CMA to non-CMA children before and after the elimination of cow's milk protein from diet (Thompson-Chagoyan et al., 2010). Even though the gut microbiota seems to differ between food allergic and healthy individuals in multiple ways, the role of these differences and whether these differences are primary or secondary for food allergy is still unknown (Dhondalay et al., 2018). Although the gut microbiota studies are promising, they have still multiple methodical issues such as the collection, storage, and analysis of samples. In addition the healthy microbiota that promotes oral tolerance is not yet discovered (West et al., 2015).

2.3.3 Nutrition

The early manifestation of food allergies has generated interest in the role of maternal and early childhood food and nutrient intake in prevention of food allergy and CMA.

For the reduction of food allergy, exclusive breastfeeding has been recommended up to child's age of 4-6 months, because of breast milk's multiple known benefits for the mother and infant (Agostoni et al., 2008; Greer et al., 2019; Muraro et al., 2014). However, the actual role of breastfeeding in the prevention of food allergy, including CMA, is still unknown. A systematic literature review stated that there is insufficient evidence about the relationship of exclusive breastfeeding and the risk of child's food allergy (Güngör et al., 2019). Further, the EAACI guidelines concluded that breastfeeding may not reduce the risk of CMA or other food allergies. Thus, no recommendation in the light of prevention of CMA cannot be made, but breastfeeding is still recommended whenever possible because of its known other benefits (Halcken et al., 2021).

When breastfeeding is not possible or sufficient, the cow's milk based infant formula is introduced in child's diet sometimes as early as in the first days of life. Very early exposure to cow's milk formula (first 3 days of life) compared to breastfeeding or amino acid based formula has been observed to increase the risk of CMA in one RCT (Urashima et al. 2019). In contrast, one prospective cohort reported that introducing cow's milk protein within the first 14 days of life was associated with a decreased risk of developing CMA, but this decreased risk was evident only when regular exposure continued until the age of 4–6 months (Katz et al., 2010). A recent RCT studied the role of regular exposure to cow's milk formula (at least 150mg cow's milk protein) during the age of 1 to 2 months and observed that early daily consumption of cow's milk protein prevented the development of CMA (Sakihara et al., 2021). Thus, it seems that the timing of the exposure to cow's milk protein may

be crucial in prevention of CMA. For the prevention of CMA, the EAACI guideline recommends to avoid supplementary feeding in breastfed infants by regular cow's milk formula for the first week of life, as the early ingestion of cow's milk protein may increase the risk of CMA (Halcken et al., 2021). In Finland, it is recommended to use commercial infant formula for all infants regardless of their age, if the breastfeeding is not possible (*Eating Together-Food Recommendations for Families with Children*, 2019).

Introduction of the solid foods to the child's diet is recommended to start between 4-6 months of age (Muraro et al., 2014). The systematic review and meta-analysis concluded that early introduction of allergenic foods is associated with reduced risk of IgE-mediated food allergy. In particular, the introduction of peanut and egg before the age of 6 months can substantially decrease the risk of developing the respective allergies (Scarpone et al. 2023). Evidence accumulated from RCTs has led to changes in guidelines. In Finland, current recommendations advise the introduction of complementary foods from the age of 4 months, and no later than 6 months, with particular emphasis on offering egg and peanut in an age-appropriate and safe form before 6 months of age to support the prevention of food allergies (Food allergy (children): current care guidelines, 2025). The explanation for the role of early nutrition for the development of food allergy has also been searched from the gut microbiota as the early nutrition affects the maturation as well as the diversity of gut microbiota (Torow & Hornef, 2017). There is also growing interest towards the use of probiotics, prebiotics, and synbiotics in the modulation of early gut microbiota and by that in food allergy prevention (Huang et al., 2017).

Multiple nutrients, such as vitamin D, folate, and polyunsaturated fatty acids (PUFA) are observed to have immunomodulatory effects, therefore their effects to the development of food allergy have gained most interest. For vitamin D there are receptors presented in majority of body cells including the immune system cells. Vitamin D have multiple effects on the immune system, it may, for instance, provoke Th2-cell reactions, suppress Th1 reactions, increase T-regulatory cell populations as well as inhibit the production of IgE (Heine et al., 2008; Hewison, 2012). The folic acid acts as a methyl donor and by that it has a role in epigenetic programming. In animal and epidemiological studies, maternal use of folic acid during pregnancy has shown to modify genes linked to the allergic inflammation, suggesting that the excessive use during late pregnancy may provoke the development of allergic disease in the offspring. However, the evidence is limited and scarce, thus no recommendations should be given (McStay et al., 2017). The immunomodulatory effects of n-3-PUFA and n-6-PUFA have observed to be partly opposite, as the n-3-PUFA are observed to

have anti-inflammatory and the n-6-PUFA to have proinflammatory effects (Miles & Calder, 2017) The antioxidant nutrients are hypothesized to both promote allergic inflammation (Allan et al., 2010) as well as prevent the development of allergic diseases (Devereux & Seaton, 2005; Seaton et al., 1994). Further, the immunomodulatory effects by antioxidants may vary between the nutrients, and are best known for Vitamin A and E (Garcia-Larsen et al., 2018). The interests of the role of these nutrients in development of CMA and other food allergies is mainly focused in nutrient intake during pregnancy or lactation and is discussed more deeply in the following sections. In conclusion, nutrition can affect the development of food allergies through the immunomodulatory changes by nutrients or foods, through their role in developing tolerance for the food antigens as well as through to changes in gut microbiota. In the prevention of food allergies and CMA, both the maternal diet during pregnancy and lactation, as well as the early nutrition of the child itself may have a role. However the evidence is still limited and currently the modification of the diet or avoidance of potential food allergens during pregnancy or lactation is not recommended for the prevention of food allergy, including CMA, in the offspring (Halcken et al., 2021).

2.3.4 Development of tolerance

In food allergy the normal development of oral tolerance towards a specific allergen fails, leading to allergic inflammation, which is characterized by increased production of allergen specific Th2-cells and allergen specific IgE antibodies. For the prevention of food allergies, the contribution of the development and maintenance of tolerance for the foreign antigens, called allergens, is crucial, but the immunological mechanisms behind it are not yet completely understood. It has been suggested that allergen specific T-reg cells play a major role in the development of tolerance. This is based on the observation that the amount of these cells increases in patients who have undergone successful allergen specific immunotherapy. The antigen specific T-reg cells are mainly produced in the peripheral lymphoid tissues, where the ingested antigen is presented to the naïve Th-cells, which then mature to the antigen specific T-reg cells. T-reg cells are capable of inhibiting the mast cell activation, naïve Th-cell conversion to the allergen specific Th2-cell, and IgE production by B-cells, and thus have a key role in food allergy prevention (Noval Rivas & Chatila, 2016). There is also growing knowledge about the role of B-reg cells for the development of tolerance. Similarly, for the T-reg cells, the amount of antigen specific B-reg cells in circulation is reported to increase with allergy resolution. The best-known effect of

tolerance induced by B-reg cells is their ability to produce cytokine IL-10, which can maintain the T-reg population and suppress the development of Th2-cells. In addition, IL-10 could be capable of directly reducing allergic inflammation by inhibiting monocyte activation and maturation. B-reg cells are also shown to produce IgG4, which competes with the allergic specific IgE, and by that prevents the degranulation of mastocytes, basophils, and eosinophils, leading to dampening of allergic inflammation (Braza et al., 2014).

It seems that the postnatal period is crucial for the development of oral tolerance, as the production of antigen specific T-reg cells are observed to begin shortly after ingestion of the antigen (Tsuji & Kosaka, 2008). However, also the prenatal time may have a role in development of tolerance, as the antigen specific T-reg cells have observed to develop already in utero (Mold et al., 2008) and the modulation of immune systems by environmental factors seems to begin before birth (Schaub et al., 2009). The maternal diet during pregnancy may participate in this modulation of fetal immune system, and multiple factors including maternal antioxidant, vitamin D, fatty acid, and dietary fiber intake/status, are suggested to have an influence on fetal immune development (West et al., 2010).

For the development of oral tolerance also the route of the introduction of the antigen for the first time is relevant. Based on the “dual allergen exposure hypothesis” exposure to an allergen through inflamed skin because of eczema leads to allergic sensitization, whereas oral exposure for allergen is protective for food allergy and enhance the development of oral tolerance (Kulis et al, 2021).

The early introduction of solid foods into the infant’s diet seems to induce the development of tolerance, however, the optimal timing for each food is still unknown. Also, several immunomodulatory factors in breast milk promote the development of oral tolerance, which need to be considered when making recommendations. Therefore it is currently recommended to start the introduction of solid foods between 4-6 months of age, without any specific delay for the allergenic foods (Muraro et al., 2014).

2.4 Maternal diet during pregnancy and development of childhood CMA and other food allergies

Because of the early appearance of CMA, the maternal nutrient intake during pregnancy may have even major role in the development of CMA in the offspring than other food allergies. Further, multiple nutrients have been observed to have

immunomodulatory effects, as discussed earlier, which may also alter the risk of CMA in the offspring. The current knowledge about the role of maternal diet during pregnancy in development of CMA in the offspring is limited, and the studies are more focused in prevention of food allergies as a group rather than exploring individual food allergies. The role of maternal dietary factors, especially the intake of PUFA and antioxidant nutrients, for the development of CMA is discussed more in depth in this chapter.

2.4.1 The role of maternal nutrition during pregnancy

The fetal immune system develops during pregnancy and for the successful pregnancy the active and responsive immune system between fetus and mother is needed (Mor et al., 2017, Rackaityte & Halkias, 2020). During pregnancy there are three different immunological stages: proinflammatory stage during implantation and placentation (wk 0-12), anti-inflammatory stage during fetal growth (wk 13-27), and again the proinflammatory stage during parturition (wk 28-40) which prepares the infant for the delivery. During proinflammatory stages the immune environment is shifted towards Th1-immune response, whereas the anti-inflammatory stage is characterized by Th2-immune environment. The placenta has a major role in this immune regulation, therefore the environmental factors can affect the immune environment in utero through the maternal circulation (Mor et al., 2017). Maternal diet is one of these environmental factors which is shown to have an impact on fetal immune system development. For example the low levels of nutrients is noticed to affect directly to the development of leukocytes (Macpherson et al., 2017). Further, the maternal nutrition may induce epigenetic changes, which can lead to altered fetal Th1/Th2 balance and thus affect the child's subsequent risk of allergic disease (Harb et al., 2017).

The knowledge about the effects of maternal nutrient intake during pregnancy and the risk of CMA or other food allergies in the offspring is still scarce. The methods used in the studies vary and most of the data is collected observationally and only a few RCTs are available. In addition, studying the role of a single nutrient is complicated because the results are also affected by the interaction with genetics as well as the whole diet and lifestyle (Pham & Bunyavanich, 2018).

For fatty acids the studies have concentrated mainly for the possible protective effect of the maternal intake of n-3-PUFA during pregnancy, however the systematic literature researches and meta-analyses of the RCTs have not observed any association between maternal use of n-3-PUFA supplementation during pregnancy

and the risk of food allergy in the offspring (Hyunh et al., 2023, Bärebring et al., 2022). Studies have included CMA as an outcome under the group of “food allergy”, but no results using CMA as an individual outcome has reported. The role of n-3-PUFA and other fatty acids are discussed in depth later.

In case of folate, folic acid and vitamin D one population-based birth cohort study has observed and increased risk of higher maternal intake of these nutrients from supplements during pregnancy for the development of CMA in the offspring (Tuokkola et al., 2016). The association between food allergy in the offspring and maternal intake of vitamin D supplementation during pregnancy has been studied in one RCT, where they did not observe association between maternal use of vitamin D supplementation and the food allergy in the offspring (Goldring et al. 2013).

In case of antioxidants, one case-control study has observed a decreased risk of higher maternal intake of vitamin A, retinol and riboflavin, and increased risk of higher maternal intake of β -carotene during pregnancy and the development of CMA in the offspring (Kuśmierek et al., 2019). The role of antioxidants nutrients is discussed more in depth later.

For the avoidance of potential food allergens during pregnancy, the systematic literature review has concluded that the evidence is uncertain having little or no effect in prevention of food allergy (Halcken et al., 2021). For CMA birth cohort studies have observed that maternal high consumption of milk during pregnancy is associated with decreased risk and low consumption during lactation with increased risk of CMA in the offspring (Järvinen et al., 2014; Tuokkola et al., 2016). The studies about association between maternal intake of nutrients during pregnancy and the risk of

Table 1 Studies about the association between maternal diet during pregnancy and the development of cow's milk allergy in the offspring

Reference, country	Study design	Subjects (n)	Controls (n)	Exposures	Exposure measurement	Outcome	Direction of the association between exposure and outcome
Tuokkola et al. 2016, Fi	Cohort	4921		Vitamin D, Folate, Folic Acid	FFQ	CMA	↑ Vitamin D, Folate, Folic Acid
Tuokkola et al 2016, Fi	Cohort	6288		Consumption of milk	FFQ	CMA	↓ Consumption of milk
Kuśmierk et al 2019, PI	Case-control	51	25	Vitamin A, B-carotene, Retinol, Riboflavin, Vitamin E, Vitamin C	FFQ	CMA	↑ B-carotene ↓ Vitamin A, Retinol, Riboflavin

Abbreviations: ↑ direct association, ↓ inverse association PI=Poland, Fi=Finland, CMA=cow's milk allergy, FFQ=food frequency questionnaire

CMA in offspring are collected in table 1. The evidence is currently mostly based on singular studies mainly done in cohort setting.

2.4.2 The role of fatty acids

The interest in the role of maternal intake of fatty acids and the development of CMA or food allergy in the offspring focuses strongly on the maternal intake of polyunsaturated fatty acids (PUFA). The two most important groups of PUFAs are n-3-PUFAs and n-6-PUFAs. The parent fatty acid for the n-6-PUFAs is linoleic acid (LA) and for the n-3-PUFAs the alpha-linolenic acid (ALA). These are essential fatty acids as they can't be produced in human body. Other n-3- and n-6-PUFAs can be produced from these "parent PUFAs" by elongating and desaturation. Alongside these essential PUFAs, the most important dietary PUFAs are of n-6-PUFAs arachidonic acid (AA) and of n-3-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). From diet the main sources of saturated fatty acids are dairy fat, palm, coconut, and peanut oil, of LA vegetable oils, of ALA flaxseed, perilla,

canola, and soybean oil, of AA animal fats, liver, eggs, and fish, and of EPA and DHA fish and fish oils (FAO, 2009). AA, EPA, and DHA can be produced from LA and ALA, but the same enzymes are responsible for this bioconversion for both series. Thus, it is crucial that the ratio of LA to ALA is balanced in diet and that these non-essential fatty acids are also received from the diet even though the dietary intake of AA, EPA, and DHA is far lower than intake of ALA and LA (Miles & Calder, 2017).

PUFAs are found in all cell membranes, and typically in immune cells the proportion of n-6-PUFAs is higher than other PUFAs. Both n-3- and n-6-PUFAs have shown to have immunological effects, possibly opposite to each other. The n-3-PUFA are observed to have anti-inflammatory and the n-6-PUFA to have proinflammatory effects. The AA on the cell membrane increases the production of the eicosanoids responsible for inflammation. Especially the production of prostaglandins (PG) and leukotrienes is enhanced and in animal studies these eicosanoids are linked to allergic inflammation. For example, PGD₂ activates eosinophils and Th₂-type responses and PGE₂ promotes production of IL4 and IL5 from naïve Th-cells and Ig switching in B-cells towards the higher production of IgE, thus, contributing the allergic inflammation. The higher intake of n-3-PUFA from diet is observed to increase their proportion on cell membranes and leading to decreased proportion of n-6-PUFA on cell membrane. As a result, the anti-inflammatory effect of the n-3-PUFA comes mainly by reducing the amount of AA on cell membrane leading to reduced production of inflammatory eicosanoids from AA. Further, the eicosanoids produced from EPA and DHA are far less inflammatory than from AA and from EPA and DHA it is possible to produce specialized pro-resolving mediators, which have anti-inflammatory effects. The simultaneous changes in dietary patterns and increased prevalence of allergic diseases together with the known immunological effects of n-3- and n-6-PUFAs have led to the hypothesis that the intake of n-3-PUFAs could prevent the development of allergic diseases (Miles & Calder, 2017).

Fatty acids could also affect the susceptibility to developing food allergy through their interactions with gut microbiota. Especially the role of short chain fatty acids (SCFA) has been discussed in the light of gut microbiome, as in addition to dietary intake of SCFA, it can be produced from dietary fiber through the fermentation by gut microbes (Venter et al., 2019). The evidence of SCFA in immune regulation comes mainly from animal studies, which have concentrated in the SCFA produced by microbes instead of the dietary intake of SCFA. In these studies the SCFA have shown to have multiple effects on immunological cells, including the ability to promote T-regulatory cells formation in gut, and by that it might have role in allergy prevention (Corrêa-Oliveira et al., 2016). Altogether, in the future the role of gut microbiome

Table 2 Individual RCTs about the association between maternal intake of n-3-PUFAs during pregnancy and development of food allergy in the offspring

Reference, country	Study design	Subjects (n)	Controls (n)	Intervention	Control	Outcome	Results	Meta-analysis where included
Dunstan et al 2003, Au	RCT	40	43	3,7g n-3-PUFA(56.0% DHA and 27.7% EPA)/day from GA 20wk until delivery	Olive oil capsules	Physician diagnosed FA and positive SPT until the age of 12 mo	ns	Bärebring 2022, Huynh 2023
Furuhjelm et al 2009, Se	RCT	52	65	1.6 g EPA and 1.1 g DHA/day from GA 25wk to 3–4 mo of bf	n-6-PUFA 2.5 g/day and n-3-PUFA 0.28 g/day	Physician diagnosed FA and positive SPT until the age of 12 mo	↓	Huynh 2023
Furuhjelm et al 2011, Se	RCT	53	63	1.6 g EPA and 1.1 g DHA/day from GA 25wk to 3–4 mo of bf	n-6-PUFA 2.5 g/day and n-3-PUFA 0.28 g/day	Physician diagnosed FA and positive SPT until the age of 24 mo	ns any food allergy ↓ IgE-mediated FA	Bärebring 2022, Huynh 2023
Palmer et al 2012, AU	RCT	368	338	0,8g of DHA and 0,1g EPA/day from GA 21 wk until delivery	Vegetable oil capsules	Physician diagnosed FA until the age of 12 mo	ns	Bärebring 2022, Huynh 2023
Palmer et al 2013, Au	RCT	368	338	0,8g of DHA and 0,1g EPA/day from GA 21 wk until delivery	Vegetable oil capsules	Physician diagnosed FA until the age of 3 yr	ns	Huynh 2023
Berman et al 2016, US	RCT	58	26	1,06g EPA and 0,274g DHA or 0,9g DHA and 0,18g EPA per day from GA 12 to 20 until delivery	Soy oil capsules	Maternal raport of FA at 3 yr (any FA)	ns	Bärebring 2022, Huynh 2023
Komulainen et al 2024, Fi	RCT	75	72	1.9g DHA and 0,22 EPA/day from early pregnancy up to 6 mo postpartum	Equal amount of medium chain fatty acids	Physician diagnosed FA at 24 months	ns	

Abbreviations: AU Australia, Se Sweden, US, united states of America, Fi Finland, ns non-significant association, ↑ direct association, ↓ inverse association, FA= food allergy

should be taken into account when studying the role of fatty acids in allergy development, as it may alter the serum fatty acid composition (Venter et al., 2019).

Based on the known immunological effects of the PUFA, the studies about the role of maternal intake of fatty acids and development of food allergy in the offspring have mostly concentrated on the intake of n-3-PUFA from diet. None of these studies have examined the association specifically for CMA alone, however, CMA has typically

been included as one of the food allergies assessed. A recent systematic literature research and meta-analysis of RCTs including 6 studies did not find an association between maternal use of n-3-PUFA supplementation and the food allergy in the offspring. When they stratified the studies based on the outcome for “early” (during the first 3 years) and “late” (beyond 3 years of age) groups, in the early group the maternal intake of n-3-PUFA supplementation was associated with decreased risk of food allergy in offspring but the association was not observed in the late group (Huynh et al., 2023). This is supported by other systematic literature research and meta-analysis of RCTs which did not observe any association between maternal use of N-3-PUFA supplements during pregnancy and food allergy in the offspring (Bärebring et al., 2022). In both meta-analyses the methodology between the individual RCTs varied including the amount and timing of the supplementations as well as the follow up time of study subjects. Further the definition of food allergy varied between the studies, and oral food tests were not used for the diagnosis. A recent RCT supports the findings of meta-analyses as it did not observe association between maternal N-3-PUFA supplementation during pregnancy and food allergy in offspring (Komulainen et al., 2023). Individual studies included in the meta-analyses are presented in table 2. Thus, the role of N-3-PUFA in prevention of food allergy remains still unclear and no studies reporting solely CMA as an outcome are done.

2.4.3 The role of antioxidants

The antioxidant nutrients are hypothesized to promote allergic inflammation by modifying immune reactions towards the Th2-type reactions (Allan et al., 2010), but also to prevent the development of allergic disease through their ability of reducing oxidative stress and damage (Devereux & Seaton, 2005; Seaton et al., 1994). The effects of antioxidant nutrients to the immune system may vary between nutrients because single nutrients may have also direct effects on the immune system in addition to their antioxidant effects. These individual effects are best known for vitamins A and E.

