Early life dietary factors on the risk of celiac disease. Associations of dietary factors with risk of celiac disease in children at genetic risk.

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2022

Document Version:
Publisher's PDF, also known as Version of record

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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To be defended at Medelhavet, Clinical Research Center, Inga Marie Nilssons gata 53, Malmö on Friday 11th of March 2022 at 9:00.

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Louisa Mearin Manrique
Professor emerita of Pediatrics, University of Leiden, the Netherlands.

Organization LUND UNIVERSITY	Document name Doctoral Dissertation
	Date of issue 11th of March 2022
Author Elin M Hård af Segerstad	Sponsoring organization

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Abstract

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The first study tested associations of daily gluten intake to age five years with incidence of CDA and CD. For every g/day increase in gluten the risk of CDA and CD increased.

The second study analyzed associations of intake of different gluten food sources to age two years and risk of CDA and CD in Swedish TEDDY children. An intake of >1/2 slice of bread at age 12 months, as well as every bottle of milk cereal drink at age 18 months, was asociated with increased risk of both study outcomes.

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The final study explored simplified dietary patterns and associations with CDA and CD. Higher adherence to *Unsaturated fats and wheat* at age nine months as well as *Potatoes and meat* at age 18 months were inversely associated with CDA and CD, whereas higher adherence to *Unsaturated fats and wheat* at 24 months was associated with increased risk of CDA and CD.

This thesis concludes that several dietary factors after infancy and weaning are associated with risk of CDA and CD in children at genetic risk. These findings should be validated in other prospective cohorts as well as in clinical trials before concluding causality.

Key words celiac disease, celiac disease autoimmunity, TEDDY, children, HLA DQ2, HLA DQ8, gluten, wheat, rye, barley, milk powder, dietary patterns, food records

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN 1652-8220		ISBN 978-91-8021-190-1
Recipient's notes	Number of pages 95	Price
	Security classification	

Signature  Date 2022-02-03

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Coverart by Cornelia Runehammar

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ISBN 978-91-8021-190-1
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

If we knew what it was we were doing,
it would not be called research, would it?
A. Einstein

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Abbreviations

AGA, anti-gliadin antibody

AGE, advanced glycation end products

APC, antigen presenting cell

CD, celiac disease

CDA, celiac disease autoimmunity

CI, confidence interval

DPG, deamidated gliadin peptide

EER, estimated energy expenditure

EMA, endomysium autoantibodies

ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition

FDR, first-degree relative

FFQ, food frequency questionnaire

IgA, immunoglobulin A

IgG, immunoglobulin G

HLA, human leucocyte antigen

HR, hazard ratio

g, grams

GF, gluten-free

kcal, kilocalories

kg, kilograms

MCD, milk cereal drink

OR, odds ratio

PCA, principal components analysis

RBA, radiobinding assay

RCT, randomized controlled trial

SD, standard deviation

SDP, simplified dietary pattern

T1D, type 1 diabetes

TEDDY, The Environmental Determinants of Diabetes in the Young

tTG, tissue transglutaminase

tTGA, tissue transglutaminase autoantibodies

Q, quartile

List of publications

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I-IV.

I. Carin Andrén Aronsson, Hey-Seung Lee, **Elin M. Hård af Segerstad**, Ulla Uusitalo, Jimin Yang, Sibylle Koletzko, Edwin Liu, Kalle Kurppa, Polly J. Bingley, Jorma Toppari, Annette G. Ziegler, Jin-Xiong She, William A. Hagopian, Marian Rewers, Beena Akolkar, Jeffrey P. Krischer, Suvi M. Virtanen, Jill M. Norris, Daniel Agardh; for the TEDDY study group. Association of Gluten Intake During the First 5 Years of Life with Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Genetic Risk. *JAMA*. 2019;322(6):514-523.

II. **Elin M. Hård af Segerstad**, Xiang Liu, Ulla Uusitalo, Daniel Agardh, Carin Andrén Aronsson for the TEDDY study group. Sources of dietary gluten in the first two years of life and associations with celiac disease autoimmunity and celiac disease in Swedish genetically predisposed children: TEDDY study. *Under review pending revisions,* 2021.