Vitamin A is received from the diet either in the form of retinol or carotenoids, and the most important sources of retinol are animal sources, such as liver and milk, whereas carotenoids are received from plants (Blomhoff & Blomhoff, 2006). Vitamin A has shown to have multiple effects on the T- and B-cells activation and proliferation. For the development of allergic diseases vitamin A could have both protective effects by promoting T-reg cell induction as well as predisposing effects by promoting Th2-cell differentiation (Mora et al., 2008).

Vitamin E is received from vegetable oils, nuts, seeds, and green vegetables and it is shown to modulate the Th1/Th2 balance at the gene expression level in animal studies (Lee & Han, 2018). Further, in vitro studies have suggested that vitamin E may have immunomodulatory effects, which possibly suppress the allergic inflammation, and these effects may begin already in utero (G Devereux et al., 2002; Wassall et al., 2013; Winkler et al., 2007).

In addition to zinc's role as an antioxidant, zinc deficiency is associated with the decreased maturation of Th-cells. Zinc deficiency has observed to decrease the production of Th1-cell interleukins, but not Th2-cell interleukins, thus, leading to imbalance in Th1/Th2 responses, favoring the Th2 immune responses. Therefore zinc deficiency could favor the allergic inflammation (Prasad, 2008).

The current evidence of the effects of maternal antioxidant intake during pregnancy on the development of allergic diseases in the offspring is mainly from epidemiological studies, and only some RCTs are done. The systematic review of the RCTs and observational studies stated that the current evidence of the maternal antioxidant nutrient intake during pregnancy is scarce and do not suggest a protective effect for the development of offspring allergic disease (Garcia-Larsen et al., 2018).

The evidence about the association between maternal intake of antioxidants and the development of food allergy in the offspring is based on observational studies, mainly from cohort studies. Three studies (Yang et al. 2023, Kuśmierek et al., 2019; West et al., 2012), has measured the maternal antioxidant intake during pregnancy by questionnaires and two studies (Miyazaki et al. 2013, Gromadzinska et al., 2018) by levels of antioxidants in blood samples during pregnancy. The antioxidant nutrients investigated have varied between the studies. Any of the studies has not find an association between maternal intake of vitamin E or zinc during pregnancy and food allergy in the offspring. For other antioxidants the results were inconsistent (table 3). One case-control study has reported CMA as an outcome and observed an increased risk between high maternal beta-carotene intake during pregnancy and CMA in offspring, decreased risk with high maternal intake of vitamin A, retinol and riboflavin and CMA in offspring, for vitamins E and C no association was observed (Kuśmierek et al., 2019).

In conclusion the knowledge of the association between maternal intake of antioxidant nutrients and development of food allergy in the offspring is limited and scarce, and only one study has used CMA as an outcome. Further, the limited number of studies had methodical variation in definition of outcome and the measurement of the intake of studied antioxidant. Thus, more studies with comparable methods are needed to assess the real association.

Table 3. Studies about the association between maternal intake of antioxidant nutrients during pregnancy and the development of food allergy in the offspring

Reference, country	Study design	Subjects (n)	Controls (n)	Exposure	Exposure measurement	Outcome	Direction of the association between exposure and outcome
West et al 2012, Au	Cohort	300		Vitamin C, Vitamin E, Zinc, β -Carotene, Copper	FFQ	IgE-mediated FA	\downarrow Copper
Gromadzinska et al, 2018, Pl	Cohort	252		Vitamin A, Vitamin E, β -Carotene	Blood sample	FA	ns
Yang et al, 2023, JP	Cohort	74 948		Zinc	FFQ	FA (parental reported) until the age of 4 yr	ns
Miyazaki et al 2023, Jp	Cohort	94 794		Selenium, Manganese, Mercury	Blood sample	FA (parental reported) until the age of 3yr	\downarrow Selenium,
Kuśmierek et al 2019, Pl	Case-control	51	25	Vitamin A, β -carotene, Retinol, Riboflavin, Vitamin E, Vitamin C	FFQ	CMA	\uparrow β -carotene \downarrow Vitamin A, Retinol, Riboflavin

Abbreviations: ns non-significant association, \uparrow direct association, \downarrow inverse association Au=Australia, Pl=Poland, FA=food allergy, CMA=cow's milk allergy

2.5 Definition, mechanisms, and occurrence of T1DM

T1DM is an autoimmune disease, where the autoimmunity towards the insulin producing beta cells in pancreas leads to a chronic deficiency of insulin production. (Atkinson & Eisenbarth, 2001). T1DM develops to a genetically susceptibility individuals. The strongest association is found with the Human Leukocyte Antigen (HLA) complex, which contains three different HLA-types from which the HLA class II is the strongest predictor of T1DM development (Atkinson, 2012). The clinical T1DM is preceded by an asymptomatic preclinical phase, where the autoantibodies related to T1DM, including islet cell antibodies (ICA), autoantibodies to insulin (IAA), glutamic acid decarboxylase antibodies (GADA), and insulinoma-associated-2 autoantibodies (IA-2A), appear in the circulation (Knip et al., 2005). These

autoantibodies are suggested to develop often early in life before the age of 2 years (Parikka et al., 2012; Ziegler et al., 2012) The development of these autoantibodies is used as a marker for the later development of T1DM, but the duration of the preclinical phase varies between individuals (Knip et al., 2010). Further, not all individuals with the autoantibodies in circulation develop clinical T1DM, and the risk of T1DM is associated with the number of the detectable autoantibodies in the circulation, where two or more autoantibodies are highly associated with the development of clinical T1DM (Knip et al., 2005).

The prevalence of T1DM has increased in western countries over the decades and the increase is predicted to continue (Patterson et al., 2009). In Finland the prevalence of T1DM is one of the highest in the world and has been increasing: in 1980 the annual incidence was 31.4/100 000 children whereas in 2005 the incidence was 64.2/100 000 in children under 15 years old. During the years 1980-2005 it was noted that the increase in incidence emphasized to the younger age groups, as the increase in incidence was highest in children under the age of 4 years (Harjutsalo et al., 2008). However, during the years 2006-2011 the incidence has plateaued and starting to decline among children under the age of 7 years (Parviainen et al., 2020). The strong genetics behind T1DM cannot totally explain this rapid change. Thus, the environmental and lifestyle factors are thought to have a major role in the development of T1DM (Knip et al., 2005). Because of the early appearance of T1DM related autoantibodies and the rapid changes in incidence especially in the early age groups, it is possible that the environmental factors early in life, including the diet in infancy, may have a significant role in development of T1DM (Knip et al., 2010).

2.6 Diagnosis and treatment of T1DM

The preclinical phase of T1DM is characterized by the increasing loss of beta cells in the pancreas, leading to the loss of insulin release, which manifests as an impaired glucose tolerance. As the loss of beta cells continues, it eventually leads to hyperglycemia, which is the base for the diagnosis of T1DM. The WHO diagnostic criteria for diabetes are: 1. fasting plasma glucose level ≥ 7.0 mmol/l, 2-hour plasma glucose level after 75g oral glucose tolerance test $\geq 11,1$ mmol/l, HbA1C ≥ 48 mmol/l, or random plasma glucose level ≥ 11.1 mmol/l in an individual with symptoms such as polydipsia, polyuria, and weight loss. If the individual does not have symptoms, repeated tests are needed, preferring the same test in which the diabetic plasma glucose level was first recognized (WHO, 2019). As in T1DM the loss of beta cells from pancreas is permanent, and causes the lack of own insulin production,

leading to blood hyperglycemia, the insulin replacement therapy is always needed as a treatment (Atkinson & Eisenbarth, 2001).

2.7 The coexistence of CMA and T1DM

The factors behind the possible associations between T1DM and CMA are diverse including at least immunological, dietary, and gut microbial aspects. When evaluating the role of CMA in the development of T1DM, the possible atopic origin of the CMA as well as the elimination of cow's milk from child's early diet should be considered. Further, because of the rapid increase in prevalence of both CMA and T1DM, these diseases might share some risk factors, which enhance the development of both allergic and autoimmune immunologic reactions.

2.7.1 The role of cow's milk and other dietary factors in development of T1DM

Environmental factors, including dietary factors, are thought to affect the development of T1DM, as the increase in incidence of T1DM has been rapid, and only a relatively small number of subjects are at genetically high risk of T1DM. Cow's milk is suggested to be one of these potential dietary risk factors. In RCT it is observed that children with T1DM have elevated humoral response to multiple cow's milk proteins (Luopajarvi et al. 2008). The underlying mechanisms proposed are that immunological reactivity or the permeability of the intestine to cow's milk proteins may be enhanced in newly diagnosed T1DM patients. Additionally, it has been suggested that children who develop T1DM consume dairy products differently (Knip 2003). For the association between T1DM and consumption of cow's milk both the introduction age of cow's milk and the amount of used cow's milk have studied as an exposure, and both T1DM as well as development of T1DM related autoantibodies are used as an outcome. Studies have included different milk types as an exposure, as dairy products are introduced in the infant's diet at different times as well as the amount of consumption of different dairy products vary during the childhood. First, cow's milk is consumed in form of infant formulas, and thus for many children it is the first foreign dietary protein introduced into the diet (Knip & Simell, 2011). Later the sour milk products are introduced in the infant's diet. After one year of age, infant formula is replaced with liquid milk. In Finland, nutritional recommendations suggest that toddlers consume 4 dl of liquid dairy products and 1 slice of cheese per day to ensure

sufficient calcium, iodine, and vitamin D intake (*Eating Together-Food Recommendations for Families with Children*, 2019). The processing of dairy products varies, and products can undergo both homogenization and heat treatments. Different types of milk are presented in figure 2. As the factors in milk that have been under interest include bovine insulin and milk protein and the procession may alter these factors, thus the procession of dairy products has been considered in one study when defining exposures (Koivusaari 2024 et. al).

Considering cohort studies, the age at which different dairy products are introduced in the diet does not appear to be associated with T1DM or the development of autoimmunity (table 4). The most studied exposure has been milk-based formula before the age of 5 months. In studies where the study cohort had a genetic risk for T1DM, the cohort studies did not find an association between early introduction of milk-based formula and the development of T1DM related autoimmunity (Virtanen et al 1998, Couper et al 1999, Ziegler et al 2003) also no association was seen for the development of type 1 diabetes in nested case-control study (Virtanen et al 2000). In studies based on the general population, two cohort studies have reported that exposure to cow's milk formula was associated with increased risk for development of T1DM related autoimmunity (Wahlberg et al 2006, Holmberg et al 2007), however for T1DM no association was seen in one cohort study (Wahlberg et al 2005). The introduction age varies between the studies (from first days of life to 5 months of age) causing methodological variation between studies. In

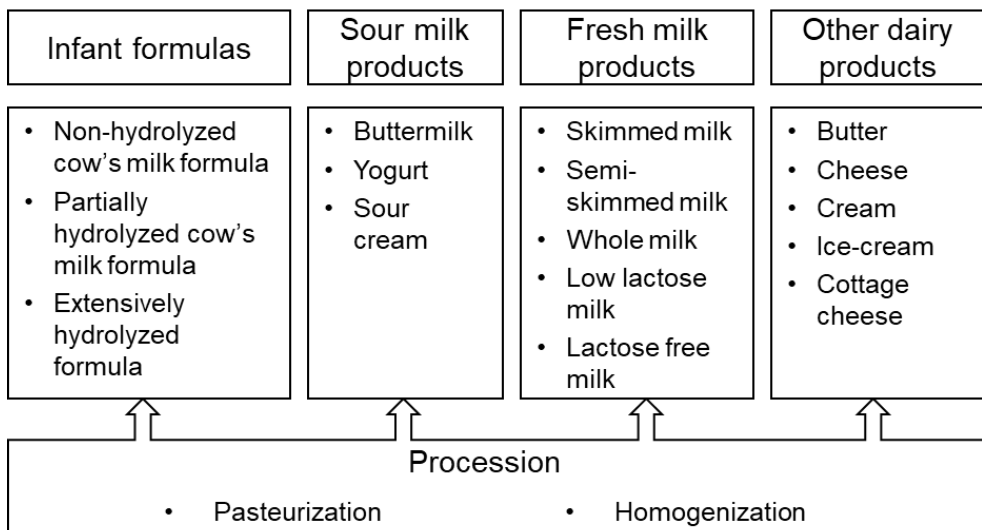


Figure 2. Different types of milk products

addition only few studies have considered the degree of hydrolysis of milk-based formula which may be significant for the risk of developing autoimmunity. In the study by Hummel et al. 2017, it was found that early exposure to extensively hydrolyzed formula compared to partially hydrolyzed formula increased the risk of developing autoimmunity, especially when exposure was early (within the first week). In the same study, no association was found with other forms of milk (cow's milk, regular cow's milk formula) compared to partially hydrolyzed formula. This result is interesting, as one RCT has studied the role of cow's milk in developing T1DM, and it did not observe any difference between the groups of children using either extensively hydrolyzed formulas or commercial formulas in development of T1DM until the age of 11.5 years (Knip, et al., 2018). It is possible that both exposures in this study elevated the risk of T1DM, and thus no difference was observed.

For amount of used cow's milk during childhood, cohort studies suggest that higher consumption of fresh dairy products (mainly liquid) is associated with an increased risk of developing T1DM related autoimmunity (table 5). Two of these studies have been conducted in Finnish study population with a higher genetic risk for T1DM (Virtanen et al 1998, Niinistö et al 2024), and one study in Swedish general population (Wahlberg et al 2006). The nested case-control studies conducted in a Finnish study population with a higher genetic risk for T1DM support the cohort studies (Virtanen et al 2012, Niinistö et al 2017). The systematic literature review and meta-analysis by Lampousi et al. 2021 stated that higher intake of cow's milk products (>3 glasses of milk per day) increases the risk of both T1DM and development of T1DM related autoimmunity (RR(T1DM) 1.81 95%CI 1.12-2.91, RR(IA) 1.25 95%CI 1.06-1.47). The studies about other dairy products are more contradictory. High consumption of fermented dairy products has been associated with both higher (Virtanen et al. 1998) and lower (Niinistö et al. 2004) risk to develop T1DM related autoimmunity in genetically high-risk study populations. In population-based study groups, no association was found (Wahlberg et al. 2006). The evidence regarding cow's milk-based formula is also contradictory; cohort studies in genetically higher-risk populations have found both lower risk (Niinistö et al. 2024) and no significant risk (Koivusaari et al. 2024), while a nested case-control study found higher risk (Virtanen et al. 2012) between the higher intake of cow's milk based formula and the development of T1DM related autoimmunity. No association has been observed between the intake of cheese and with the development of T1DM related autoimmunity (Virtanen et al. 2012, Koivusaari 2020, Niinistö 2024). One factor behind the inconsistent findings may be related to milk processing. Koivusaari et al. studied the association of differently processed milks with the development of

Table 4 Studies about the association between the introduction age of different milk types and development of T1DM or T1DM related autoimmunity

Reference, country	Study design	Subjects (n)	Genetical susceptibility for T1DM (yes/no)	Controls (n)	Outcome	Timing of exposure (before the age of)	The direction of the association between outcome and different types of milk products					
							Cow's milk formula	Cow's milk based formula	Hydrolyzed cow's milk based formula	Extensively-hydrolyzed cow's milk based formula	Dairy products	
Holmberg et al, 2007, Sw	cohort	3788	no		IAA	4 months		↑				
Uusitalo et al, 2018, Fi	cohort	7563	yes		IAA	5 months	ns		ns		ns	
Virtanen et al 1998, Fi	cohort	725	yes		IAA	2 months		ns				
Virtanen et al 2006, Fi	cohort	3565	yes		IAA		ns					
Savilahi & Saarinen 2009, Fi	cohort	6209	no		T1DM	2 months			ns	ns	ns	
						1 week			ns	ns	ns	
Couper et al 1999, Au	cohort	317	yes		IAA		ns		ns			ns
Wahlberg et al 2006, Sw	cohort	7208	no		IAA	2 months		↑				
Virtanen et al 2000, Fi	nested case-control	33	yes	254		2 months		ns				
Wahlberg 2005, Sw	cohort	6000	no		IAA	2 months		ns				
Ziegler 2003, De	cohort	1610	yes		IAA	3 months		ns				
Frederiksen 2013, US	cohort	1835	yes		T1DM		ns					
Hummel 2017, US, Fi, De, Sw	cohort	8676	yes		IAA	3 months	ns		ns	ns	ns	ns
						7 days			ns			↑
Hakola 2018, Fi	cohort	5915	yes		IAA	4 months	ns					

Abbreviations: IAA=T1DM related autoantibodies, n/s non-significant association, ↑ direct association, Au=Australia, De=Germany, Fi=Finland, Sw=Sweden

Table 5 Studies about the association between the amount of used cow's milk products and development of T1DM or T1DM related autoimmunity

Reference, country	Study design	Subjects (n)	Genetical susceptibility for T1DM (yes/no)	Controls (n)	Outcome	The direction of the association between outcome and different types of milk products						
						Cow's milk products	Liquid fresh cow's milk	Cow's milk based formula	Hydrolyzed formula	Sour milk products	Cheese	
Virtanen et al 1998, Fi	cohort	725	yes		IAA		↑				↑	
Wahlberg et al 2006, Sw	cohort	7208	no		IAA		↑	ns			ns	
Niinistö et al, 2024, Fi	cohort	2069	yes		IAA		↑	↓	ns		↓	ns
Virtanen et al 2012, Fi	nested case-control	232	yes	803	IAA	↑	↑	↑			ns	ns
Virtanen et al 2000, Fi	nested case-control	33	yes	254	T1DM		↑				↑	
Niinistö 2017 et al, Fi	nested case-control	240	yes	480	IAA		↑					

Abbreviations: IAA=T1DM-related autoantibodies, n/s non-significant association, ↑ direct association, ↓ inverse association, Fi=Finland, Sw=Sweden

T1DM related autoimmunity, finding that non-fermented milk products, fermented milk products, and homogenized milk products were associated with a higher risk of developing T1DM related autoimmunity, while the degree of pasteurization did not appear to affect the level of risk. Thus, it is possible that also the processing alters the risk between consumption of milk products and development of T1DM.

2.7.2 The immunological factors behind T1DM, CMA, and allergic diseases

The immunological reactions behind allergic diseases and T1DM are different and partly controversial. Allergic inflammation is characterized by presence of Th2-cells, whereas Th1-cells are thought to have a crucial role in provoking the autoimmune reaction in T1DM. Both Th-cells mature from the naïve CD4⁺ Th-cell under the influence of different cytokines. The cytokines IL-12, IFN- α , and IFN- γ provoke the maturation of Th1-cells, whereas the cytokines IL-2 and IL-4 lead to the maturation of Th2-cells. Further, the mature Th1-cell produces IFN- γ , which inhibits the production of IL-4 leading to the suppression of the maturation of Th2-cells. The same is seen other way around, as the mature Th2-cell produces IL-4, which inhibits the production of IFN- γ (Annunziato & Romagnani, 2009). Based on this reciprocal counter-regulation between Th1 and Th2 cells, the Th1/Th2-paradigm was presented, which suggests that allergic and autoimmune diseases could not manifest simultaneously (Coffman, 2005; Sornasse et al., 1996).

The pathophysiology behind these diseases is later discovered to be more complex, as Th2 cells have shown to have role in autoimmunity and Th1 cells in allergic inflammation. Further, new Th-cells have been discovered, for example Th17, which have been linked to both the development of T1DM and allergic diseases. In addition Treg cells have suggested to have a role in maintaining the tolerance and thus preventing the development of both allergic and autoimmune diseases (Shah, 2012). The new knowledge about the pathophysiology behind these diseases and the lack of consistent epidemiological observations of the inverse association between T1DM and allergic diseases suggests the Th1/Th2-paradigm to be an oversimplification.

The epidemiological studies about the association of allergic diseases and T1DM have reported conflicting results. The systematic literature review and meta-analysis of observational studies observed a slight inverse association between asthma and T1DM, but not for allergic rhinitis or atopic eczema (Cardwell et al., 2003). However, in this meta-analysis the results from individual studies were scarce and the evidence

was mainly from case-control studies. The cohort studies about the association between allergic diseases and T1DM are presented in table 6. Only one of these cohort studies has studied CMA and observed an increased risk between food allergy against egg, cow's milk and/or nuts and T1DM related autoantibodies (Wahlberg 2011), in same study the increased risk was also observed for wheezing during first year of life and eczema. However, three cohort studies have studied the association between eczema and T1DM and observed that eczema decreased the risk of T1DM (Thomsen 2011, Schmitt 2016, Krischer et al, 2020.). For asthma both increased (Hsiao 2015) and decreased risk (Krischer et al, 2020) for T1DM have observed in cohort studies. Further, one case-cohort study observed that the previous asthma increased the risk of T1DM, whereas previous T1DM decreased the risk of asthma, suggesting that the sequential appearance of the diseases may affect to the direction of the risk (Metsälä et al 2018). The association between CMA and T1DM is still undiscovered as this has been studied by only one cohort study.

Table 6 The epidemiological studies about the association of allergic diseases and T1DM

Reference, country	Study design	Subject (n)	Controls (n)	Exposure	Outcome	Data sources	The direction of the association between allergic outcome and T1DM (disease order not taken into account unless otherwise specified)							
							Asthma	Wheeze	Eczeema	Rhinitis	Hay fever	Food allergy		
Kero et al, 2001, Finland	Cohort	60 254		Asthma	T1DM	r	ns							
Wahlberg et al 2011, Sweden	Cohort	7208		Wheezing, eczema, rhinitis, allergy against egg, cow's milk, fish and nuts/almonds	Positivity for IA-2A or GADA	q,bs		↑		↑				↑ (egg, cm, fish, nuts)
Thomsen et al, 2011, Denmark	Cohort	54 530 twins		Atopic dermatitis, asthma, hay fever	T1DM	r, q	ns			↓				ns
Schmitt et al, 2016, Germany	Cohort	655 815		atopic derarntitis	T1DM	r								↓
Hsiao et al, 2015, Taiwan	Cohort	17 725		asthma	T1DM	r		↑						
Metsälä et al, 2018, Finland	Case-cohort	9541 children with T1DM 82473 children with asthma	171 138	T1DM or asthma	Asthma for children with T1DM, T1DM for children with asthma	r		↓ (prior T1DM)		↑ (prior asthma)				
Krischer et al, 2020, multinational	Cohort	2159 children with genetical susceptibility for T1DM		Positivity for IAA, GADA or IA-2A	Asthma, allergic rhinitis, eczema			↓		↓				ns

Abbreviations: r=register, q=questionnaire, b=blood sample, ns non-significant association, ↑ direct association, ↓ inverse association

3 AIMS OF THE STUDY

The aim of this study was to investigate the association between maternal intake of antioxidants and fatty acids during pregnancy and development of CMA in the offspring. As well as investigate the association between CMA and development of T1DM.

The study aims presented more precisely with the information of the part-study in which this was investigated:

- Investigate the association between maternal antioxidant intake during pregnancy and development of cow's milk allergy in the offspring until the age of 3 years (Study I)
- Investigate the association between maternal fatty acid intake during pregnancy and development of cow's milk allergy in the offspring until the age of 3 years (Study II)
- Investigate the association between cow's milk allergy and development of type 1 diabetes until the age of 16 years (Study III)

4 SUBJECTS AND METHODS

4.1 Study design, data sources and collection

4.1.1 Maternal dietary factors during pregnancy and development of CMA in the offspring

We collected the data for studies I and II from the DIPP Nutrition study which is executed in frame of the type 1 Diabetes Prediction and Prevention (DIPP)-study. DIPP-study is an ongoing multidisciplinary birth cohort study which is carried out in Finland in three university hospital areas: Turku, Tampere and Oulu since 1994, aimed to research the factors affecting the development of T1DM. From the study areas all born children with a parental written consent of approval for the study are screened for the HLA-conferred susceptibility for T1DM from cord blood. Children with moderate or high genes are requested to participate in the study (14% of the children).

The DIPP Nutrition study is a prospective population-based cohort study which began in September 1996 in Oulu and in August 1997 in Tampere University Hospital. The study is aimed to research the effects of perinatal and early nutritional factors in development of T1DM related autoantibodies, T1DM, childhood allergies and asthma, and obesity. We obtained the data for studies I and II from the study period of August 1997 and September 2004, when the maternal dietary data for pregnancy was collected. We included children with the available information of personal identity code (n=6288) in the study. The maternal dietary data from pregnancy and the CMA status of children was available for 4921 children (78.3%). The children with disabilities or diseases, whose parents were of non-Caucasian origin, or did not understand Finnish, English, or Swedish were excluded. All children still at follow up at the age of 5 years, also including children without dietary data, were asked to participate in the Asthma and Allergy sub-study. The flow chart of the cohort of

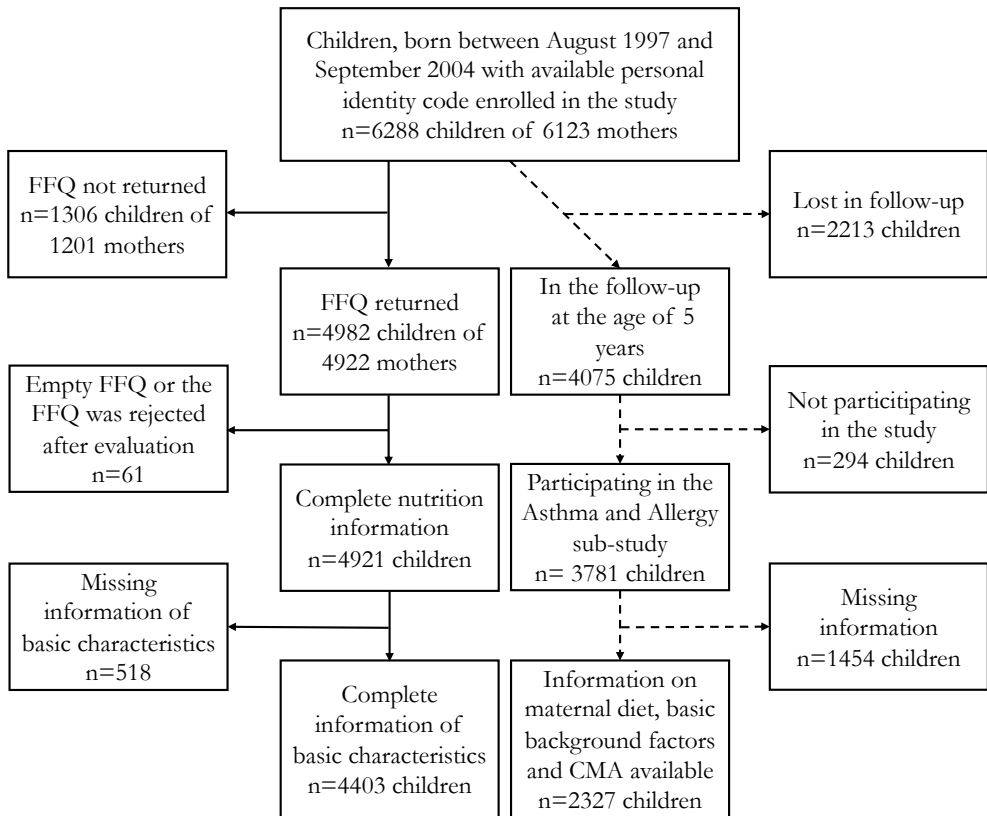


Figure 3 The flow chart of the study participants in the studies I and II. FFQ, food frequency questionnaire, CMA, cow's milk allergy

children included in the studies I and II from the DIPP nutrition study as well as from the Asthma and Allergy sub-study is presented in the figure 3.

4.1.2 Association between type 1 diabetes and cow's milk allergy

The study III is based on nationwide register data from four nationwide registers (table 7), and we applied a case-cohort design to study the association between prior CMA and the risk of subsequent T1DM. The initial birth cohort consists of all children born in Finland between 1.1.1986 and 31.12.2008. From this initial birth cohort, we identified all children diagnosed with T1DM before the age of 16 years or the end of 2009 as cases (n=7754). From the initial birth cohort, we selected a random 10% sample for each birth year as a reference cohort (n=137 798). Because of the study

Table 7 The description of registers used in the study of the association between cow's milk allergy and type 1 diabetes (study III)

Register	Obtained data	Maintaining authority
Population Register	Selection of initial and reference cohort	Social Insurance Institution
Special Reimbursement Register	Date of special reimbursement for the costs of drugs or infant special formulas	Social Insurance Institution
Drug Prescription Register	Purchases of drugs and special infant formulas	Social Insurance Institution
Medical Birth Register	Maternal background and perinatal factors	Finnish Institute for Health and Welfare

design 859 children with T1DM were included in the random sample and therefore in the reference cohort. CMA was considered as exposure diseases.

4.2 Definition of cow's milk allergy and type 1 diabetes

In studies I and II we defined CMA based on a valid special reimbursement for the special infant formula and complemented it by the parental information of child's CMA. We collected the parental information by validated questionnaire fulfilled by parents in the study visits at the child's age of 6 and 12 months and annually thereafter until the age of 9 years (Tuokkola et al., 2010; Tuokkola et al., 2008).

In Finland all the special reimbursements for the costs of medicine or special formulas are applied from the Social Insurance Institution (SII) by the written statement of the physician, in case of T1DM and CMA by pediatrician. The statement describes used diagnostic protocols and the individual course of the diseases. For all diseases there is specific criteria assessed by the SII, which need to be fulfilled to be able to receive the special reimbursement. These criteria are evaluated from the written statement by independent specialists in SII. The special reimbursement for the costs of medicine or special formulas needed in the treatment of the disease is not dependent of the child's place of residence or treatment, or social economic status.

In study III the special reimbursement for special infant formula was our main definition for CMA. In all studies I, II and III we collected the information of the special reimbursement from the Special Reimbursement Register maintained by the SII. We

used the unique personal identity code to link the data from different sources (Gissler & Haukka, 2004). The reimbursement for special infant formula is granted for all Finnish children with diagnosed CMA up to child's age of two years. For CMA the criteria for the special reimbursement have changed in 1994 and 2005. Before the year 1994, the grant was based on the examination done by pediatrician in a children's hospital ward. Since 1994 the criteria have included the need of symptoms suggestive of CMA and disappearance of these symptoms during an elimination diet and this is fulfilled with the need of either positive skin prick test for cow's milk protein, elevated S-IgE values for cow's milk protein or oral food challenge for cow's milk. For majority of the children the diagnosis was made based on oral food challenge (Kaila et al., 2000). In 2005 the oral food challenge came mandatory for all children over the age of 1 year.

In study III our definition for T1DM was a valid special reimbursement for the costs of insulin, and at least one purchase of the insulin (Anatomical Therapeutic Chemical (ATC) code 10) from the pharmacy after the grant of the special reimbursement. Our main definition for CMA was a valid special reimbursement for the special infant formulas for at least 6 months (n=4228). As this is open for the possible false positive diagnosis, we used strict CMA criteria where the main criterion was fulfilled with the need of at least three formula purchases from the pharmacy after the grant of the special infant formula (n=2665). For both CMA and T1DM we collected the information about the start and end date of the reimbursement and the SII disease code. We used the start date of special reimbursement as a proxy for a diagnosis date for both CMA and T1DM.

4.3 Dietary methods (I-II)

We assessed the maternal diet during pregnancy by validated 181-item semi quantitative food frequency questionnaire (FFQ). The FFQ was designed to assess the diet of one month period during the 8th month of pregnancy, which is the month prior the maternity leave. In the validation study the FFQ fulfilled after delivery was validated to be as representable as the FFQ fulfilled at the end of 8th month of pregnancy. Further the FFQ was validated against food records which were kept two times for five days during the 8th month of pregnancy (Erkkola, 2001). In the validation study, Pearson correlations with food records for antioxidants were 0.37 for vitamin A, 0.71 for retinol, 0.53 for β -carotene, 0.22 for vitamin E, 0.65 for vitamin C, 0.45 for zinc, and 0.46 for selenium, and for fatty acids were SFA 0.55, MUFA 0.34, total PUFA 0.47, total n-3-PUFA 0.39, total n-6-PUFA 0.49. The FFQ was designed to reflect the

Finnish food consumption habits. The food consumption was assessed by the frequency of food items and mixed dishes used (not at all, less than once a month, times used monthly, weekly or daily) and were reported by the common portion sizes (plateful, deciliter, glass, natural units). Further, the information about where the food was usually eaten, as well as the individual habit of type of fats used in cooking, salads, and baking (oils, butter, margarine) were queried. Information about the use of vitamin and/or nutrient supplements, including the type and brand and manufacturer's name, the amount (per day/week) and the timing (pregnancy weeks) of used supplementation was also collected. The FFQ was mailed to the mothers after delivery and returned at the study visit at child age of 3 months. All the FFQs were checked by the trained study nurse. The FFQs with 10 or more missing values or form inadequately filled in were rejected from the analysis (n=53, 1.1%).

We calculated the daily intake of studied nutrient by the Finnish food composition database, FINELI (National Institute for Health and Welfare Nutrition Unit, 2009), by an inhouse software of Finnish Institute of Health and Welfare. The data was double entered to the database. The FINELI food consumption database is constantly updated and the nutrient values for the foods are collected mainly from the chemical analyses. The nutrient value of individual course is calculated by the recipes, which are based on the Finnish cookbooks, also personal recipes can be used if they are available. In the recipes, the loss of nutrients during the cooking process is considered. In addition, the individual habit of used fats in cooking were considered. During the study time the FINELI database was updated to reflect the changes in the Finnish food consumption habits. The changes in recipes were based on the FINDIET 1997 (National Public Health, 1998) and FINDIET 2002 (Männistö S, Ovaskainen M, 2003) studies, which are national dietary surveys providing information about food consumption of women aged 22-44 years. Therefore, we used two versions of FINELI-databases, one for study years 1997-2002 and one for years 2003-2004. We obtained the information about the nutrient content of used supplements from the National Food Administration, manufacturers, and from the Finnish pharmacopeia for supplements registered as drugs.

4.4 Potential confounding factors

For studies I and II we collected the information on pregnancy and delivery complications, gestational age, birth weight and height, the number of mother's earlier deliveries and maternal smoking during pregnancy from the medical birth records of the delivery hospitals. For study III we collected the information of child's

birth year, sex, gestational age, delivery type, birth weight and height, as well as the number of mother's earlier deliveries, maternal age and socioeconomic status during delivery, and maternal smoking during pregnancy from the Medical Birth Register.

In studies I and II we asked the information of maternal and paternal vocational education, age and place of residence at the recruitment. We queried the information about breastfeeding from the parents at the study visits at the child's age of 6 months, 1, 2 and 3 years. For children participating in the Asthma and Allergy sub-study at the age of five years, we asked for information on parental history of asthma and allergic rhinitis, and the child's animal contacts during the first year of life from the parents.

In study III we collected information on maternal asthma and diabetes from the Special Reimbursement Register. We included child's asthma as a potential confounding factor with three different definitions, because of the findings of increased risk of T1DM for children with asthma (Metsälä et al., 2018) and in children using anti-asthmatic drugs (Metsälä et al., 2020) from the same cohort as the present study. Our main definition for asthma was a special reimbursement for the costs of anti-asthmatic drugs. The SII criteria for this reimbursement are that the diagnosis is based on recurrent wheezing episodes verified by pediatrician or pulmonary function tests showing obstruction. In addition, long-lasting inhaled corticosteroids as a treatment is needed. In sensitivity analysis we used two additional definitions for asthma 1. the main criteria or at least one purchase of any anti-asthmatic drug (ATC code R03) from the pharmacy and 2. at least one purchase of any anti-asthmatic drug from the pharmacy.

4.5 Statistical analyses

In studies I and II we used χ^2 -test to analyze the differences in background factors between children with and without CMA. We analyzed the association between the maternal dietary factors and CMA in the offspring by logistic regression. To estimate the regression coefficients, the possible reliance among siblings was taken into account with generalized estimating equations (GEE) with the sandwich estimator of variance (Liang & Zeger, 1986). We used logarithmic transformation for the nutrients, and we adjusted the nutrients for the energy intake by the residual method (Willett, 1998). Further, we standardized the nutrients so that the OR is presented per 1 standard deviation increment of the particular intake of the nutrient. We calculated the total intake of nutrient as a sum of the intake from food and supplements. We analyzed both total intake and the intake from food alone separately, if the intake from food was meaningful. We analyzed the nutrients as continuous variables. First, we

conducted the unadjusted analysis. For study I we did this among children with the available information of variables used in the first adjusted model, whereas in study II we did the unadjusted analysis among whole study population. Second, we conducted the adjusted analysis. The variables included in the first adjusted model were study center, sex, birth weight of the child, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, number of older siblings, season of birth, urbanity of living environment, and length of breastfeeding. We selected the confounding factors for the adjusted analyses based on previous knowledge (Botha et al., 2019; Faure et al., 2006; Koplin et al., 2008; Tatsumi et al., 2014; Vassallo et al., 2010) and their association with CMA in the present data. We considered these analyses as main analyses. We conducted sensitivity analyses among children participating in the Asthma and Allergy sub-study. The variables included in this sensitive analysis were all the variables used in the adjusted main analysis and the maternal and paternal history of asthma or allergic rhinitis, and child's visits to a stable and pet keeping during the first year of life. In study I we repeated the main analyses (unadjusted and adjusted) in this sub-population to evaluate the role of smaller sample size. In study II we also analyzed the nutrients as quartiles, where the first and last quartile were compared to the combined middle quartile. We made only unadjusted quartile analyses, as we did not observe any significant associations. In both studies I and II we analyzed the interaction of maternal history of asthma or allergic rhinitis and the intake of nutrients. If the interaction was significant ($p < 0.05$), we conducted the analyses separately for the mother's with and without history of allergic rhinitis or asthma. In the analyses we used SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) in study I and SAS version 9.3 (SAS Institute Inc., NC, USA) and IBM SPSS statistics for Windows, version 27 (IBM corp., Armonk, NY, USA) in study II.

In study III we analyzed the association between CMA and T1DM by weighted cox regression with inverse probability weighting to consider the case-cohort study setting. We considered CMA, T1DM and asthma as time-dependent variables, where the date of grant for the special reimbursement or the purchase of the anti-asthmatic drugs from pharmacy were used as a proxy for the diagnosis date. The cohort was followed up until the diagnosis of T1DM, age of 16 years, end of year 2009, or death, whichever came first. We conducted unadjusted, partially adjusted, and fully adjusted analyses. The variables included in the fully adjusted model were child's sex, birth decade, and asthma as well as maternal asthma, diabetes, smoking during pregnancy, and number of previous pregnancies. We selected these based on previous knowledge and their associations with T1DM and CMA in the current data

(Metsala et al., 2010; Metsälä, et al., 2020; J. M. Norris et al., 2020). We considered these analyses as main analyses. In addition, we conducted multiple sensitivity analyses in the subgroup of children born between 1995-2005, as the information of the drug and special infant formula purchases was available since year 1994. First, to evaluate the impact of this smaller sample size we conducted the main analyses in this subgroup (n=83 852, n(T1DM)=3383, n(CMA)=3065). Second, to evaluate the possible misclassification of CMA, we conducted the main analyses by using the strict CMA criteria (n(CMA)=2665). Third, we conducted these analyses by using two additional asthma criteria. In all models we included the interaction term between child's asthma and CMA to evaluate whether the association between CMA and T1DM is modified by child's asthma. We handled missing data by complete case analysis. In the analyses we used STATA, version 14, (RRID:SCR_012763) software.

4.6 Ethical issues

For studies I and II all families have given their written approval of the study and have informed that they can withdraw from the study at any time without need of an explanation. The ethics reviews were conducted by the respective committees of the hospital districts of Oulu and Tampere. The study was conducted in accordance with the declaration of Helsinki.

The study III was based on information which was collected originally for administrative purposes. No direct contact with the participants was needed. For the register-based studies no written consent from the participants nor their parents are needed according to the Finnish law. To use the administrative collected information for scientific study purposes, the authorities maintaining the registers need to approve the study and also ethical approval is needed. The study was approved by the Institutional Review Board of the Finnish Institute for Health and Welfare, and by the institutions keeping the registers after hearing The National Data Protection Authority.

5 RESULTS

5.1 Characteristics of the study population

In studies I and II we identified 409 children with CMA and the cumulative incidence of CMA by the age of 3 years was 9.1%. In study III we identified 7754 children with T1DM, and the reference cohort included 137 798 children. The mean follow-up time for the children with T1DM was 7.4 years (SD 4.1), and for children without T1DM 11.2 years (SD 5.0). In study III we identified 4228 children with CMA and the mean diagnosis age was 7.1 months (SD 3.9). Of these children 262 had both CMA and T1DM with the mean diagnosis age of 7.2 months (SD 4.2). For all children with both CMA and T1DM, CMA was diagnosed prior to T1DM. The cumulative incidence of CMA in children with T1DM was 3.4% and children without T1DM 2.9%. The subgroup of children born between years 1995-2008, consisted of 3383 children with T1DM and the reference cohort included 80 866 children. In this subgroup we identified 3065 children with CMA and the cumulative incidence of CMA in children with T1DM was 3.8% and children without T1DM 3.1%.