III. **Elin M. Hård af Segerstad**, Hye-Seung Lee, Carin Andrén Aronsson, Jimin Yang, Ulla Uusitalo, Ingegerd Sjöholm, Marilyn Rayner, Kalle Kurppa, Suvi M. Virtanen, Jill M. Norris, Daniel Agardh, The TEDDY study group. Daily Intake of Milk Powder and Risk of Celiac Disease in Early Childhood: A Nested Case Control Study. *Nutrients* 2018;10(5):550-559.

IV. **Elin M. Hård af Segerstad**, Xiang Liu, Carin Andrén Aronsson, Ulla Uusitalo, Jimin Yang, Jill Norris, Suvi M. Virtanen, Edwin Liu, Kalle Kurppa, Sibylle Koletzko, Annette G. Ziegler, Jorma Toppari, William A. Hagopian, Marian Rewers, Richard A. McIndoe, Beena Akolkar, Jeffrey K. Krischer, Daniel Agardh, for the TEDDY study group. Dietary patterns during early childhood confer different risk of celiac disease autoimmunity and celiac disease in children at genetic risk: TEDDY study. *Manuscript,* 2021.

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Populärvetenskaplig sammanfattning

Syftet med denna avhandling är att undersöka kostens betydelse för uppkomsten av celiaki (glutenintolerans) hos barn med ärftlig risk för sjukdomen.

Celiaki är en livslång sjukdom som oftast uppstår under barndomen. Kroppens immunförsvar reagerar på födoämnet gluten, vilket är ett protein som finns i vete, råg och korn. Den immunologiska reaktionen mot gluten leder till att tunntarmens slemhinna stöts bort med ett sämre näringsupptag som följd.

Celiaki drabbar personer som bär på en kombination av arvsanlag som finns på kromosom 6, så kallade HLA-gener. HLA kodar för viktiga funktioner i immunförsvaret. Den kombination av HLA som är kopplad till celiaki finns hos ca 40% av den europeiska befolkningen, men bara ca 1% drabbas av sjukdomen.

Under mitten av 1980-talet ökade nyinsjuknandet i celiaki dramatiskt hos barn under två år i Sverige. Denna unika ökning skedde samtidigt som de nationella kostråden för spädbarn ändrades till att gluten skulle introduceras i barns kost vid sex månader, i stället för som tidigare vid fyra månaders ålder. Samtidigt ökade barnmatsproducenter innehållet av gluten i välling och gröt. När kostråden vid mitten av 1990-talet ändrades tillbaka till att gluten skulle introduceras vid fyra månaders ålder under skydd av amning, minskade nyinsjuknandet av celiaki. Dessa snabba ändringar i förekomst av celiaki i befolkningen talar för att omgivningsfaktorer påverkar risken för att få celiaki. Även om erfarenheten från de nationella ändringarna av kostrekommendationerna pekar mot kostens betydelse är förekomsten av celiaki hos svenska barn idag fortfarande bland den högsta i världen.

Hypoteserna som testades i denna avhandling var om mängden gluten som barn äter har betydelse för deras risk att utveckla celiaki. Vidare undersöktes om intag av olika typer av gluteninnehållande livsmedel och sädesslag, samt om intag av mjölkpulver i barnmatsprodukter påverkar risken av att utveckla celiaki. Slutligen undersöktes om olika kostmönster hos små barn har betydelse för deras risk att utveckla celiaki.

Studierna som denna avhandling bygger på utgår från insamlade data från den multinationella studien The Environmental Determinants of Diabetes in the Young (TEDDY). Barn från USA, Sverige, Finland och Tyskland, undersöktes för HLA-gener vid födseln och de barn som bar på riskgener som är kopplade till typ 1 diabetes och celiaki bjöds in till en 15 år lång uppföljning med bland annat insamling av matdagböcker, information om livshändelser och regelbunden blodprovstagning i syfte att undersöka vilka faktorer som kan vara involverade vid uppkomsten av typ 1 diabetes och celiaki.

Resultaten som presenteras i denna avhandling visar att ju mer gluten som barn med ärftlig risk äter under de fem första levnadsåren, desto större är deras risk för att utveckla celiaki. Det verkar inte ha någon betydelse från vilket sädesslag som gluten intas, men att bröd, gröt och välling verkar öka risken. Däremot verkar inte intag av mjölkpulver under de två första levnadsåren påverka risken för barnet att få celiaki. Vidare påvisades ett samband mellan flera olika kostmönster i tidig ålder och celiaki. Gemensamt för de kostmönster som minskade risken för celiaki var att de utgjordes av mycket potatis, havre, ris och andra glutenfria sädesslag, rotfrukter och kött.