In studies I and II the children with CMA were more often male and less often had pets inside the home during the child's first year of life. Further, the mothers were more often non-smokers, had higher vocational education and both mothers and fathers more often had been affected by allergic rhinitis and/or asthma. Additionally, in study I the mothers of children with CMA more often used vitamin supplements containing vitamin A, C, and E during pregnancy (table 8). In study III the children with CMA were more often male (58% vs. 51%) and had asthma (19% vs. 5%), and their mothers more often had asthma (6% vs 3%). and were non-smokers (87% vs 78%). In study III the children with T1DM were more often male (55% vs 52%), had asthma (7.3% vs 5.2%) and their mothers more often had diabetes (2.4% vs 0.41%) and asthma (2.9% vs 2.8%), had fewer pregnancies (≥ 2 deliveries 22% vs 24%), and were more often non-smokers (81% vs 78%). No difference was observed for the child's birth year, gestational age, mode of delivery, maternal age at delivery, or maternal socioeconomic status

Table 8 The characteristics of the study population in studies I and II

	Study I			Study II		
	All participants	Cow's milk allergy	P-value	All participants	Cow's milk allergy	P-value
	(n=4403) n (%)	(n=409) n (%)		(n=4921) n (%)	(n=448) n (%)	
Male sex	2329 (52,9)	248 (60,6)	0,001	2590 (52,6)	270 (60,3)	0,001
Season of birth			0,421			0,256
Spring (Apr–May)	806 (18,3)	66 (16,1)		904 (18,4)	73 (16,3)	
Summer (Jun–Aug)	1169 (69,6)	104 (25,4)		1310 (26,6)	112 (25,0)	
Autumn (Sep–Nov)	990 (22,5)	92 (22,5)		1091 (22,2)	98 (21,9)	
Winter (Dec–Mar)	1439 (32,7)	147 (35,9)		1616 (32,8)	165 (36,8)	
Maternal age at delivery			0,051			0,017
<25	811 (18,4)	56 (13,7)		927 (18,8)	61 (13,6)	
25–29	1547 (35,1)	149 (36,4)		1714 (34,8)	159 (35,5)	
30–34	1282 (29,1)	134 (32,8)		1433 (29,1)	149 (33,3)	
>35	763 (17,3)	70 (17,1)		847 (17,2)	79 (17,6)	
Maternal vocational education			<0,001			<0,001
No professional education	272 (6,2)	19 (4,7)		296 (6,2)	20 (4,5)	
Vocational school or course	1191 (27,1)	75 (18,3)		1300 (27,1)	83 (18,9)	
Upper secondary vocational education	1915 (43,5)	202 (49,4)		2082 (43,4)	220 (50,0)	
Academic education	1025 (23,3)	113 (27,6)		1114 (23,2)	117 (26,6)	
Missing information				129	8	
Maternal smoking status during pregnancy			0,005			0,010
No	3972 (90,2)	385 (94,1)		4278 (90,0)	406 (93,5)	
Yes	431 (9,8)	24 (5,9)		474 (10,0)	28 (6,5)	
Missing information				169	14	
Mode of delivery			0,883			0,520
Ceasarean section	539 (12,2)	51 (12,5)		633 (13,0)	62 (13,9)	
Vaginal	3864 (87,7)	358 (87,5)		4253 (87,0)	383 (86,1)	
Missing information				35	3	
Urbanity of the place of living			0,539			0,162
Rural	549 (12,5)	44 (10,8)		609 (12,5)	45 (10,1)	
Semi-urban	423 (9,6)	41 (10,0)		467 (9,6)	50 (11,2)	
Urban	3431 (78,0)	324 (79,2)		3809 (78,0)	350 (78,7)	
Missing information				36	3	
Maternal asthma or allergic rhinitis			<0,001			<0,001
No	1331 (54,1)	107 (43,2)		1410 (54,2)	112 (43,1)	
Yes	1130 (45,9)	141 (56,9)		1190 (45,8)	148 (56,9)	
Missing information	1942	161		2321	188	
Paternal asthma or allergic rhinitis			<0,001			0,001
No	1466 (60,5)	123 (50,4)		1546 (60,5)	129 (50,8)	
Yes	956 (39,5)	121 (49,6)		1008 (39,5)	125 (49,2)	
Missing information	1981	165		2367	194	
Pets inside home first year			0,011			0,004
No	1720 (67,6)	190 (74,8)		1817 (67,7)	201 (75,6)	
Yes	821 (32,3)	64 (25,2)		865 (32,3)	65 (24,4)	
Missing information	1862	155		2239	182	
Maternal use of vitamin A supplements during pregnancy			0,032			
No	4124 (93,7)	373 (91,2)				
Yes	279 (6,3)	36 (8,8)				
Maternal use of vitamin C supplements during pregnancy			0,007			
No	2986 (67,8)	253 (61,9)				
Yes	1417 (32,2)	156 (38,1)				
Maternal use of vitamin E supplements during pregnancy			0,001			
No	2874 (65,3)	237 (58,0)				
Yes	1529 (34,7)	172 (42,1)				

5.2 Association between maternal antioxidant and fatty acid intake during pregnancy and development of cow's milk allergy in the offspring

The mean total and dietary intake of antioxidant nutrient and the total intake of fatty acids are shown in table 9. In study I the maternal total intake of beta-carotene was associated with an increased risk of CMA in the offspring (OR 1.10 95% CI 1.02-1.20) after adjustment for study center, sex, birth weight of the child, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, number of older siblings, season of birth, urbanity of living environment, and length of breastfeeding. Any of the other antioxidant nutrients studied were not associated with the development of CMA in the offspring (figure 4). In study II the maternal intake of any of the studied fatty acids were not associated with the development of CMA in the offspring after same adjustments as in study I (Figure 4). The results were similar when solely the intake from food was analyzed.

The results from the unadjusted analysis and from the sensitive analysis when further adjusted for maternal and paternal history of allergic rhinitis or asthma and the child's animal contacts during first year of life were substantially similar. In unadjusted analysis the direct association was observed for the total intake of beta-carotene (OR 1.12 95% CI 1.03-1.21), and for the dietary intake of beta-carotene (OR 1.11 95% CI 1.03-1.20) and vitamin E (OR 1.11 95% CI 1.00-1.22). In the sensitivity analyses, the direct association was observed for the total intake of beta-carotene (OR 1.12 95% CI 1.00-1.26), as well as for zinc (OR 1.13 95% CI 1.00-1.27). Additionally, the intake of selenium from food (OR 0.85 95% CI 0.74-0.98) was associated with decreased risk of CMA in the offspring. In study I these associations were observed also when adjusted only for the putative confounding factors in this subpopulation. Further, we did not observe any significant associations between maternal intake of fatty acids and the risk of CMA in the offspring either for the upper or lower quartile when compared to mid-half.

In study I we observed an interaction between maternal history of allergic rhinitis or asthma for the total intake of vitamin E, and in study II for total intake of alfa-linolenic acid. For other antioxidant nutrient or fatty acids we did not observe this interaction. The total intake of vitamin E was associated with increased risk of CMA in the offspring only in mothers with history of allergic rhinitis or asthma. The total intake of alfa-linolenic acid was associated with a decreased risk of CMA in the offspring only in mothers without such a history (Table 10).

Table 9 Maternal daily intake of nutrients from diet and supplements (total) and from diet during pregnancy (Mean values and standard deviations)

Nutrient	Mean	SD
Vitamin A total (RAE*, µg)	1362.5	832.6
Vitamin A diet (µg)	1344.8	826.3
Vitamin C total (mg)	221.2	144.5
Vitamin C diet (mg)	197.8	116.3
Vitamin E total (mg)	13.1	8.6
Vitamin E diet (mg)	11.8	8.3
β-Carotene total (µg)	4479.7	3825.9
β-Carotene diet (µg)	4280.2	3706.0
Retinol total (µg)	917.8	715.1
Retinol diet (µg)	916.6	713.2
Selenium total (µg)	91.4	29.3
Selenium diet (µg)	84.6	25.4
Zinc total (mg)	18.9	6.6
Zinc diet (mg)	16.8	4.9
SFA total (g)	43.7	16.9
Myristic acid total (14:0) (g)	4.7	2.0
Palmitic acid total (16:0) (g)	20.7	7.7
Stearic acid total (18:0) (g)	10.5	4.3
MUFA total (g)	35.7	12.6
Sum of 18:1 isomers total (g)	32.9	11.7
PUFA total (g)	13.8	5.1
n-3 PUFA total (g)	2.9	1.2
α-Linolenic acid total (18:3n-3) (g)	2.5	1.0
EPA (20:5n-3) total (mg)	82.3	63.9
DHA (22:6n-3) total (mg)	223.9	152.7
n-6 PUFA total (g)	10.6	4.0
Linoleic acid total (18:2n-6) (g)	10.3	3.9
Arachidonic acid total (20:4n-6) (mg)	128.2	53.6
γ-linolenic acid total (18:3n-6) (mg)	56.8	31.7
Conjugated linoleic acid total (mg)	180.9	80.6
Trans fatty acids total (g)	1.7	0.87
n-6/n-3 ratio	3.7	0.84
Linoleic acid / α-Linolenic acid ratio	4.3	1.0

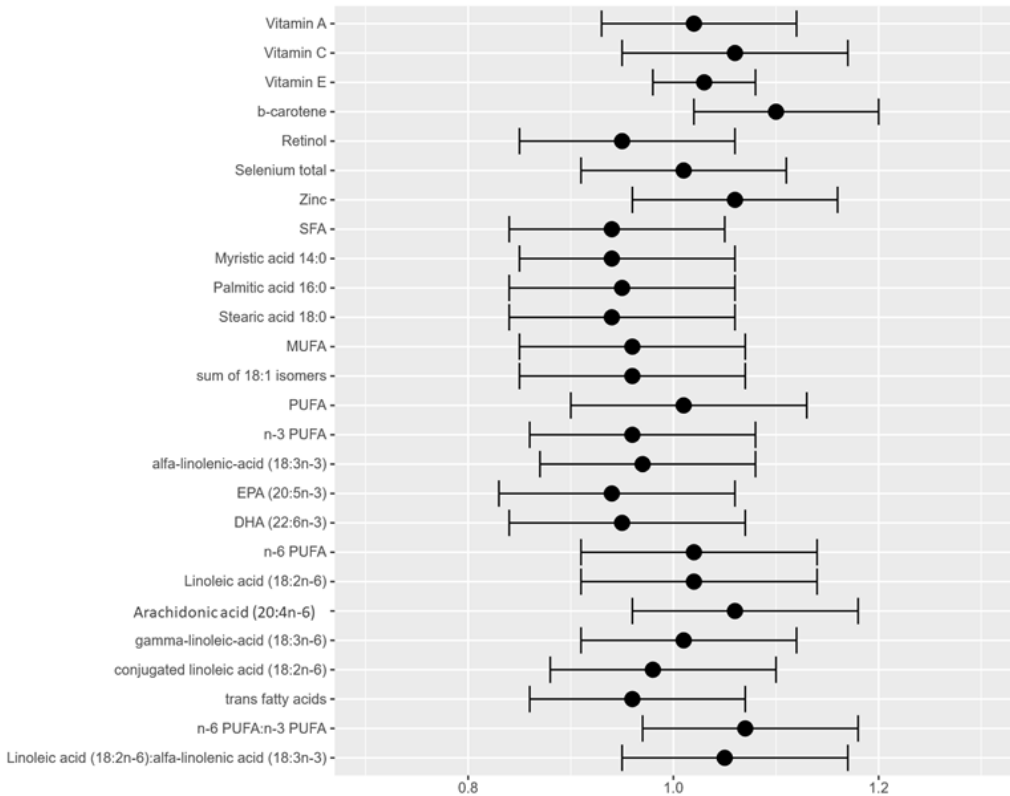


Figure 4 The association between maternal intake of antioxidants and fatty acids during pregnancy and the risk of cow's milk allergy by the age of 3 years in the offspring. Data points are adjusted odds ratios with 95% confident intervals (bars). The odds are presented per one standard deviation increment of the particular nutrient. The association has been analyzed by logistic regression

Table 10 The association between maternal intake of antioxidant nutrients and fatty acids during pregnancy and the risk of cow's milk allergy analyzed separately for the mothers with and without history of allergic rhinitis or asthma. The odds ratios are presented per one standard deviation increment of the particular nutrient. The association has been analyzed by logistic regression.

	Mothers with history of allergy		Mothers without history of allergy †		p-value
	OR	95%CI	OR	95%CI	
Vitamin E	1.61	1.14-2.28	0.98	0.85-1.12	0.013
Alfa-linolenic acid	1.05	0.88-1.26	0.72	0.56-0.93	0.012

† Adjusted for study center, sex, birth weight of the child, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, number of older siblings, season of birth, urbanity of living environment, and length of breastfeeding, paternal history of allergic rhinitis or asthma and the child's animal contacts during first year of life

5.3 Association between T1DM and CMA

In the main analyses among children born between 1986-2008 CMA was associated with increased risk of T1DM when adjusted for all the potential confounding factors including, sex, birth decade, asthma, maternal asthma and diabetes, smoking during pregnancy, number of previous deliveries (HR 1.17; 95%CI 1.02-1.34) (Figure 5). We observed this association also in unadjusted as well as in partly adjusted models.

In the sensitivity analyses, made among children born between 1995-2008, we did not observe an association between CMA and T1DM after adjustment for all the potential confounding factors (Figure 5). In unadjusted model the CMA was associated with increased risk of T1DM (HR 1.21; 95%CI 1.02-1.43). The change from the main criteria of CMA to the strict criteria of CMA did not change the results substantially.

In sensitive analysis with the strict CMA criteria, we used two additional criteria for asthma. The change in asthma criteria did not change the result substantially. In the fully adjusted model, the results were: a valid special reimbursement for asthma medication or at least one purchase of any anti-asthmatic drug from pharmacy

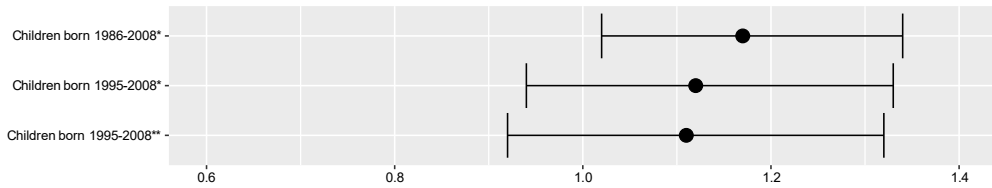


Figure 5 The association between cow's milk allergy and type 1 diabetes in a register-based case-cohort study. The association has been analyzed by a weighted Cox proportional hazard regression using inverse probability weighting to account for the case-

(HR=1.14; 95% CI 0.96-1.35), and for at least one purchase of any anti-asthmatic drug from pharmacy (HR=1.14; 95% CI 0.96-1.35).

6 DISCUSSION

6.1 Methodological considerations

6.1.1 Study design and population

Studies I and II are prospective birth cohort studies, where the data is collected before the development of diseases. This study design enables us to examine multiple exposures and outcomes simultaneously. By cohort study the possible risk and protective factors for the outcome can be determined, enabling to study these by trials. The prospective design diminishes the bias caused by reverse causation. The limitations are that the study may be expensive and time consuming to execute. Further, because of the long study time the drop out of study subjects is common. A major strength of the present study was the relatively large cohort of mothers and children. Unfortunately, at the child's age of 5 years only 2327 children were still at follow up, and therefore the results of the sensitivity analyses might be distorted by dropout. One of the major limitations is that all children had high or moderate HLA-conferred susceptibility for T1DM, representing about 14% of children in Finland. As these infants might have higher gut permeability (Vaarala et al., 2008), it may explain the higher incidence of CMA in studies I and II, than previously reported prevalences from Finland (Pyrhönen et al., 2009; K M Saarinen et al., 1999). Therefore, the generalization of our results to the unselected pediatric population calls for precaution.

The study III is a large register-based case-cohort study with sufficient power to evaluate the association between T1DM and CMA. The use of case-cohort study design reduces the selection and information bias, as the cases and non-cases are from the same sample and the exposure is assessed blinded. In the present study the number of cases was relatively large, and the information in all the registers was collected prospectively, thus the recall bias should be minimized. Because of the high incidence of T1DM in Finland and consequently in the study population, the generalization of the results to the population with low incidence of T1DM should be made cautiously.

6.1.2 Data sources

We collected the data for the studies I and II prospectively and independently of the disease status of the child, which reduces the recall bias. Further, data was available for the large amount of background and early life factors, which enabled considering multiple confounding factors in analyses, including the maternal and paternal history of allergic rhinitis and/or asthma. Unfortunately, we could not control the child's diet, thus the residual confounding cannot be ruled out.

The registers that we used for the study III are shown to have good coverage as well as validity. The information between registers was linked by personal identity code, which improves the completeness of the information collected for the study (Gissler & Haukka, 2004). In study III a major strength of using nationwide register-based data is the avoidance of reporting, recall and participating bias. Further, we were able to consider multiple perinatal and maternal background factors as confounding factors. One of the limitations is that the information for these registers is collected for administrative purposes and therefore do not have information of all factors important for the study. Thus, we could not control all the important confounders, such as paternal T1DM and history of allergic diseases, or the child's diet during early life. Therefore, the results might be affected by the residual confounding.

6.1.3 Food frequency questionnaire

FFQ is useful method to assess the diet of large groups and is often used in nutritional epidemiological studies. The FFQ has some limitations in general, as the respondents typically overestimate the intake of healthy food and underestimate the intake of unhealthy food. Further, for the respondent it might be hard to memorize the amount and frequency of food consumed (Cade et al., 2002).

In studies I and II we collected data of the maternal diet during pregnancy retrospectively by the validated FFQ specifically designed for the study. The FFQ was designed to represent the mother's diet during 8th pregnancy month and thus presents an estimate of the whole diet during pregnancy. The FFQ has some limitations. The FFQ was mailed to the mothers after delivery, because the recruitment of mothers was after delivery, and is therefore open for recall bias. After all, the FFQ has been validated to be suitable to represent the diet of a pregnant woman when compared to the two 5-day food diaries filled in at the beginning and the end of the 8th pregnancy month. Further, it showed acceptable reproducibility

when compared to the FFQs filled in at the beginning and the end of the 8th pregnancy month (Erkkola, 2001). Thus, this possible recall bias should be minor. In the validation study the FFQ was observed to slightly overestimate the intake of nutrients, therefore we adjusted the nutrient intakes for the energy intake to improve the accuracy. In the validation study, the use of food supplements was not considered, therefore their validity remains unclear in the present study. In study II this should not have major relevance, as the results were substantially similar if the use of supplement were considered or not. For study II one of the strengths was that the FFQ considered the individual use of fats in cooking, improving the accuracy of the intake of fats.

6.1.4 Endpoint and exposure measurement

The register-based information of CMA in studies I, II and III is possibly open for misclassification of children with CMA. Based on criteria for the special reimbursement at the study time, it was possible that the diagnosis of CMA was made based on either high IgE-levels or positive skin prick test for cow's milk protein in addition to the disappearance of symptoms suggestive of CMA during the elimination diet. However, it is previously reported that after all majority of the diagnoses were based on food challenge (Kaila et al., 2000), thus this problem should be minor. In addition, the definition may exclude children who get diagnosed after the age of 1 year, as after this the special infant formula is possible to replace with plant-based milks, for example with oat milk, and thus there is no need for the special reimbursement for the special infant formulas. As typically the symptoms of CMA begin before the age of 1 year, this problem should be minor (Høst, 1994; Vandenplas, 2017).

In studies I and II we fulfilled the register-based information with parental report of child's CMA. The parental report is previously reported to represent well or extremely well the physician diagnosed CMA (Tuokkola et al., 2010; Tuokkola et al., 2008). Thus, the misclassification of cases should be minor.

In the sensitivity analyses of study III, we used strict criteria of CMA including at least 3 purchases of infant formulas from the pharmacy. This may exclude the children diagnosed near the age of 1 year. However, the analyses were done in this subpopulation with both the main and strict criteria of CMA, and there was no significant difference in results. Further, the incidence of CMA in the study population was 3.0%, which is similar to previously reported from Finland (Pyrhönen et al., 2009;

Saarinen et al., 1999). Suggesting that misclassification of children with CMA should be minor.

In study III the special reimbursement register based information of T1DM should be reliable as the fulfilment of the criteria is evaluated by two independent specialists. In addition, the admittance is not dependent of the social status or place of residence. It is possible that children with type 2 diabetes are included in the study, as the criteria for the special reimbursement is same for all types of diabetes in need of insulin. However, the need of insulin in type 2 diabetes before the age of 16 years is rare (Zeitler et al., 2018), thus this should not affect the results.

6.2 Association between maternal intake of antioxidants and fatty acids during pregnancy and CMA in the offspring

6.2.1 Maternal intake of antioxidant nutrient during pregnancy

Our results that maternal intake of beta-carotene during pregnancy increased the risk of CMA in the offspring is supported by mechanistical studies but is partly in contrast with epidemiological findings.

The anti-inflammatory effects of nutrients might be lost with higher doses. Thus, it is suggested that high intake of antioxidant nutrients may suppress the production of Th1 type cytokines, and through cross-regulation lead to excessive production of Th2 cytokines (Murr et al., 2005). Therefore, the strong inflammatory suppression may promote Th2 responses and thus promote allergic inflammation (Gostner et al., 2015).

The epidemiological evidence of the association between maternal intake of antioxidant nutrients during pregnancy and the development of food allergy in the offspring is limited and scarce. Only one study has used CMA as an outcome, and partly supports our findings, as they observed that maternal higher intake of beta-carotene increased the risk of CMA in the offspring, and no association was observed for vitamin E and C. However, they observed an inverse association between maternal intake of vitamin A, retinol, and riboflavin and the development of CMA, which is in contrast with our findings (Kuśmierek et al., 2019). The studies about food allergy mainly supports our findings, as they have not observed an association between maternal intake of beta carotene, vitamin A, vitamin C, vitamin E and the

development of food allergy in the offspring (Gromadzinska et al., 2018; West et al., 2012).

The maternal allergy may modulate the immunological environment in utero, and by that modify the immunological effects of nutrients during pregnancy (Cook-Mills, 2015). This may explain our result that maternal intake of vitamin E during pregnancy was associated with higher risk of CMA only in mothers with history of allergy but not in mothers without such a history.

6.2.2 Maternal intake of fatty acids during pregnancy

We did not observe an association between maternal intake of fatty acids and CMA in the offspring. Only the maternal intake of alfa-linolenic acids was inversely associated with CMA in the offspring of mothers without history of allergic rhinitis and/or asthma, but not in mothers with such a history.

Our results are supported by two RCTs, which observed no association between maternal n-3-PUFA supplementation during pregnancy and food allergy in the offspring (Dunstan et al., 2003; Palmer et al., 2012, 2013). One RCT is in apparent contrast with our results, as they observed protective effect of maternal n-3-PUFA supplementation during pregnancy on the development of food allergy in the offspring (Furuhjelm et al., 2009, 2011). Two recent systematic literature review and meta-analyses of RCTs supports our finding, as they did not find an association between maternal use of n-3-PUFA supplementation and the food allergy in the offspring (Huynh et al., 2023, Bärebring et al., 2022) The methodical differences between studies, including timing and dosage of n-3-PUFA supplementation, may explain the scarce results as the crucial timing of fetal immune system development is not yet discovered and the association might be dose dependent.

It is possible that maternal allergy modulates the immunological environment in utero (Cook-Mills, 2015), which may partly explain our result that the protective effect of alfa-linolenic acid for the development of CMA was only observed for the mothers without history of allergy. Thus, these changes caused by maternal history of allergy may overpower the protective effect of the intake of alfa-linolenic acid during pregnancy.

6.3 Association between CMA and T1DM

We observed that child's CMA was associated with an increased risk of T1DM. To our knowledge this was first study to report an association between CMA and later T1DM in children. The evidence of the role of other food allergies or food allergies in general for the development of T1DM is scarce and limited, studies reporting both increased risk for T1DM (Wahlberg et al., 2011) and null associations (Caffarelli et al., 2004). For other allergic diseases, such as atopic eczema, more studies exist with results of all null (Gazit et al., 2008; Meerwaldt et al., 2002), direct (Fsadni et al., 2012) and inverse (The EURODIAB Substudy 2 Study Group, 2000; Stene & Joner, 2004) associations. Based on this scarce evidence, the Th1/Th2 paradigm is suggested to be an oversimplification, which is supported by our results as well. After the presentation of original Th1/Th2 paradigm, new Th-cells, such as Th17 has been discovered (Annunziato & Romagnani, 2009). Thus, the immunopathology behind these diseases might be more complex and cannot solely be explained by reciprocal counter-regulation between Th1- and Th2-cells.

Our results do not support the previous findings of the direct association between early cow's milk consumption and development of T1DM (Virtanen et al 1998, Niinistö et al 2024, Wahlberg et al 2006, Virtanen et al 2012, Niinistö et al 2017), as in Finland the special reimbursement for special infant formula correlates well with the strict cow's elimination diet (Tuokkola et al., 2010). Our results are partly supported by the RCT which did not observe a difference in development of T1DM between groups of children weaned to the extensively hydrolyzed formula or commercial formula (Knipet al., 2018).

The shared risk factors behind the diseases may explain our result of the possible co-occurrence of T1DM and CMA. The changes in environmental factors, including decrease of infectious diseases and microbial exposures, may cause adverse immunological reactions through their alterations in gut microbiota (Matamoros et al., 2013; Okada et al., 2010). For both CMA and T1DM these alterations in gut microbiota are suggested to have a role in disease development (Huang et al., 2017; Knip & Siljander, 2016). Further, the gut microbiota of children with CMA is observed to differ from the gut microbiota of healthy controls (Thompson-Chagoyan et al., 2010). Thus, these changes in gut microbiota, reflecting the changes in environmental and dietary factors, may partly explain our results. However, the studies of gut microbiota have multiple methodological differences and limitations including sample analysis, collection and storage, and low number of study subjects.

Thus, more studies are needed to discover the actual effects of the changes in gut microbiota for the development of CMA or T1DM.

Our observation that CMA was associated with an increased risk of T1DM in children without asthma, suggests that child's asthma should be considered in future studies. However, the reason for this finding is not clear. The systematic literature review and meta-analysis suggested a slight inverse association between asthma and T1DM (Cardwell et al., 2003). However, the results of individual studies are scarce, and one study observed that the sequential appearance of diseases had an influence for the association (Metsälä et al., 2018). Further, the asthma medication might have role, as it is observed to be associated with increased risk of T1DM (Metsälä et al., 2020).

7 CONCLUSIONS

Based on our main findings of the present study the following conclusions were drawn.

- There was no association between the maternal intake of fatty acids or majority of the antioxidant nutrients during pregnancy and the development of CMA in the offspring.
- Cow's milk allergy may be a risk factor for type 1 diabetes.
- The association between maternal intake of antioxidant or fatty acids during pregnancy and the cow's milk allergy in the offspring might be affected by the maternal history of allergy.

This study provided new information of the role of maternal diet during pregnancy and the development of CMA in the offspring, which is valuable when making new dietary recommendations for pregnant women. Further, this study provided completely new information on the association between CMA and development of T1DM. The identification of possible new groups of children in high risk for T1DM helps targeting the prevention strategies in future.

8 FUTURE DIRECTIONS

The results of our study revealed a gap in the knowledge of the risk factors for CMA, such as maternal diet during pregnancy. As well as a gap in knowledge of the association between CMA and T1DM. The future research topics which are raised from our study are:

- Examination of the role of maternal dietary factors during pregnancy for the development of CMA in the offspring in different study settings and populations.
- Examinations of the reasons why maternal allergy affects the association between maternal dietary factors during pregnancy and the development of CMA in the offspring.
- Examinations of the association between CMA and T1DM in a large prospective setting.
- Examinations of the biological mechanisms and reasons behind the association between CMA and T1DM.

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PUBLICATIONS

PUBLICATION

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Maternal antioxidant intake during pregnancy and the development of cows' milk allergy in the offspring

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Maternal antioxidant intake during pregnancy and the development of cows' milk allergy in the offspring

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Abstract

Cows' milk allergy (CMA) is the most common food allergy in young children, and it is often the first manifestation of atopic diseases. Accordingly, very early environmental factors, such as maternal diet during pregnancy, may play a role in the development of CMA, but the evidence is limited. The aim of this study was to investigate the association between maternal intake of antioxidant nutrients during pregnancy and the subsequent development of CMA in the offspring in a prospective, population-based birth cohort within the Finnish Type 1 Diabetes Prediction and Prevention Study. Maternal dietary information during pregnancy was collected with a detailed, validated FFQ. The maternal dietary information and the information on putative confounding factors were available for 4403 children. Information on diagnosed CMA (n 448) was obtained from a medical registry and queried from the parents up to child's age of 3 years. The Finnish food composition database was used to calculate the average daily intake of nutrients. Logistic regression was applied for statistical analyses, and the nutrient intakes were adjusted for energy intake. OR are presented per 1 SD increment of the particular nutrient intake. Maternal total and dietary intake of β -carotene was associated with an increased risk of CMA in the offspring when adjusted for the putative confounding factors (total OR 1.10, 95% CI 1.02, 1.20; dietary OR 1.10; 95% CI 1.01, 1.19). Using dietary supplements containing antioxidants in addition to a balanced diet may not confer any additional benefits.

Key words: Antioxidants; Cows' milk allergy; Maternal diet; Pregnancy

Cows' milk allergy (CMA) is a common food allergy, affecting 2–6% of Finnish children under the age of 3–4 years^(1,2). It is often the first manifestation of allergic diseases. Maternal

nutrition during pregnancy among other environmental factors has been implicated to play a role on the development of allergic diseases in the offspring⁽³⁾. Because CMA usually

Abbreviations: CMA, cows' milk allergy; DIPP, Diabetes Prediction and Prevention; Th, T helper.

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manifests during infancy, these early exposures may be of importance in the development of CMA. Antioxidant nutrients have been shown to exert immunological effects, and they could potentially influence the development of allergic diseases⁽⁴⁾. Two diverse hypotheses have emerged: epidemiological evidence suggests that a diet lower in antioxidants is associated with an increase in allergic diseases⁽⁵⁾, whereas the mechanistic hypothesis points towards antioxidants leading to the suppression of T helper type 1 (Th1) cytokines and thus a higher susceptibility to allergic diseases (reviewed by Allan *et al.*⁽⁶⁾). In addition to antioxidative effects, the nutrients may have other immunoregulatory pathways. For example, retinoic acid is involved in regulatory T-cell formation and thus the development of oral tolerance⁽⁷⁾. The most recent meta-analysis on maternal nutrition during pregnancy and allergic diseases in the child states that the current evidence is in favour of protective association between vitamin E as well as for Zn and childhood wheezing, but is inconclusive against other allergic diseases. For other antioxidant nutrients, the associations were even more contradictory⁽⁸⁾.

The knowledge of maternal dietary factors affecting the risk of CMA in the offspring is limited. One study reported that maternal intake of vitamin D from the diet during pregnancy was associated with decreased risk and intake of folate with increased risk of CMA in the offspring⁽⁹⁾. To our knowledge, associations between antioxidant nutrient intake during pregnancy and the development of CMA in the offspring have not been reported before. The aim of this study was to investigate the associations between maternal intake of antioxidant vitamins and minerals during pregnancy both from diet and supplements and the subsequent development of CMA in the offspring.

Subjects and methods

Subjects

The Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study is a multidisciplinary prospective population-based birth cohort study⁽¹⁰⁾. The study is conducted in three university hospitals in Finland (Turku, Oulu and Tampere) and after parental informed consent, all newborn infants from these areas were screened for human leucocyte antigen (HLA)-conferred susceptibility to type 1 diabetes from cord blood samples. Infants who carry HLA genotypes conferring high and moderate risk for type 1 diabetes (14% of those screened) were invited to participate in the study. The children with severe congenital abnormalities or diseases, or whose parents were of non-Caucasian origin or did not understand Finnish, Swedish or English were excluded. The study was conducted in accordance to the Declaration of Helsinki. The local Ethical Committees approved the study. All families have given their written informed consent.

The DIPP Nutrition study is a part of the main DIPP study comprising children born in the Oulu and Tampere areas. The present study comprised 6288 children born between October 1997 and September 2004. Both maternal dietary data during pregnancy and the information of child's CMA were available for 4921 children (78.3%) from a total of 4861 pregnancies. Complete information on background factors used in the current

analyses in the pregnancy cohort was available for 4403 children (Fig. 1).

When the children were 5 years of age, an Asthma and Allergy sub study (including parental history of allergy and child's animal contacts) was performed. All children at follow-up, also those who did not have maternal dietary data, were invited. Of the 4075 children (65% of the 6288 children) still at follow-up at 5 years, 3781 children (93% of those 4075 invited) participated in the Asthma and Allergy sub study. Of these children, 2327 children had information also on maternal diet, basic background factors and cows' milk allergy, enabling a sub-group analysis with information on parental allergies included in the current study. The flow chart of the cohort with maternal dietary data and the information of child's CMA is presented in Fig. 1.

Dietary assessment

Maternal diet during pregnancy (eighth month) was assessed by a 181-item semi-quantitative FFQ, which has been validated against food records (two times 5-d food record) in a setting that reflects the present study⁽¹¹⁾. The validation study did not take into account the nutrient intake from supplements. In the validation study, Pearson correlations with food records were 0.37 for vitamin A, 0.71 for retinol, 0.53 for β -carotene, 0.22 for vitamin E, 0.65 for vitamin C, 0.45 for Zn and 0.46 for Se⁽¹¹⁾. The FFQ was designed to represent the entire diet over the eighth pregnancy month. As in Finland mother's pregnancy leave begins right after eighth pregnancy month, it is likely to be the most representative month of the average nutrient intake during the whole pregnancy. The mother's received the FFQ via mail after delivery, and they returned it at the 3-month study visit.

A trained study nurse checked the FFQ when returned. The FFQ was specifically designed to reflect Finnish food consumption habits, and it assessed the consumption frequency of foods or food groups (not at all, number of times per d, week or month) as common serving sizes, such as a glass, a plateful or decilitre. The individual habits of fat used in cooking and baking were taken into account. The food consumption data were double entered. The FFQ was rejected if there were ten or more missing frequencies or the form was inadequately filled in (n 53, 1.1%). Daily intakes of vitamins A, C and E, β -carotene, retinol, Se and Zn were calculated with the use of the Finnish food composition database, Fineli[®]⁽¹²⁾, by an in-house software of the Finnish institute for health and welfare. The detailed content of the FFQ and data processing has been described elsewhere^(11,15). During the study time, the recipe compositions were updated in order to reflect the changes in food consumption habits and changes in the food market. That is why two versions of the database were used: the first version for the study years 1997–2002, and the second version for the years 2003–2004. The changes in recipes were mainly based on food consumption information of women aged 25–44 years from the national dietary surveys, FINDIET 1997⁽¹⁴⁾ and FINDIET 2002⁽¹⁵⁾. Recording of the FFQ and the accuracy of the nutrient database of the Finnish institute for health and welfare were checked at dietary analyses. The FFQ also included a question about dietary supplements, asking about the type, brand name and manufacturer's name, as well as the amount of each supplement per d or per week, and the

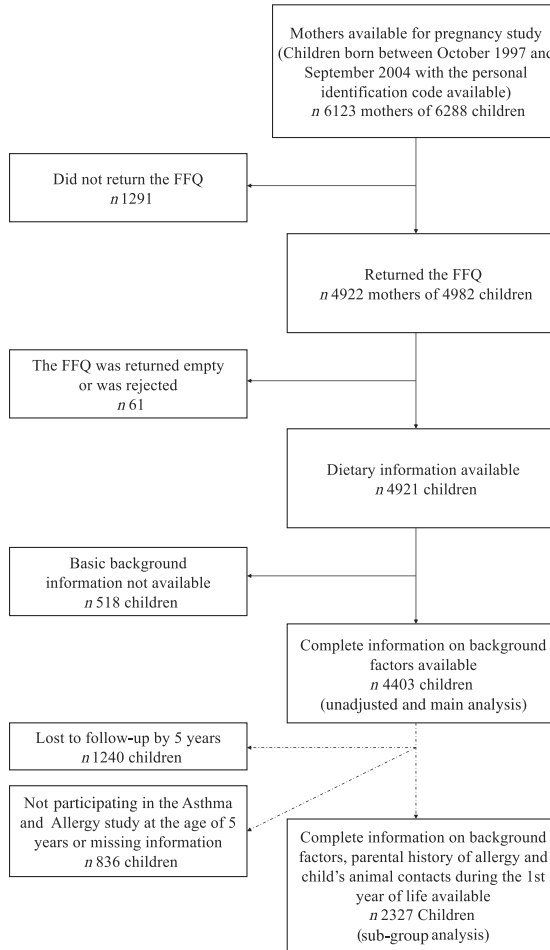


Fig. 1. Flow chart of the study cohort.

pregnancy weeks during which the supplements were used. The nutrient contents of the dietary supplements were obtained from the National Food Administration, manufacturers and from the Finnish pharmacopoeia for supplements registered as drugs.

Endpoints

Information on CMA, obtained from the registers of the Social Insurance Institution, complemented with parental reports, was used as the endpoint. The register-based information on CMA was based on a granted special reimbursement for the costs of special infant formulas needed in the management of diagnosed CMA (ICD-10 codes L27.2 or K52.2). The special

reimbursement is entitled to all Finnish infants up to age 2 years irrespective of infants/parents socio-economic status, place of residence or place of treatment. An application and a certificate from a paediatrician, stating that the CMA diagnosis has been made according to specified medical criteria, are required. Paediatricians have agreed on the criteria for the diagnosis, which, during the study period, was usually a response to an elimination diet and an open challenge, and rarely a response to an elimination diet with a positive skin prick test or a specific IgE. Only in some rare cases, a double-blind placebo-controlled challenge was performed. In addition, CMA of the children was queried with open questions from parents at the age of 6 months and 1 and 2 years, and with a structured, validated questionnaire at the age of 3 years^(16,17).

Background factors

Families were asked for information on maternal and paternal vocational education, age and place of residence at the recruitment. Information on pregnancy and delivery complications, gestational age, birth weight and height, earlier deliveries and maternal smoking during pregnancy was received from the medical birth records of the delivery hospitals. Information about breast-feeding was asked from the parents in the dietary questionnaires. Among those families who participated in the Asthma and Allergy study at the child's age of 5 years, the parents were asked for their asthma and allergic rhinitis background, and child's animal contacts during the first year of life⁽¹⁸⁾.

Statistical methods

The differences in background factors between children with and without CMA were analysed by using the χ^2 test. Logistic regression was applied to study the associations between maternal antioxidant nutrient intakes and the risk of CMA in the offspring. The possible reliance among siblings was accounted for by using the generalised estimating equations with the sandwich estimator of variance to estimate regression coefficients in logistic regression analysis⁽¹⁹⁾. Selection of variables included in adjusted models was based on previous evidence^(20–24) as well as their association with CMA in the present study. The nutrient intakes were adjusted for energy intake by the residual method⁽²⁵⁾ after logarithmic transformation. The total intake of each nutrient was calculated as the sum of intake from foods and supplements. The intake of nutrients from food alone and from food and supplements together was analysed if the nutrient intake from supplements was meaningful. Nutrient intake variables were used as continuous explanatory variables in the analyses, and first, the unadjusted analysis was conducted. The variables included in the first adjusted model were study centre, sex, birth weight of the child, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, number of older siblings, season of birth, urbanity of living environment and length of breast-feeding. These analyses were considered as main analyses. In addition, we made analysis in a sub group of children who participated in the Asthma and Allergy study at the child age of 5 years. In this analysis, the adjusted model included all those variables included in the main adjusted model as well as the information about maternal history of allergy, paternal history of allergy, visits to a stable and pet keeping during the child's first year of life. Due to the significant drop out of children at the follow-up at 5 years of age, when the Asthma and Allergy sub study was performed, we also repeated the main analyses in this sub group to evaluate whether the results, adjusted for allergy variables, are due to the reduced number of subjects or confounding by the allergy variables. Interaction between maternal history of allergy (mother has allergic rhinitis or asthma) and nutrient intakes was tested, and analyses were done separately for mothers with history of allergy and mothers without history of allergy if the interaction was significant ($P < 0.05$). SAS version 9.3 (SAS Institute Inc.) was used in the analysis.

Results

The mean total and dietary intake of the antioxidant nutrients is shown in Table 1. The cumulative incidence of CMA was 9.3% by the age of 3 years. The background factors associated with an increased risk of CMA were male sex, high parental education level, parental allergic history and maternal use of vitamin supplements containing vitamins A, C and E during pregnancy (Table 2). Maternal smoking and having pets inside the home during the child's first year of life were, in turn, associated with a decreased risk of CMA.

Maternal total intake of β -carotene and intake of β -carotene and vitamin E from food were associated with an increased risk of CMA in the offspring in the unadjusted model (Table 3). In the main analysis, after adjusting for putative baseline confounders, the associations remained for intake of β -carotene, both total (OR 1.10; 95% CI 1.02, 1.20) and from food (OR 1.10; 95% CI 1.01, 1.19) (Table 3).

In the sub-group analysis, after adjusting for putative baseline confounders, parental history of allergy and child's animal contacts during the first year of life, the intake of Se from food (OR 0.85; 95% CI 0.74, 0.98) was associated with a decreased risk, and the total intake of Zn (OR 1.13; 95% CI 1.00, 1.27) and β -carotene (OR 1.12; 95% CI 1.00, 1.26) with an increased risk of CMA in the offspring. These associations were also observed when adjusted only for putative baseline confounders (online Supplementary 1).

An interaction between maternal history of allergy and the intake of antioxidant nutrients was observed for the total intake of vitamin E ($P_{\text{for interaction}} = 0.013$) when adjusted for all the potential confounding factors, including paternal history of allergy. The total intake of vitamin E was associated with an increased risk of CMA in mothers with a history of allergy (OR 1.61; 95% CI 1.14, 2.28), but not in mothers without such a history (OR 0.98; 95% CI 0.85, 1.12). For other nutrients, no interactions with maternal allergic history were observed (data not shown).

Discussion

In this large population-based birth cohort study, we observed that maternal total and dietary intake of β -carotene during

Table 1. Maternal daily intake of nutrients from diet and supplements (total) and from diet during pregnancy (n 4403) (Mean values and standard deviations)

	Mean	SD
Vitamin A total (RAE*, μg)	1362.5	832.6
Vitamin A diet (μg)	1344.8	826.3
Vitamin C total (mg)	221.2	144.5
Vitamin C diet (mg)	197.8	116.3
Vitamin E total (mg)	13.1	8.6
Vitamin E diet (mg)	11.8	4.3
β -Carotene total (μg)	4479.7	3825.9
β -Carotene diet (μg)	4280.2	3706.0
Retinol total (μg)	917.8	715.1
Retinol diet (μg)	916.6	713.2
Se total (μg)	91.4	29.3
Se diet (μg)	84.6	25.4
Zn total (mg)	18.9	6.6
Zn diet (mg)	16.8	4.9

* Retinol equivalent (RAE) = 1 μg of retinol = 12 μg of β -carotene.