Eftersom avhandlingen utgår från en observationsstudie så kan slutsatser om orsak och verkan för de samband som observerats mellan kost och celiaki inte dras. Resultaten behöver bekräftas i andra studier på barn med ärftlig risk för celiaki innan kostråd på samhällsnivå eventuellt kan revideras. Baserat på resultaten från denna avhandling har två nya interventionsstudier startats i syfte att kartlägga orsakssamband och om celiaki kan förhindras genom kostförändringar.

Introduction

Celiac disease (CD) is an immune mediated disorder leading to villous atrophy in the small intestine (1). Another feature of CD is the development of autoantibodies against tissue transglutaminase (tTGA) (2). The driving antigen in CD is gluten, which is a protein found in wheat, rye, and barley (3). A gluten-free (GF) diet leads to recovery of the intestinal mucosa and normalization of tTGA levels in the majority of patients (4).

To this day, a life-long strict GF diet with no more than 10 mg gluten/day remains the sole treatment of CD and reintroduction of gluten in the diet leads to relapse of the disease (5, 6). The GF diet is composed of foods naturally free of gluten, including meat, eggs, dairy, vegetables, fruits, oats, buckwheat, quinoa, and millet. The burden of a GF diet may have a negative impact on quality of life as well as nutritional status, especially if GF diet products are expensive and scarce (7).

The discovery of celiac disease

The Dutch pediatrician Willem-Karel Dicke was the first to discover an association of wheat intake with clinical malabsorption syndrome in children. In the 1930's, his studies on different diets in affected pediatric patients suggested that bread and husk were causing their symptoms (8, 9). After further studies on the dietary management of his patients, he concluded that gliadins, the alcohol soluble fraction of gluten proteins found in wheat and rye, were causing fat malabsorption and stunted growth in affected children (8, 10). Some years later, medical doctor Charlotte M Andersson and her colleagues described the histopathological intestinal changes typical for CD in untreated patients, which were reversed on a wheat-free diet (11). In the early 1960's, researchers discovered that many CD patients had detectable serum antibodies against gliadin (AGA) (12). This led to the hypothesis that the immune system could be involved in the pathogenesis.

Pathogenesis of celiac disease

In CD, there is an abnormal T-cell response to specific gliadin peptides in gluten (3, 13). In the small intestine, gluten is partially degraded to peptides (14). Gliadin activates the release of zonulins which increase gut permeability, thus allowing gluten residues to be absorbed into the intestinal lumen (15). In the lumen, glutamine in gliadin peptides is deamidated to glutamate by the enzyme tTG, rendering them negatively charged (16). Deamidated gliadin peptides (DGPs) are phagocytized by antigen presenting cells (APC) and presented in the clefts of human leucocyte antigen (HLA) DQ2 and DQ8 molecules.

The APC presents the bound deamidated gliadin to naïve helper T-cells. In CD patients, only certain epitopes are recognized by T-cells, leading to their activation (3). About 40 T-cell epitopes have been described, but these are believed to only account for part of all celiac-specific peptides (17). Typical for T-cell epitopes are that they are good substrates for tTG and that they bind exclusively to DQ2 and DQ8 (3).

T-cells are activated to either natural killer T-cells or to helper T-cells. Natural T-killer cells induce inflammation by the release of cytokines leading to destruction of enterocytes and mucosal villus atrophy. T-helper cells activate B-cells that start to produce antibodies to DGP, tTGA, as well as to endomysium autoantibodies (EMA) (3, 16, 18) (Figure 1).

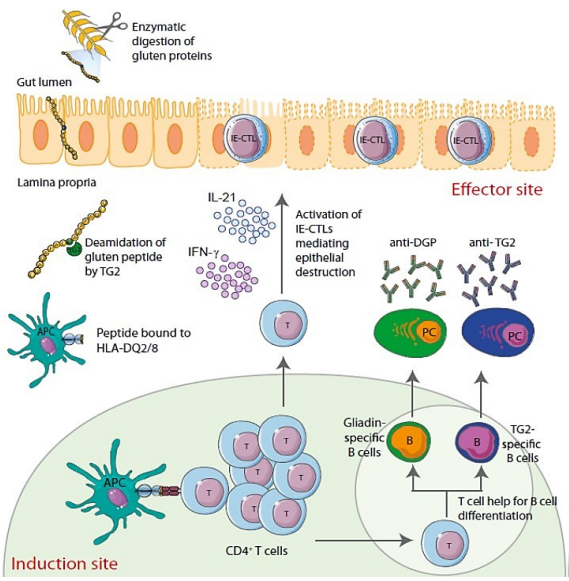


Figure 1 Key events in CD pathogenesis, from Christophersen et al, Trends is Molecular Medicine, 2019 (18).