**Table 2.** Distribution of background characteristics of all children who participated in the study and for cows' milk-allergic children (cases) (Numbers and percentages)

	Cows' milk allergy*						P †
	All participants (n 4403)		Non-cases (n 3994)		Cases (n 409)		
	%	n	%	n	%	n	
Sex							
Boys	52.9	2329	52.1	2081	60.6	248	0.001
Girls	47.1	2074	47.9	1913	39.4	161	
Season of birth							
Spring (April–May)	18.3	806	18.5	739	16.1	66	0.421
Summer (June–August)	26.6	1169	26.7	1065	25.4	104	
Autumn (September–November)	22.5	990	22.5	898	22.5	92	
Winter (December–March)	32.7	1439	32.4	1292	35.9	147	
Age of the mother at delivery (years)							
<25	18.4	811	18.9	755	13.7	56	0.051
25–29	35.1	1547	35.0	1398	36.4	149	
30–34	29.1	1282	28.7	1148	32.8	134	
>35	17.3	763	17.4	693	17.1	70	
Maternal vocational education							
No professional education	6.2	272	6.3	253	4.7	19	<0.001
Vocational school or course	27.1	1191	27.9	1116	18.3	75	
Upper secondary vocational education	43.5	1915	42.9	1713	49.4	202	
Academic education	23.3	1025	22.8	912	27.6	113	
Maternal smoking status during pregnancy							
No	90.2	3972	89.8	3587	94.1	385	0.005
Yes	9.8	431	10.2	407	5.9	24	
Mode of delivery							
Section	12.2	539	12.2	488	12.5	51	0.883
Vaginal	87.8	3864	87.8	3506	87.5	358	
Urbanity of the place of living							
Rural	12.5	549	12.6	505	10.8	44	0.539
Semi-urban	9.6	423	9.6	382	10.0	41	
Urban	78.0	3431	77.8	3107	79.2	324	
Maternal use of vitamin A supplements during pregnancy‡							
No	93.7	4124	93.9	3751	91.2	373	0.032
Yes	6.3	279	6.1	243	8.8	36	
Maternal use of vitamin C supplements during pregnancy‡							
No	67.8	2986	68.4	2733	61.9	253	0.007
Yes	32.2	1417	31.6	1261	38.1	156	
Maternal use of vitamin E supplements during pregnancy‡							
No	65.3	2874	66.0	2637	58.0	237	0.001
Yes	34.7	1529	34.0	1357	42.1	172	
Maternal asthma or allergic rhinitis§							
No	54.1	1331	55.3	1224	43.2	107	<0.001
Yes	45.9	1130	44.7	989	56.9	141	
Missing information		1942		1781		161	
Paternal asthma or allergic rhinitis§							
No	60.5	1466	61.7	1343	50.4	123	<0.001
Yes	39.5	956	38.3	835	49.6	121	
Missing information		1981		1816		165	
Pets inside home during the first year of life§							
No	67.7	1720	66.9	1530	74.8	190	0.011
Yes	32.3	821	33.1	757	25.2	64	
Missing information				1707		155	

* Cumulative incidence of cows' milk allergy by the age of 3 years.

† Comparison with the χ^2 test comparing distributions of cows' milk allergy across the categories.

‡ Including the use of multivitamin supplements.

§ Information was collected from the Asthma and Allergy study, which was performed at the child's age of 5 years.

pregnancy was associated with an increased risk of CMA in the offspring up to child's age of 3 years.

A major strength of our study is that the data were collected prospectively from a large number of mothers and children. Furthermore, a major advantage is the good quality of dietary information, which was gathered by a detailed, validated

FFQ. Cross-classification in quintiles by food consumption and nutrient intake was acceptable for all nutrients.

The sub-group analysis, done within children participating in the Asthma and Allergy study, enabled us to take into account the parental history of allergy and the child's animal contacts during the first year of life as potential confounding factors. In this sub-

Table 3. Risk of cows' milk allergy in the offspring by 3 years of age associated with maternal daily intake of energy and nutrients during pregnancy* (Odds ratios and 95 % confidence intervals)

	Unadjusted (n 409†/4403‡)		Adjusted analysis§ (n 409†/4403‡)	
	OR	95 % CI	OR	95 % CI
Energy	0.97	0.88, 1.08	0.98	0.88, 1.09
Fat	0.93	0.83, 1.03	0.95	0.85, 1.07
Protein	0.97	0.88, 1.08	0.94	0.84, 1.04
Carbohydrates	1.07	0.96, 1.19	1.07	0.95, 1.20
Vitamin A total	1.02	0.93, 1.11	1.02	0.93, 1.12
Vitamin A diet	1.01	0.93, 1.11	1.02	0.93, 1.12
Vitamin C total	1.07	0.97, 1.17	1.06	0.95, 1.17
Vitamin C diet	1.03	0.93, 1.14	1.02	0.91, 1.14
Vitamin E total	1.04	0.99, 1.09	1.03	0.98, 1.08
Vitamin E diet	1.11	1.00, 1.22	1.06	0.96, 1.18
β -Carotene total	1.12	1.03, 1.21	1.10	1.02, 1.20
β -Carotene diet	1.11	1.03, 1.20	1.10	1.01, 1.19
Retinol total	0.93	0.84, 1.04	0.95	0.85, 1.06
Retinol diet	0.94	0.84, 1.04	0.95	0.85, 1.06
Se total	1.03	0.94, 1.14	1.01	0.91, 1.11
Se diet	0.94	0.85, 1.04	0.93	0.84, 1.03
Zn total	1.09	0.99, 1.19	1.06	0.96, 1.16
Zn diet	1.02	0.92, 1.13	0.99	0.89, 1.11

* Both total intake and intake from food sources alone are reported for those nutrients which are also derived from supplements.

† Number of children with cows' milk allergy.

‡ Number of children in the analysis.

§ Adjusted for study centre, sex, birth weight of the child, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, number of older siblings, season of birth, urbanity of living environment and length of breast-feeding.

|| OR are presented per 1 so increment of the particular nutrient intake.

group analysis, we did not observe difference between the results when the adjustment was made only for putative confounding factors and when further adjusted for parental history of allergy and the child's animal contacts during the first year of life. This suggests that these factors do not have a significant confounding role in our study group. However, the difference observed between the main and the sub-group analyses suggests that the drop out had distorted the sub-group results.

A limitation of the present study is that the study subjects were selected based on HLA-conferred susceptibility to type 1 diabetes, representing about 14 % of all newborn infants in Finland. These infants may have an increased intestinal permeability⁽²⁶⁾, which may explain why the incidence of CMA in our study was higher than that previously reported in Finland⁽¹⁾. Therefore, our results may not be fully generalisable to the unselected paediatric population. Estimating the nutrient intake by the FFQ has some limitations. First, as the mothers received the FFQ after delivery and were asked to retrospectively report their diet during the eighth month of pregnancy, it is open for a recall bias. However, in the validation study, the FFQ filled after delivery was considered to be equally representative of the diet during the eighth month of pregnancy as FFQ filled during the eighth month of pregnancy; therefore, this recall bias should be minor. Second, the FFQ is reported to slightly overestimate the nutrient intake; however, the adjustment of nutrient intakes for energy intake should diminish this problem⁽¹¹⁾. Third, as nutrient intake from supplements was not calculated in the validation study, the validity of the intake from supplements in the present study remains unclear. Fourth, in the validation study, the Pearson correlation coefficient for vitamin E was low (r 0.22), and therefore, the observed associations for vitamin E might be diminished and should be interpreted cautiously. We took several possible

confounders into account in order to specifically study the effects of the selected antioxidant nutrients, to avoid the bias in results from some other lifestyle factors, which are associated with the intake of these nutrients. Unfortunately, the diet of the children themselves was not controlled for. However, because we examined a large number of dietary variables in our analyses, it is possible that the significant association may only be due to chance. A further limitation is that we only had information about the age at diagnosis, and the onset of symptoms of CMA was not known, although these two should coincide.

To our knowledge, this is the first study to examine the association between antioxidant nutrient intake during pregnancy and the development of CMA in the offspring. Only one previous study has used food allergy as an outcome when studying the associations of maternal antioxidant intake with offspring allergic diseases. The authors suggested that vitamin C and Cu could be associated with a reduced risk of food allergy, but for vitamin E, β -carotene or Zn, no association was seen⁽²⁷⁾. Our observation on the association between maternal total and dietary intake of β -carotene and the increased risk of CMA in the offspring is in line with some mechanistic studies^(28,29), but is not supported by epidemiological findings^(30,31).

Evidence from epidemiological studies on the influences of maternal antioxidant intake during pregnancy on allergic outcomes in childhood is scarce, studies reporting mostly protective or non-significant associations. The most recent meta-analysis observed a protective association only for the maternal intake of vitamin E and Zn during pregnancy and wheezing in the offspring⁽⁸⁾. In addition, two previous studies have reported that high maternal plasma Se concentration during pregnancy⁽³⁰⁾ or in cord blood⁽³¹⁾ was associated with a decreased risk of wheezing in the offspring. However, wheezing is a condition



different from CMA and may not always be of atopic origin. The effect of oxidative stress on the pathogenesis of pulmonary manifestations of atopic disease is likely to differ from that of CMA. On the other hand, mechanistic studies have implied a possible predisposing effect of antioxidant nutrients on allergic diseases. The anti-inflammatory benefits of the antioxidant nutrient may be lost with higher doses because of the too strong inflammatory suppression which could lead to Th1 suppression promoting Th2 responses associated with allergic diseases^(28,29). Murr *et al.* have suggested that increased antioxidant intake could suppress cytokines, namely interferon- γ , leading to Th1 differentiation⁽³²⁾. This suppression would then, due to cross-regulation, promote the development of a Th2 phenotype. In addition, previous *in vitro* studies have shown that vitamin A and its derivatives may favour Th2 immune responses⁽³³⁾.

In Finland, the mothers who use dietary supplements receive higher amounts of antioxidant nutrients from their diet have a higher education and smoke less compared with mothers who do not use any supplements⁽³⁴⁾. This may have affected our results as those mothers with overall healthier lifestyle may seek help for their child's symptoms more actively, which may increase the child's likelihood of receiving a CMA diagnosis. Even though some associations prevailed after adjusting for maternal education, it is possible that our finding on maternal antioxidant nutrient intake and higher risk of CMA in the offspring is not causal.

Because allergic diseases are strongly hereditary, we examined the associations between nutrients and CMA in children separately for mothers with and without a history of allergy. We observed that maternal intake of vitamin E during pregnancy was associated with a higher risk of CMA only in the offspring of mothers with a history of allergy. However, this observation might be affected by the selection bias and therefore needs to be interpreted cautiously. Even so, it is possible that the maternal allergy influences the immunological environment *in utero*; therefore, the maternal allergy may modify the immunological effects of nutrients during pregnancy⁽³⁵⁾. Further studies are needed to explore the significance as well as the immunological mechanisms behind this finding.

In conclusion, these results are the first to link maternal antioxidant nutrient intake during pregnancy with the development of CMA in the offspring. For majority of studied antioxidant nutrients, we did not observe an association between maternal intake and CMA in the offspring. Thus, the maternal use of supplements containing antioxidant nutrients during pregnancy does not seem to have any additional benefits. A sufficient and balanced intake of antioxidant nutrients is best achieved by adhering to the dietary guidelines for pregnant women.

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S. M. V., J. Tuokkola and M. Kaila were responsible for the current study design and concept. J. I., M. Knip, J. Toppari and R. V. are members of the steering committee of the DIPP study. S. M. V. designed the DIPP nutrition study, and within the DIPP nutrition study, the allergy study was designed by S. M. V. and M. Kaila. J. Tuokkola and S. M. V. conducted the research. H.-M. T. and H. T. were responsible for the statistical analysis. R. V. was responsible for the clinical work in Oulu, and M. Knip was responsible of the clinical work in Tampere. J. Tuokkola and A. L. wrote the first version of the article with equal contribution. J. M., M. Kaila and S. M. V. participated in the writing process. S. M. V. and M. Kaila. had the primary responsibility for the final work with equal contribution. All the authors participated in the critical revision of the manuscript and have accepted the final version.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit <https://doi.org/10.1017/S0007114520003633>

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PUBLICATION II

Maternal energy-adjusted fatty acid intake during pregnancy and the development of cows' milk allergy in the offspring

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Maternal energy-adjusted fatty acid intake during pregnancy and the development of cows' milk allergy in the offspring

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Abstract

Cows' milk allergy (CMA) is one of the earliest manifestations of allergic diseases. Early dietary factors, like maternal diet during pregnancy, may play a role in the development of allergic diseases in the offspring. We aimed to investigate the association between maternal intake of fatty acids during pregnancy and the risk of CMA in the offspring. Our study was conducted in a population-based cohort, the Finnish Type 1 Diabetes Prediction and Prevention study. We collected the maternal dietary data by a validated FFQ. We obtained the information on CMA in the study participants (n 448) from registers and from the parents. Dietary data and information on CMA were available for 4921 children. We used logistic regression in the analyses, and fatty acid intakes were energy adjusted. The maternal intake of SFA, MUFA, PUFA, n -3 PUFA, n -6 PUFA, trans fatty acids, ratio of n -3 PUFA to n -6 PUFA or ratio of linoleic acid to α -linolenic acid was not associated with the risk of CMA in the offspring when adjusted for perinatal factors, background factors, parental history of asthma or allergic rhinitis and infant animal contacts. The intake of α -linolenic acid was associated with a decreased risk (OR 0.72; 95% CI 0.56, 0.93) of CMA in the offspring of mothers without a history of allergic rhinitis or asthma. In conclusion, the maternal intake of fatty acids during pregnancy is not associated with the risk of CMA in the offspring.

Key words: Milk hypersensitivity; Pregnancy; Diet; Fatty acid

Allergic diseases are among the most common chronic diseases, especially in developed countries⁽¹⁾. One of the earliest manifestations of allergic diseases is cows' milk allergy (CMA), which affects 2–6% of children in Finland^(2,3).

In developed countries, the dietary intake of n -6 PUFA has increased, and simultaneously, the intake of n -3 PUFA has decreased⁽⁴⁾. This changed ratio of intake of n -6/ n -3 PUFA

has led to a more pro-inflammatory environment, as the n -6 PUFA are observed to promote allergic inflammation by releasing allergy promoting eicosanoids from the cells, whereas n -3 PUFA reduce the allergic inflammation by damping the release of pro-inflammatory factors from the cells⁽⁵⁾. Because the fatty acid status of the fetus and the newborn infant may be modulated by maternal fatty acids, and that the development

Abbreviations: CMA, Cows' milk allergy; DIPP, Diabetes Prediction and Prevention; RCT, randomised controlled trial.

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of the immune system starts already *in utero*, it has been implicated that maternal fatty acid intake may affect the risk of development of allergic diseases in children⁽⁶⁾.

A systematic review of epidemiological studies and meta-analysis of randomised controlled trials (RCT) has suggested a protective role of higher intake of *n*-3 PUFA and fish during pregnancy for allergic diseases, especially for eczema, wheeze and asthma⁽⁷⁾. However, some null results have also been reported^(8–11), and one of the studies had used sensitisation for cows' milk protein as an outcome⁽¹⁰⁾. The RCT of the role of maternal supplementation of *n*-3 PUFA in pregnancy have reported both inverse⁽¹²⁾ and null results^(13,14) in relation to the risk of food allergy in the offspring. However, none of these RCT used CMA as an outcome. Thus, the role of maternal fatty acid intake during pregnancy for the development of CMA in the offspring remains unclear.

The aim of our study was to examine the maternal intake of different fatty acids during pregnancy and the development of CMA in the offspring. To our knowledge, this is the first study to assess this association. We hypothesised that maternal higher intake of *n*-3 PUFA decreases and higher intake of *n*-6 PUFA increases the risk of CMA in the offspring.

Methods

Study population

We obtained the data from the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) nutrition study, which is a multidisciplinary prospective population-based birth cohort study done in a framework of the ongoing DIPP study. The DIPP study carried out in Finland in the area of Turku, Tampere and Oulu University Hospitals aimed at generating novel insights into the pathogenesis of type 1 diabetes⁽¹⁵⁾. All newborn infants in these areas have been invited for screening of their human leucocyte antigen -conferred susceptibility for type 1 diabetes. Children carrying genotypes conferring high or moderate disease risk (14 % of the infants) are invited to enrol in the follow-up study. Children with severe congenital abnormalities or diseases, whose parents were of non-Caucasian origin, or did not understand Finnish, English or Swedish, were excluded from the DIPP study.

The DIPP nutrition study is conducted among children born in the area of Oulu and Tampere University Hospitals. The present study comprises children born between August 1997 and September 2004 (*n* 6288), with available personal identity code. Altogether, 4921 children had information on maternal diet during pregnancy and child's CMA by the age of 3 years (Fig. 1, Tables 1–3). In addition to the dietary data, we collected information on parental history of allergic rhinitis or asthma from the Asthma and Allergy sub-study, where all children still in follow-up at the age of 5 years (*n* 4075) were invited to participate (*n* 3781). Altogether, 2327 children had information on maternal diet during pregnancy, basic background factors, child's CMA by the age of 3 years and parental history of allergic rhinitis or asthma (Table 3:2. Adjustment column).

The present study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures involving

patients were approved by the respective ethical committees of the hospital districts of Oulu and Tampere. Written informed consent was obtained from all parents, and they were informed that they can withdraw from the study at any time without further explanations.

Dietary data

The mothers completed a validated 181-item semi-quantitative FFQ, which was designed to reflect the diet during the 8th month of pregnancy (1 month preceding the start of the Finnish maternity leave). The FFQ was validated against 10-d food records⁽¹⁶⁾. The FFQ was mailed to the mothers after delivery and returned at the child's 3-month study visit. The use of food ingredients and dishes was reported as common serving sizes and the frequency of use as no use, daily, weekly or monthly use. The individual variation of used fats in cooking, baking and salad dressings was also queried. Further, the information about the vitamin and mineral supplements used during the whole pregnancy, including supplement's brand name and the amount of used supplements (tablets, drops, spoonfuls or millilitres), was collected. The FFQ that were filled in inadequately or contained more than ten missing values were excluded (*n* 53, 1.1 %). The data processing of the FFQ has been described earlier⁽¹⁶⁾. Briefly, we double entered the dietary data into the database. We used Finnish Food Composition Database 'Fineli'⁽¹⁷⁾ and in-house software of the Finnish Institute of Health and Welfare to calculate the estimate of the daily average of the studied fatty acids for each mother.

The fatty acids investigated in the present study were SFA, MUFA, PUFA, *n*-3 PUFA, *n*-6 PUFA and trans fatty acids. In addition, we investigated the ratios of *n*-3 PUFA to *n*-6 PUFA and linoleic acid to α -linolenic acid. In the validation of the FFQ against two 5-d food records, the Pearson correlations were SFA (0.55), MUFA (0.34), PUFA (0.47), *n*-3 PUFA (0.39) and *n*-6 PUFA (0.49)⁽¹⁶⁾.

Confounding factors

At the time of enrolment, we asked from the parents their age, occupation, education level and the place of residence. We collected the information on the child's sex, delivery type, gestational age, pregnancy and delivery complications, birth weight and height, mother's earlier deliveries and maternal smoking during pregnancy from the Medical Birth Registers of Tampere and Oulu University Hospitals. We asked about breast-feeding from parents at study visits at the child's age of 6 months, 1, 2 and 3 years. Among children who participated in the Asthma and Allergy sub-study, we collected the information of maternal and paternal history of allergic rhinitis or asthma, pet keeping and contacts to the farm animals during the child's first year of life by the parental questionnaire completed at the 5-year study visit.

Endpoints

Our definition of CMA was based on information obtained from the special reimbursement register maintained by the Social Insurance Institution of Finland and linked using personal

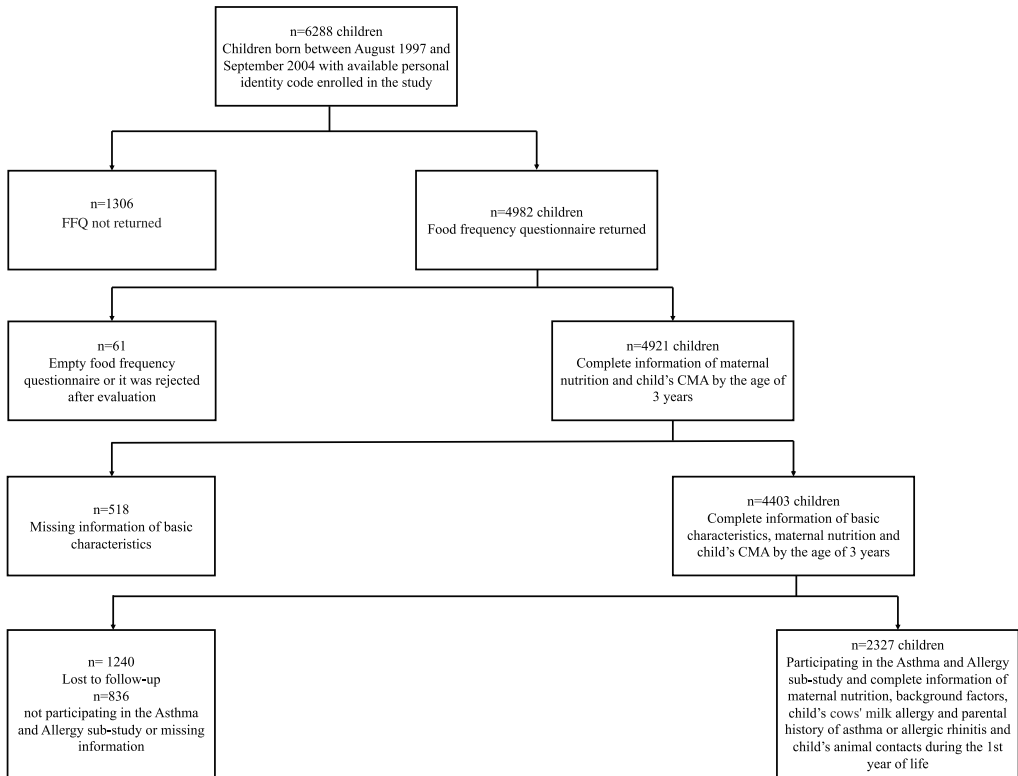


Fig. 1. Flow chart of the participants. CMA, cows' milk allergy.

identity codes⁽¹⁸⁾. We complemented the register data with a parental report on CMA queried by the validated questionnaire, which was filled in at study visits, when the child was 6 and 12 months old and annually thereafter, until the age of 9 years^(19,20).

From the register, we obtained the information about a valid special reimbursement for the costs of special infant formula needed in the treatment of diagnosed CMA (ICD-10 codes L27.2 or K52.2). In Finland, every child with CMA, diagnosed by a paediatrician, is entitled for this reimbursement up to the age of 2 years, irrespective of the family's socio-economic status or place of residence. At the time of the present study, the CMA diagnosis in Finland was usually based on an open oral food challenge⁽²¹⁾.

Statistical methods

We analysed the difference in background factors between children with and without CMA by the χ^2 test. We selected the variables used in the adjusted models based on previous knowledge⁽²²⁻²⁴⁾ and their association with CMA in the present study. We analysed the association between maternal fatty acid intake and the risk of CMA in the offspring by logistic regression.

The possible reliance among siblings was accounted for using the generalised estimating equations with the sandwich estimator of variance to estimate regression coefficients in logistic regression.

After the logarithmic transformation, we adjusted the nutrients for energy intake by the residual method⁽²⁵⁾ and we used standardised scores in the analyses. We also divided the energy-adjusted dietary intake into quartiles, and the first and last quartiles were compared with the combined mid quartiles in our analysis. We analysed fatty acid intakes from food and from food and supplements, if the supplemental intake was meaningful. Our first adjusted model included study centre, sex, birth weight, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, season of birth, number of older siblings, length of breast-feeding and urbanity of the living environment as covariates. We made the unadjusted and the first adjusted model among children with information on maternal diet during pregnancy, child's CMA status and basic background characteristics (n 4921). We made the second adjusted analysis among children participating in the Asthma and Allergy sub-study (n 2327). The variables used in the second adjusted model were all those in the first adjusted model in



Table 1. Distribution of background characteristics among the whole study population (*n* 4921) and cases with cows' milk allergy (Numbers and percentages, *n* 448)

	All participants <i>n</i> 4921		Cows' milk allergy		<i>P</i> *
	%	<i>n</i>	%	<i>n</i>	
Sex					0.001
Boys	52.6	2590	60.3	270	
Girls	47.4	2331	39.7	178	
Season of birth					0.256
Spring (April–May)	18.4	904	16.3	73	
Summer (June–August)	26.6	1310	25.0	112	
Fall (September–November)	22.2	1091	21.9	98	
Winter (December–March)	32.8	1616	36.8	165	
Age of the mother at delivery (years)					0.017
< 25	18.8	927	13.6	61	
25–29	34.8	1714	35.5	159	
30–34	29.1	1433	33.3	149	
> 35	17.2	847	17.6	79	
Maternal vocational education					<0.001
No professional education	6.2	296	4.5	20	
Vocational school or course	27.1	1300	18.9	83	
Upper secondary vocational education	43.4	2082	50.0	220	
Academic education	23.2	1114	26.6	117	
Missing information		129		8	
Maternal smoking status during pregnancy					0.010
No	90.0	4278	93.5	406	
Yes	10.0	474	6.5	28	
Missing information		169		14	
Mode of delivery					0.520
Caesarean section	13.0	633	13.9	62	
Vaginal	87.0	4253	86.1	383	
Missing information		35		3	
Urbanity of the place of living					0.162
Rural	12.5	609	10.1	45	
Semi-urban	9.6	467	11.2	50	
Urban	78.0	3809	78.7	350	
Missing information		36		3	
Duration of breast-feeding (months)					0.103
< 3	21.2	991	17.2	74	
3.0–6	18.8	879	20.5	88	
> 6	60.1	2813	62.2	267	
Missing information		238		19	
Duration of gestation (weeks)					0.070
≤ 38.9	25.0	1219	26.1	116	
39–39.9	23.9	1165	22.5	100	
40–40.8	24.8	1210	29.1	129	
≥ 40.9	26.2	1279	22.3	99	
Missing information		48			
Birth weight in quartiles (g)					0.112
780–3219	24.5	1198	21.8	97	
3220–3571	25.5	1245	23.4	104	
3572–3889	24.5	1195	24.9	111	
3890–5620	25.5	1248	29.9	133	
Missing information		35			
Number of previous pregnancies					0.282
0	46.9	2286	44.5	198	
1–2	44.9	2186	48.3	215	
> 2	8.2	400	7.2	32	
Missing information		49			
Maternal asthma or allergic rhinitis†					<0.001
No	54.2	1410	43.1	112	
Yes	45.8	1190	56.9	148	
Missing information		2321		188	
Paternal asthma or allergic rhinitis†					0.001
No	60.5	1546	50.8	129	
Yes	39.5	1008	49.2	125	
Missing information		2367		194	



Table 1. (Continued)

	All participants <i>n</i> 4921		Cows' milk allergy		
	%	<i>n</i>	Cases <i>n</i> 448		<i>P</i> *
			%	<i>n</i>	
Pets inside home during the first year of life†					
No	67.7	1817	75.6	201	0.004
Yes	32.3	865	24.4	65	
Missing information		2239		182	
Visits to stable during the first year of life†					
No	81.9	2183	84.0	221	0.348
Yes	18.1	482	16.0	42	
Missing information		2256		185	

* Comparison done with χ^2 test, comparing the distribution of the cows' milk allergy across the categories.

† Information collected only from the children participating in the Asthma and Allergy sub-study at the age of 5 years.

Table 2. Maternal daily intake of fatty acids during pregnancy from diet and supplements together (Mean values and standard deviations, *n* 4921)

Fatty acid	Mean	SD
SFA, g	43.7	16.9
Myristic acid 14:0, g	4.7	2.0
Palmitic acid 16:0, g	20.7	7.7
Stearic acid 18:0, g	10.5	4.3
MUFA, g	35.7	12.6
Sum of 18:1 isomers, g	32.9	11.7
PUFA, g	13.8	5.1
<i>n</i> -3 PUFA, g	2.9	1.2
α -Linolenic acid (18:3 <i>n</i> -3), g	2.5	1.0
EPA (20:5 <i>n</i> -3), mg	82.3	63.9
DHA (22:6 <i>n</i> -3), mg	223.9	152.7
<i>n</i> -6 PUFA, g	10.6	4.0
Linoleic acid (18:2 <i>n</i> -6), g	10.3	3.9
Arachidonic acid (20:4 <i>n</i> -6), mg	128.2	53.6
γ -Linolenic acid (18:3 <i>n</i> -6), mg	56.8	31.7
Conjugated linoleic acid (18:2 <i>n</i> -6), mg	180.9	80.6
Trans fatty acids, g	1.7	0.87
Ratios:		
<i>n</i> -6: <i>n</i> -3	3.7	0.84
Linoleic acid (18:2 <i>n</i> -6): α -linolenic acid (18:3 <i>n</i> -3)	4.3	1.0
Energy, kJ	11 700	3440

addition to maternal and paternal history of allergic rhinitis or asthma, and both visits to a stable and pets inside the home during the study participant's first year of life. We tested the interaction of maternal history of allergic rhinitis or asthma and fatty acids among the population participating in the Asthma and Allergy sub-study. If the interaction was significant (*P*-value < 0.05), we studied the association separately among mothers with and without a history of allergic rhinitis or asthma using the fully adjusted model. Missing data were addressed using complete case analysis. SAS version 9.3 (SAS Institute Inc.) and IBM SPSS Statistics for Windows, version 27 (IBM corp.) were used in the analysis.

Results

We identified 448 children with CMA (9.1%). Children with CMA were more often male, had less often pets inside the home

during the first year of life, and their mothers were more often non-smokers during pregnancy and had higher vocational education. Further, their mothers and fathers were more often affected by asthma or allergic rhinitis (Table 1). Children who participated in the Asthma and Allergy sub-study had older, more educated and less frequently smoking mothers when compared with non-participants⁽²⁶⁾.

The maternal intake of studied fatty acids is shown in Table 2. Maternal total intake of SFA, MUFA, PUFA, *n*-3 PUFA, *n*-6 PUFA, trans fatty acids, ratio of *n*-3 PUFA to *n*-6 PUFA or ratio of linoleic acid to α -linolenic acid was not associated with the risk of CMA in the offspring (Table 3). When fatty acid intakes and ratios were analysed as quartiles, we did not observe significant associations between either the lower or the higher quartile as compared with the mid-half and CMA (data not shown).

We observed an interaction between maternal history of allergic rhinitis or asthma and the maternal intake of α -linolenic acid (*P*-value = 0.012). For mothers without a history of allergic rhinitis or asthma (*n* 1265), α -linolenic acid was associated with a decreased risk (OR 0.72; 95% CI 0.56, 0.93) of CMA in the offspring (*n* 237), but not in mothers with a history of allergic rhinitis or asthma (*n* 1062, OR 1.05; 95% CI 0.88, 1.26).

Discussion

We did not observe an association between maternal intake of fatty acids and the development of CMA in the offspring. When we took the maternal history of allergic rhinitis or asthma into account, the maternal intake of α -linolenic acid was inversely associated with the risk of CMA in the offspring of the mothers without a history of allergic rhinitis and asthma, but not in mothers with such a history.

To our knowledge, this is the first study to report the association between maternal intake of fatty acids during pregnancy and the development of CMA in the offspring. The associations between maternal intake of fatty acids during pregnancy and development of allergic rhinitis, asthma, eczema and wheeze in the offspring have been previously reported from the same cohort as in the present study^(11,27). Higher maternal intake of α -linolenic acid during pregnancy was associated with a decreased risk of asthma⁽²⁷⁾ and allergic rhinitis in the

**Table 3.** Associations between maternal daily intake of energy-adjusted fatty acids during pregnancy with the risk of cows' milk allergy in the offspring by the age of 3 years. The OR are presented per 1 standard deviation increment of the particular fatty acid. Both the dietary intake and total intake (dietary + supplement) of each fatty acid were analysed if the supplemental intake was meaningful (Odd ratios and 95 % confidence intervals)

Fatty acid	Unadjusted <i>n</i> 448*/4921†		Adjustment 1‡ <i>n</i> 409*/4403‡		Adjustment 2§ <i>n</i> 234*/2327†	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
SFA total	0.88	0.79, 0.98	0.94	0.84, 1.05	0.96	0.82, 1.13
Myristic acid 14:0 total	0.89	0.80, 0.99	0.94	0.85, 1.06	1.00	0.86, 1.16
Palmitic acid 16:0, total	0.89	0.80, 0.99	0.95	0.84, 1.06	0.95	0.81, 1.12
Stearic acid 18:0, total	0.89	0.81, 0.99	0.94	0.84, 1.06	0.94	0.80, 1.10
MUFA, total	0.94	0.85, 1.05	0.96	0.85, 1.07	0.91	0.78, 1.08
Sum of 18:1 isomers, total	0.95	0.85, 1.05	0.96	0.85, 1.07	0.91	0.77, 1.07
PUFA, total	1.03	0.93, 1.14	1.01	0.90, 1.13	0.95	0.82, 1.12
<i>n</i> -3 PUFA, food	0.98	0.89, 1.09	0.96	0.86, 1.07	0.92	0.79, 1.06
<i>n</i> -3 PUFA, total	0.98	0.89, 1.09	0.96	0.86, 1.08	0.92	0.79, 1.07
α -Linolenic acid (18:3 <i>n</i> -3), total	0.99	0.89, 1.09	0.97	0.87, 1.08	0.93	0.80, 1.07
EPA (20:5 <i>n</i> -3), food	0.97	0.88, 1.08	0.95	0.85, 1.06	0.95	0.80, 1.11
EPA (20:5 <i>n</i> -3), total	0.96	0.87, 1.07	0.94	0.83, 1.06	0.95	0.79, 1.13
DHA (22:6 <i>n</i> -3), food	0.98	0.88, 1.08	0.95	0.85, 1.07	0.94	0.79, 1.12
DHA (22:6 <i>n</i> -3), total	0.97	0.87, 1.08	0.95	0.84, 1.07	0.94	0.79, 1.13
<i>n</i> -6 PUFA, total	1.04	0.94, 1.15	1.02	0.91, 1.14	0.97	0.82, 1.14
Linoleic acid (18:2 <i>n</i> -6), total	1.04	0.94, 1.15	1.02	0.91, 1.14	0.97	0.82, 1.14
Arachidonic acid (20:4 <i>n</i> -6), food	1.07	0.97, 1.18	1.06	0.96, 1.18	0.97	0.83, 1.14
Arachidonic acid (20:4 <i>n</i> -6), total	1.07	0.97, 1.18	1.06	0.96, 1.18	0.97	0.83, 1.14
γ -Linolenic acid (18:3 <i>n</i> -6), food	0.96	0.86, 1.06	0.99	0.89, 1.11	1.03	0.90, 1.19
γ -Linolenic acid (18:3 <i>n</i> -6), total	0.97	0.88, 1.07	1.01	0.91, 1.12	1.04	0.91, 1.20
Conjugated linoleic acid (18:2 <i>n</i> -6), total	0.92	0.83, 1.02	0.98	0.88, 1.10	1.03	0.89, 1.19
Trans fatty acids, total	0.92	0.84, 1.02	0.96	0.86, 1.07	0.94	0.81, 1.09
Ratios:						
<i>n</i> -6 PUFA: <i>n</i> -3 PUFA, food	1.06	0.97, 1.17	1.07	0.97, 1.18	1.11	0.96, 1.27
<i>n</i> -6 PUFA: <i>n</i> -3 PUFA, total	1.06	0.97, 1.17	1.07	0.97, 1.18	1.10	0.96, 1.27
Linoleic acid (18:2 <i>n</i> -6): α -linolenic acid (18:3 <i>n</i> -3)	1.05	0.96, 1.16	1.05	0.95, 1.17	1.09	0.95, 1.25

* Number of children with cows' milk allergy.

† Number of children in the analysis.

‡ Adjusted for study centre, sex, birth weight, maternal age and education, maternal smoking during pregnancy, duration of gestation, mode of delivery, season of birth, number of mother's previous deliveries, length of breast-feeding and urbanity of the living environment.

§ Adjusted additionally for the parental history of allergic rhinitis and asthma, pets inside home during the child's first year of life and visits to the stable during the child's first year of life. This additional information was collected when the child was 5 years old.

offspring⁽¹¹⁾, but for other fatty acids the findings were more inconsistent.

Our results are supported by the RCT where no associations were observed between maternal supplementation of *n*-3 PUFA during pregnancy and the risk of food allergy in the offspring^(13,14). However, a protective effect has been observed in one RCT⁽¹²⁾. The inconsistent results might be explained by the methodological differences between the studies. The dosage of *n*-3 PUFA supplementation has varied, and it is possible that there exists a dose-dependent association. In addition, the variable timing of the supplementation may have resulted in inconsistencies, as the crucial timing of the immune development in infants is yet to be determined.

Our results are in apparent contrast to the recent systematic review concluding that maternal fish oil supplementation during pregnancy may reduce the risk of sensitisation for egg and peanut in the offspring⁽²⁸⁾. However, in epidemiological studies null results have been observed when studying the association between maternal dietary intake of fatty acids and food sensitisation in the offspring^(29,30), one study reporting the sensitisation to cows' milk proteins⁽¹⁰⁾. As egg and peanuts are consumed only in small quantities, and maybe not as early in life as cows' milk products, the maternal intake of fatty acids may have a greater

role in the prevention of egg and peanut allergy. Further, the association between sensitisation and food allergy varies, and it is possible to have sensitisation without clinical food allergy, as well as food allergy without sensitisation⁽³¹⁾.

Our result that α -linolenic acid was inversely associated with the development of CMA only in mothers without a history of allergic rhinitis or asthma may be explained by the possibility that mothers transfer the risk of allergy to their offspring⁽³²⁾. Thus, it is possible that the maternal history of allergy overpowers the protective effect of α -linolenic acid.

The major strength of our study is prospectively collected data from a relatively large sample size, which minimise the selection bias. Our endpoint was based on register-based information and complemented with a parental questionnaire which is validated to represent exceptionally well the physician diagnosed CMA^(19,20). Further, our food consumption data had good coverage and were collected by validated FFQ, specifically designed for the present study. In addition, the FFQ took into account the individual habit of used fats, which increases the accuracy of the intake of specific fatty acids.

The major limitation of our study is the restriction of the participants to those with human leucocyte antigen conferred susceptibility to type 1 diabetes. As previously reported, these



infants may have increased intestinal permeability⁽³³⁾; also, the knowledge of the risk of type 1 diabetes may alter the behaviour of the family, and the parents may seek medical advice more eagerly leading to receiving the diagnosis of CMA more often. These factors may explain the higher incidence of CMA in our study population compared with what is previously reported in Finland^(2,3). Therefore, the generalisability of our results to the general paediatric populations may be limited. In addition, we did not have data on the duration between the onset of symptoms of CMA and the date of diagnosis or start of the elimination diet, even though these should be coincidental. The FFQ was designed to represent the total diet during the 8th month of pregnancy and thus presents an estimate of the diet during the whole pregnancy. In the validation study, the FFQ was observed to slightly overestimate the nutrient intake; this should be ameliorated by the usage of energy-adjusted nutrient intakes. As the FFQ was mailed to the participants after the delivery, it is open for recall bias. However, the FFQ was validated in the same design as the present study was performed and was found to be suitable to measure the maternal diet during pregnancy and has shown acceptable reproducibility and validity⁽¹⁰⁾; thus, the risk of recall bias should be minor. The use of food supplements was not taken into account in the validation study. As our results were substantially similar for the fatty acid intakes from food and from food and supplements together, this should not have major relevance in our study. The fact that we did not have data on the child's dietary intake may result in some residual confounding.

In conclusion, the present study provides novel information about the association between maternal intake of fatty acids during pregnancy and the development of CMA in the offspring. We did not observe an association between the maternal intake of fatty acids and the development of CMA in the offspring, except the maternal intake of α -linolenic acid which was associated with decreased risk of CMA in the offspring of mothers without a history of allergic rhinitis or asthma. Thus, additional benefits may not be expected for the prevention of CMA in the offspring by advising the pregnant women to use supplements containing fatty acids in addition to a healthy and balanced diet.

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S. M. V., M. K., A. L. and J. M. were responsible for formulating the research questions. J. L., M. K., J. T. and R. V. are members of the steering committee of the DIPP study. S. M. V. designed the DIPP nutrition study and within the DIPP nutrition study the allergy study was designed by S. M. V. and M. K. S. M. V. and M. K. were responsible for carrying out the study. R. V. was responsible for the clinical work in Oulu, M. K. was responsible for the clinical work in Tampere. A. L. and H.-M. T. were responsible for analysing the data. A. L. wrote the first version of the article. J. M., M. K., and S. M. V. participated in the writing process. S. M. V. and M. K. had the primary responsibility for the final work with equal contribution. All the authors participated in the critical revision of the manuscript and have accepted the final version.

All authors declare that there are no conflicts of interest.

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PUBLICATION
III

**Cow's milk allergy in infancy and later development of type 1 diabetes–
nationwide case-cohort study**

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Cow's milk allergy in infancy and later development of type 1 diabetes—nationwide case-cohort study

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Abstract

Background: It is suggested that early intake of cow's milk could be a risk factor for type 1 diabetes (T1DM). Further, the different immunological background, gives a suggestion of an inverse relationship for the occurrence of these diseases. The aim of this study was to explore the association between cow's milk allergy (CMA) and the risk of T1DM in a register-based case-cohort study.

Methods: Data were obtained from Finnish nationwide health registers. The study included all children born in Finland between January 01, 1986 and December 31, 2008 and diagnosed with T1DM before the age of 16 years ($n = 7754$). A 10% random sample from each birth year cohort was selected as a reference cohort ($n = 137,798$). T1DM, CMA, and asthma were defined based on valid special reimbursements for the costs of drugs/special formulas needed in the treatment of the diseases. Child's sex, birth decade, asthma, maternal diabetes and asthma, smoking during pregnancy, and previous deliveries were considered as confounding factors. Time-dependent, weighted Cox regression was applied for statistical analyses.

Results: Children with CMA had an increased risk of developing T1DM in fully adjusted model (HR = 1.17; 95% CI 1.02–1.34), but the association was no longer observed when including the use of special infant formulas in the definition of CMA in the sensitivity analysis (HR = 1.11; 95% CI 0.92–1.32). CMA was associated with an increased risk of T1DM in children without asthma (HR = 1.27; 95%CI 1.10–1.47), but not in children with asthma (HR = 0.80; 95% CI 0.92–1.27).

Conclusion: Children with CMA may have an increased risk of T1DM.