Biomarkers in celiac disease

The discoveries of CD specific antibodies have improved clinical diagnostics and facilitated for performing large scale screenings. In the 1990's, AGA and EMA were routinely used as diagnostic tools for selecting patients for further investigation with intestinal biopsy as well as for evaluating adherence to the GF diet in CD patients (19).

In 1997, tTG was discovered to be the major autoantigen in CD (20). tTG is a calcium-dependent enzyme found in several human tissues, including in the lamina propria of the gastrointestinal mucosa. IgA-tTG was further found to have similar diagnostic performance compared with EMA (21).

In 2004, development of DGP assays showed that IgG-DGP had higher diagnostic performance compared with AGA. (22). Although IgG-DGP was found to have lower specificity than IgA-tTG, antibodies against DGP performed even better in children younger than age two years.

Levels of IgA-tTGA correlate well with the severity of mucosal damage (23-25) and are normalized within a year on GF diet in most children with CD (26).

Clinical presentation of celiac disease

Intestinal villi increase the surface area of the gastrointestinal tract to optimize nutrient absorption. Therefore, malabsorption increases with the degree of villous atrophy in CD. The classical presentation of CD in young children includes bloating, diarrhea, constipation, abdominal distention, and pain, stunting and poor weight gain (27). Older children and adolescents often present with impacted growth or delayed puberty and iron deficiency anemia. Extra-intestinal manifestations of CD include headache, anxiety, depression, and apathy. However, screening studies have shown that many patients only have minor symptoms or may even be asymptomatic (15, 27, 28).

The diverse clinical presentation of CD has led to the analogue of an iceberg (**Figure 2**) (29). The tip above the surface represents patients with classical symptoms associated with CD. Below the surface lures patients presenting with vague symptoms or who are virtually asymptomatic. Potential or latent CD are individuals with elevated tTGA levels but with normal mucosa.

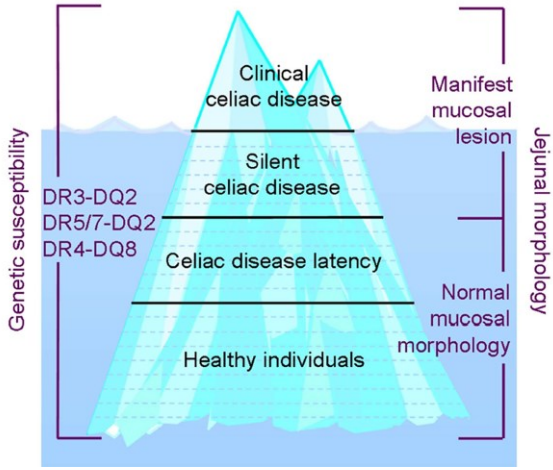


Figure 2 The celiac iceberg and spectrum of the disease. Figure from Lionetti and Catassi, International Reviews of Immunology, 2011 (29).

Diagnostic criteria of celiac disease

In 1970, the European Society for Paediatric Gastroenterology (today named European Society for Paediatric Gastroenterology, Hepatology and Nutrition, ESPGHAN) published the first diagnostic guidelines for CD. To fulfil the diagnosis, an intestinal biopsy was required to confirm villous atrophy, a second biopsy to confirm mucosal healing after gluten elimination, and a third biopsy to show relapse of the disease after gluten challenge. In addition, the patient should have complete relief of symptoms as a clinical response to the GF diet (30).

In 2012, ESPGHAN revised the diagnostic criteria of CD (28). An intestinal biopsy to confirm diagnosis in symptomatic cases with presenting high levels of tTGA was no longer required because of the high concordance between autoantibody levels and villous atrophy. Instead, diagnosis of CD could be determined if tTGA levels were more than ten times (> 10 x) the upper level of normal limit, confirmed by EMA positivity, HLA DQ2 or DQ8 as well as with complete relief of symptoms and normalization of tTGA on a GF diet (28). If tTGA <10 x normal, or EMA is negative, intestinal biopsies should be performed to confirm the diagnosis. In asymptomatic patients, intestinal biopsies should always be considered to confirm the diagnosis.