KEYWORDS

child, cohort studies, diabetes mellitus type 1, milk, milk hypersensitivity

1 | INTRODUCTION

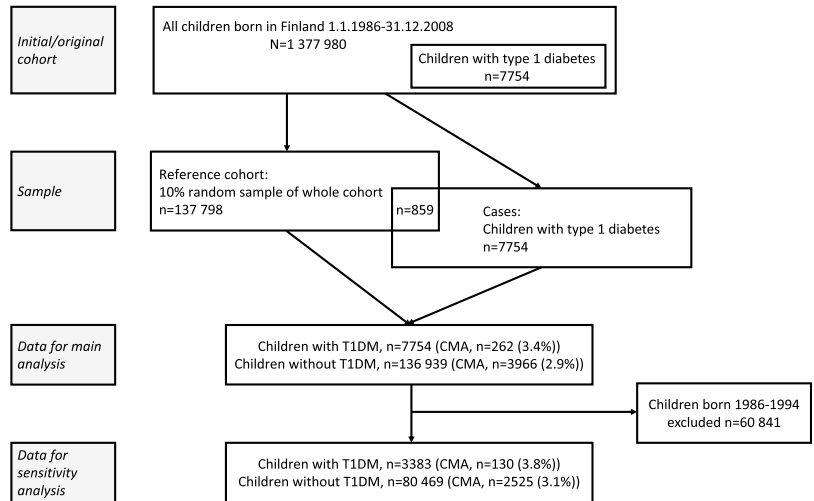
In Finland, the incidence of type 1 diabetes (T1DM) as well as the prevalence of cow's milk allergy (CMA) under the age of 5 years are one of

Johanna Metsälä and Suvi M. Virtanen are considered as joint senior author.

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FIGURE 1 Flowchart of the participants. T1DM, type 1 diabetes; CMA, cow's milk allergy



the highest in Europe.^{1,2} There are at least two background factors that connect these conditions, immunology and cow's milk.

The immunological reactions behind T1DM and allergic diseases are different and to some extent opposite. The autoimmunity process in T1DM involves T-helper type 1 cells (Th1) and Th17, whereas the allergic inflammation is characterized by Th2 immune response. Based on the reciprocal counter-regulation between Th1 and Th2 cells,³ the Th1/Th2 paradigm suggests an inverse relationship between autoimmune and allergic diseases. In the meta-analysis of observational studies, the inverse association between T1DM and allergic diseases was only observed for asthma, but not for allergic rhinitis or atopic dermatitis. Further, for each outcome the individual studies reported null, direct and inverse associations.⁴ For food allergies, both direct⁵ and null⁶ associations have been reported, suggesting that the Th1/Th2 paradigm might be an oversimplification.

On the other hand, early cow's milk exposure has been suggested to play a role in the development of T1DM.⁷⁻⁹ Findings from prospective cohort studies are consistent in that cow's milk intake during childhood is directly associated with development of pre-T1DM and/or T1DM in children.¹⁰⁻¹⁴ However, the age at introduction of cow's milk during infancy does not seem to be associated with T1DM risk.¹⁵⁻¹⁸ A randomized trial found no reduction in the risk for T1DM in the 11.5 years follow-up time when comparing weaning to an extensively hydrolyzed infant formula with weaning to a commercial cow's milk-based formula during the first 6-8 months of life (HR 1.1 95% CI 0.8-1.5).¹⁹ However, a recent finding from a prospective cohort study suggests that early feeding of extensively hydrolyzed formula is directly associated with pre-T1DM.¹⁵ Based on the current evidence, the role of CMA or the early use of cow's milk in development of T1DM remains unclear. Further, to best of our knowledge, the association between CMA and T1DM has not been reported before.

The aim of this study was to examine the association between CMA and the risk of T1DM in a case-cohort study design using Finnish nationwide registers. Based on the Th1/Th2 paradigm and the

cow's milks suggested role as a risk factor for T1DM, we hypothesized that children with CMA have a decreased risk for developing T1DM.

2 | MATERIALS AND METHODS

2.1 | Data sources and study population

The data for the present study were collected from four nationwide registers: the Population Register, the Special Reimbursement Register (information on special reimbursement for insulins available since 1964, and for special infant formulas since 1986), and the Drug Prescription Register (available since 1994) maintained by the Social Insurance Institute of Finland, and the Medical Birth Register (available since 1987), maintained by the Finnish Institute for Health and Welfare. The unique personal identity codes included in all registers were used to link, the information from different registers.²⁰

The initial cohort included all children born in Finland between January 1, 1986 and December 31, 2008 ($n = 1,377,980$). From this initial birth cohort, we identified all children with T1DM before the age of 16 years or the end of year 2009 as cases from the Special Reimbursement Register ($n = 7754$). For a reference cohort, we selected a 10% random sample for each initial birth year cohort from the Population Register ($n = 137,998$). Due to the study design, altogether 859 children with T1DM were also included in this random sample and thus included in the reference cohort (Figure 1).

2.2 | Definition of T1DM and CMA

We defined T1DM and CMA based on the information about the valid special reimbursement for the costs of insulins or special infant formulas needed in the treatment of these diseases. This information was

obtained from the Special Reimbursement Register maintained by the Social Insurance Institute. In Finland, this special reimbursement is granted by the Social Insurance Institute based on a written statement by a physician describing the individual course of the disease and the diagnostic protocol. For each disease there are specific medical criteria defined by the Social Insurance Institute. To evaluate the fulfillment of these criteria, a clinical specialist in the Social Insurance Institute further assesses the physician's statement. The admittance for the special reimbursement is not dependent of the child's place of residence, socioeconomic status or place of treatment. From the register we extracted information about the start and end date of the special reimbursements and the Social Insurance Institute's disease code. The date of admittance of the special reimbursement was used as a proxy for the date of diagnosis. T1DM was defined as a valid special reimbursement and at least one insulin (Anatomical Therapeutic Chemical [ATC] code A10) purchase after the special reimbursement.

We considered CMA as the exposure disease. In Finland, the special reimbursement for special infant formulas is granted for children with CMA up to age of 2 years. During the observation period The Social Insurance Institute criteria for this special reimbursement included a clinical examination by a pediatrician and disappearance of symptoms suggestive for CMA during the elimination diet for cow's milk. In addition, either positive skin prick test for cow's milk protein or elevated cow's milk protein specific serum IgE-value or positive oral food challenge was required. At the time, the double-blind, placebo-controlled food challenge was not required. Instead, for the majority of children the diagnosis was based on open food challenge.²¹

Our main definition for CMA was a valid special reimbursement for special infant formulas at least for 6 months. As this definition is potentially open to false positive diagnosis, we also used a strict CMA definition where at least three special infant formula purchases after the special reimbursement were required.

In Finland, the drugs and special infant formulas are sold exclusively from pharmacies and the drugs are dispensed on prescription only. From the Drug Prescription Register we collected the information of the date of purchase and for drugs the ATC code (insulins (ATC code A10) and anti-asthmatic drugs (ATC code R03)) and for special infant formula the product code. The Drug Prescription Register has recorded all reimbursable purchases since 1994.

2.3 | Confounding factors

The information on maternal age at delivery, smoking during pregnancy, socioeconomic status, the number of mother's previous deliveries, mode of delivery, as well as gestational age, child's birth year, sex, birth weight and birth length were derived from the Medical Birth Register. Additionally, the information on maternal asthma and diabetes were collected from the Special Reimbursement Register.

Based on our recent findings on increased risk of T1DM in children with asthma²² and in children using anti-asthmatic drugs,²³ we considered child's asthma as a potential confounding factor with three

different definitions. Our main definition for asthma was a valid special reimbursement for the costs of anti-asthmatic drugs, data obtained from the Special Reimbursement Register. The criteria by the Social Insurance Institute for this special reimbursement is that the asthma diagnosis is made based on either pulmonary function tests showing obstruction or recurrent obstructive episodes verified by pediatrician and the need of long-lasting drug therapy with inhaled corticosteroids. As this definition likely excludes children with only mild asthma or only intermittent drug treatment, we used two additional, more wider definitions in the sensitivity analyses: (1) a valid special reimbursement for asthma or at least one purchase of any anti-asthmatic drug (ATC code R03) without the special reimbursement, and (2) at least one purchase of any anti-asthmatic drug (ATC code R03).

2.4 | Statistical analysis

To analyze the association between CMA and the risk of T1DM we used a weighted Cox proportional hazard regression using inverse probability weighting to account for the case-cohort design. We considered CMA and asthma as time-dependent variables and the start date of the special reimbursement or the date of the first purchase of any anti-asthmatic drug were used as a proxy for the start of the disease. We followed-up the study population until the appearance of T1DM, age of 16 years, end of year 2009, or death whichever came first. We estimated unadjusted, partially adjusted, and one fully adjusted model. The fully adjusted model included the information of child's sex, birth decade and asthma as well as maternal asthma, diabetes, smoking during pregnancy, and number of previous pregnancies. These variables were selected based on previous knowledge and their associations with T1DM and CMA in the present data.²⁴⁻²⁶ These analyses were considered as the main analyses.

We conducted multiple sensitivity analyses in a subgroup of children born 1995–2008, as the information on drug and special infant formula purchases were available since 1994. First, we repeated the main analyses to evaluate the potential effect of a smaller sample size. Second, we conducted the analyses using the strict CMA definition to evaluate the potential misclassification of CMA. Third, we conducted analyses using the two additional definitions for asthma.

To examine whether the association between CMA and the risk of T1DM was modified by child's asthma, an interaction term between CMA and asthma was included in the models. Missing data were handled by complete case analysis. Analyses were performed using STATA, version 14, (RRID:SCR_012763) software.

2.5 | Ethical approval

The Institutional Review Board of the Finnish Institute for Health and Welfare, and the institutions keeping the registers, after hearing The National Data Protection Authority, have approved the study.

TABLE 1 The characteristics of the study population

Characteristics	Children with T1DM (n = 7754); n (%)	Children without T1DM (n = 136,939); n (%)
Mean follow-up time (years), mean (SD)	7.4 (4.1)	11.2 (5.0)
Male sex	4226 (55)	70,059 (52)
Child's birth year		
1986–1989	1821 (23.5)	24,351 (17.8)
1990–1999	4610 (59.5)	61,388 (44.8)
2000–2008	1323 (17.1)	51,200 (37.4)
Child's asthma	564 (7.3)	7141 (5.2)
Child's cow's milk allergy	262 (3.4)	3966 (2.9)
Gestational age in weeks		
<37	452 (5.8)	7103 (5.2)
37–41	6519 (84.1)	116,887 (85.4)
≥42	292 (3.8)	6087 (4.5)
Missing information	491 (6.3)	6862 (5.0)
Maternal age at delivery (years), mean (SD)	29.5 (5.1)	29.6 (5.3)
Maternal smoking during pregnancy		
No	6251 (81)	107,448 (78)
Yes	856 (11)	20,056 (15)
Missing information	647 (8.3)	9435 (6.9)
The number of previous deliveries		
0	2976 (38)	53,262 (39)
1	2570 (33)	44,133 (32)
≥2	1722 (22)	32,874 (24)
Missing information	486 (6.3)	6670 (4.9)
Mode of delivery		
Vaginal	6013 (77.6)	109,688 (80.1)
Cesarean section	1274 (16.4)	20,864 (15.2)
Missing information	467 (6.0)	6387 (4.7)
Maternal socioeconomic status		
Upper-white collar	872 (11.3)	15,779 (11.5)
Lower-white collar	2542 (32.8)	39,255 (28.7)
Blue collar	891 (11.5)	15,825 (11.6)
Others	863 (11.1)	16,936 (12.4)
Missing information	2586 (33.3)	49,144 (35.8)
Maternal diabetes	184 (2.4)	557 (0.41)
Maternal asthma	221 (2.9)	3802 (2.8)

3 | RESULTS

We identified 7754 children with T1DM, and our reference cohort consisted of 137,798 children. The mean follow-up time for children with T1DM was 7.4 years (SD 4.1) and for children without T1DM 11.2 years (SD 5.0). Altogether we identified 4228 children with CMA, diagnosed at the mean age of 7.1 months (SD 3.9). For all

children with both CMA and T1DM ($n = 262$), CMA was diagnosed before T1DM, with the CMA diagnosed at the mean age of 7.2 months (SD 4.2). The children with T1DM were more often male, had asthma, and more often their mothers had diabetes, were non-smokers during pregnancy, and had fewer previous pregnancies (Table 1). Children with CMA were more often male (58% vs. 51%) and had asthma (19% vs. 5%) and more often their mothers were non-smokers (87% vs. 78%) and had asthma (6% vs. 3%).

In our main analyses in the children born between 1986 and 2008, CMA was associated with an increased risk of T1DM in unadjusted analysis (HR = 1.29; 95% CI 1.13–1.46) and the increased risk remained in the fully adjusted model including the child's sex, birth decade, and asthma as well as maternal asthma, diabetes, smoking during pregnancy, and number of previous pregnancies (HR = 1.17; 95% CI 1.02–1.34) (Table 2).

In the sensitivity analysis we repeated the main analysis among children born between 1995–2008 and the association between CMA and T1DM was present in the unadjusted model, but no longer in the fully adjusted model (HR = 1.12; 95% CI 0.94–1.33) (Table 2). When further using the strict CMA definition, these results did not change substantially (Table 2). Similarly, when using the two additional definitions for asthma as a confounding factor and the main CMA definition, the results were not substantially different (a valid special reimbursement or at least one purchase of any anti-asthmatic drug: full adjusted HR = 1.14; 95% CI 0.96–1.35, at least one purchase of any anti-asthmatic drug: full adjusted HR = 1.14; 95% CI 0.96–1.35).

We observed an interaction between child's CMA and asthma (p for interaction = 0.002). In the separate analyses according to asthma, we observed that CMA was associated with an increased risk of T1DM in children without asthma (HR = 1.27; 95%CI 1.10–1.47), but not in children with later asthma (HR = 0.80; 95% CI 0.92–1.27) when adjusted for child's sex, birth decade, maternal asthma, diabetes, smoking during pregnancy, and number of previous pregnancies.

4 | DISCUSSION

In the present study, we showed that CMA was associated with an increased risk of T1DM after adjusting for several potential confounding factors. However, when applying stricter CMA criteria, the association between CMA and the risk of T1DM was no longer observed.

The major strength of our study is the use of nationwide register-based data with a good coverage. Further, the prospectively collected information and the relatively large number of cases minimize the recall bias. In addition, our criteria for T1DM should be reliable, because the criteria for the special reimbursement is evaluated by two independent specialists and the admittance is not dependent of the patient's socioeconomic status or the place of residence/treatment. Although the criteria for admittance are same for all types of diabetes in need of insulin, to our knowledge, the use of insulin is rare for types other than T1DM in children under 16 years of age.^{27,28} Thus, the misclassification of cases should be minor. Other

TABLE 2 The main results of the association between CMA and the development of T1DM in children born in Finland between 1986 and 2008 and the results of the sensitive analysis of children born between 1995 and 2008 in a register based case-cohort study

	Children born in 1986–2008; <i>n</i> = 144,693 (T1DM <i>n</i> = 7754)		Children born in 1995–2008; <i>n</i> = 83,852 (T1DM <i>n</i> = 3383)			
	CMA ^a (<i>n</i> = 4228)		CMA ^a (<i>n</i> = 3065)		CMA ^b (<i>n</i> = 2665)	
	HR	95% CI	HR	95% CI	HR	95% CI
Unadjusted	1.29	1.13–1.46	1.21	1.02–1.43	1.20	1.00–1.43
Adjusted for sex and birth decade	1.25	1.09–1.42	1.20	1.02–1.42	1.19	0.99–1.42
Adjusted for sex, birth decade, asthma	1.18	1.04–1.35	1.16	0.98–1.38	1.15	0.96–1.38
Adjusted for sex, birth decade, asthma, maternal asthma and diabetes, smoking during pregnancy, number of previous deliveries	1.17	1.02–1.34	1.12	0.94–1.33	1.11	0.92–1.32

Abbreviations: CMA, cow's milk allergy; T1DM, type 1 diabetes.

^aCMA defined based on a valid special reimbursement for at least 6 months.

^bCMA defined based on a valid special reimbursement for at least 6 months and at least three formula purchases from the pharmacy.

strengths include our possibility to take into account several potential confounders, including two new, recently identified factors: child's asthma and child's use of anti-asthmatic drugs.^{22,23} However, we may have missed some potentially important confounders, like diet and paternal factors,^{8,29–31} thus the residual confounding cannot be ruled out.

A major limitation of the present study is that the definition for CMA may be open for misclassification of the children. Although it is possible that the child has been granted for the special reimbursement without an oral food challenge, in practice majority of the children diagnosed with CMA had undergone food challenge before the diagnosis.²¹ During the observation period, the food challenge was usually done as open food challenge, which is further open for misclassification, especially for the non-IgE-mediated CMA for which the double-blind, placebo-controlled food challenge would be more accurate.³² We tried to disentangle the potential misclassification by applying a strict CMA definition, which included also information on purchased special infant formulas. When using this strict CMA definition, the association between CMA and the risk of T1DM was no longer seen. However, these results were similar to those obtained from the models using the main CMA definition in the same birth year-restricted subgroup, indicating that the disappearance of the association might be because of smaller sample size rather than inaccuracy in definition of CMA. Further, we may have missed some children receiving the CMA diagnosis after the age of 1 year, because the special formula is possible to be replaced by other milk substitutes after the age of 1 year. However, for majority of the children the symptoms start rapidly after the first exposure for cow's milk formula and thus the appearance of CMA after the age of 1 year is rare.^{33,34} In addition, the prevalence of CMA in children without T1DM was approximately 3% regardless of used definition for CMA, which is similar in comparison to previously reported prevalence in Finland.^{35–37} Thus, we consider that the probable inaccuracy in the definition of CMA did not cause major bias to our study. Because of the high incidence of T1DM in Finland, the generalizability of our results to pediatric populations with low incidence of T1DM calls for precaution.

To best of our knowledge, the association between CMA and T1DM has not been reported before. Further, studies concerning the coexistence of any/other food allergies and T1DM are scarce, and both increased risk for T1DM⁵ and null associations have been reported.⁶ For other allergic diseases, like atopic eczema, more studies exists and both direct³⁸ and inverse^{39,40} as well as null associations^{41,42} have been reported.

As the results from studies concerning the coexistence of allergic diseases and T1DM are scarce it is suggested that the Th1/Th2 paradigm might be an oversimplification, which is supported by our results as well. After the introduction of this original paradigm other kinds of Th-cells, including Th17 cells, have been recognized⁴³. Thus, the immunopathology behind these diseases might be more complex than previously thought.

In Finland the special reimbursement for special infant formula correlates well with the strict cow's milk elimination diet.⁴⁴ Therefore our finding about the possible co-occurrence of CMA and T1DM does not support the previous findings about the direct association between childhood cow's milk consumption and the development of T1DM and prevention of T1DM by eliminating cow's milk from infants diet.^{8,9} Our result is rather supported by a recent RCT, which did not observe any difference in the development of T1DM related autoantibodies nor T1DM when comparing groups weaned either extensively hydrolyzed infant formula or commercial formula.¹⁹

Our observation of the possible co-occurrence of CMA and T1DM might be explained by the shared risk factors behind the diseases. The increased prevalence of autoimmune and allergic diseases in western countries have been explained by the decrease in number of infectious diseases and microbial exposures.⁴⁵ In addition, previous studies have observed that these changes in environmental factors may alter the diversity of gut microbiota, which may lead to adverse immunological reactions.⁴⁶ For both, T1DM and CMA, studies have reported that changes in the gut microbiota, leading to dysbiosis, could be involved in the development of the diseases.^{47,48} Furthermore, the gut microbiota of children with CMA is observed to differ before and after the cow's milk protein elimination when compared to healthy controls.⁴⁹ These changes in gut microbiota, reflecting the

western lifestyle or the elimination of cow's milk protein from diet, may at least partly explain our result of the direct association between CMA and the risk T1DM.

We observed that CMA was associated with an increased risk of T1DM in children without asthma, but not in children with later asthma. The meta-analysis have observed a slight inverse association between asthma and T1DM,⁴ however individual studies are reporting all inverse, diverse and null results.⁵⁰ Further, we have recently reported that the direction of the association between asthma and T1DM in childhood is dependent by the sequential appearance of the diseases: prior diagnosed asthma increased the risk of subsequent development of T1DM, but prior T1DM decreased the subsequent development of asthma.²² Also, we have reported that the use of anti-asthmatic drugs increased the risk of development of T1DM, thus the medication may have a role in the association between these diseases.²³ The explanation behind our finding is not clear, but at least it suggests that in future the asthma should be taken into account when studying the association between CMA and T1DM.

In conclusion, our results suggest that children with CMA may have an increased risk of T1DM. This finding supports that the Th1/Th2 paradigm might be oversimplification. Further, it is possible that dietary factors related to CMA, elimination of cow's milk or use of special infant formula in early childhood, may have a role in development of T1DM, but further studies are needed to confirm this.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.


AUTHOR CONTRIBUTIONS

Suvi M. Virtanen and Annamari Lundqvist designed the initial case-cohort setting. Suvi M. Virtanen, Annamari Lundqvist, Johanna Metsälä, Lauri J. Virta, Minna Kaila and Mika Gissler contributed to the planning of the data collection. Johanna Metsälä and Suvi M. Virtanen planned and Johanna Metsälä performed the statistical analysis. Anni Lamminsalo wrote the first version of the manuscript. All authors contributed to interpretation of data, revised the article critically for important intellectual content, and approved the final version to be published.

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