In 2020, ESPGHAN again revised the diagnostic criteria to no longer require HLA to confirm diagnosis (31). Furthermore, asymptomatic children with tTGA levels >10 x the upper level of normal limit may also be diagnosed using the no-biopsy approach.

Upper endoscopy is recommended for taking intestinal biopsies, with at least one biopsy taken from the bulb and at least four from the duodenum. Histopathology are categorized according to the Marsh-Oberhuber classification where Marsh >1 is deemed to be CD (32) (Table 1).

Table 1 The Marsh-Oberhuber classification of intestinal biopsies in CD. Adopted from Dickson et al, *Journal of Clinical Pathology*, 2006 (32).

	Marsh 0	Marsh 1	Marsh 2	Marsh 3a	Marsh 3b	Marsh 3c	Marsh 4
IEL count*	<30/100	>30/100	>30/100	>30/100	>30/100	>30/100	<30/100
Crypt hyperplasia	-	-	+	+	+	+	-
Villus athrophy	-	-	-	Mild	Moderate	Total	Total

* Number of intraepithelial lymphycytes (IEL) per 100 enterocytes.

Definition of celiac disease autoimmunity

CD specific antibodies are found in children before clinical CD has developed (33-35), however not all children persistently tTGA positive develop CD. Some have transient or fluctuating tTGA without mucosal damage, as shown in screenings following children at genetic risk on a gluten-containing diet (34, 36, 37). However, longitudinal studies have demonstrated that many children with persistent tTGA and normal mucosal findings will later develop CD (33, 38). According to the Oslo definitions for CD, persistently confirmed tTGA or EMA should be referred to as CDA (27), a term widely adopted as an outcome in research on CD.

Role of HLA in celiac disease

There is a strong influence of genetics in CD. The risk of developing CD in an individual is increased to 10-20% if a family member is affected (38, 39). Moreover, the concordance of CD among monozygotic twins is 75% if one of the siblings has the disease (40, 41). The strongest genetic association with CD is linked to HLA.

The HLA DQ2 and/or DQ8 haplotypes are found in nearly all CD patients of which DQ2 is present in more than 90% of cases (16). The DQ2 haplotype is either encoded by HLA-DQA1*05:01-DQB1*02:01 (also abbreviated DQ2.5) or DQA1*02:01-DQB1*02:01 (also abbreviated DQ2.2). DQ8 constitutes of the HLA-DQA1*03:01 and DQB1*03:02 alleles (1).

Both DQ2 and DQ8 are frequently found in the general population, however with global differences (42). In the Caucasian population, about 40-60% are carriers of either DQ2 and/or DQ8 (2, 36, 43) compared with 1/3 of the population in India (44). DQ2 is more prevalent in North Africa compared with the southern parts (42).

There is a gene dose effect of HLA risk genotypes on the risk of CD. The DQ2.5/DQ2.5 genotype confers the highest risk in CD, followed by the DQ2.5/DQ2.2 and DQ2.5/DQ8 genotypes (14). In two birth cohorts, 10% and 11% of children homozygous for DQ2 were diagnosed with CD by the age of five years, compared with 3% and 6% of children heterozygous for DQ2, respectively (36, 38).

There is an overlap of shared genetic risk in CD and T1D, conferred to HLA DQ2 and DQ8 genotypes. In type 1 diabetes (T1D), a higher risk is observed in children carrying HLA DQ8, than in those with HLA DQ2 (45).

In addition to HLA DQ2/DQ8, more than 40 non-HLA genes have been associated with CD (46). The overall genetic risk is estimated to approximately 50%, of which non-HLA risk haplotypes explain 15% of the disease risk.

Epidemiology

A mass-screening in four European countries a decade ago found a 1% (95% CI 0.9, 1.1) prevalence of CD with country differences (47). In a later systematic review with meta-analysis, the pooled global prevalence of positive CD serology was 1.4% (95% CI 1.1, 1.7%) and biopsy-proven CD 0.7% (95% CI 0.5%, 0.9%) with lower prevalence in South America and North Africa, and higher prevalence in Europe, Oceania, and Southeast Asia. (48). In Sweden and in some parts of the Colorado, the prevalence of screening detected CD has been reported to be as high as 3% (36, 49).

CD is most likely underdiagnosed due to lack of population-based screenings. Globally it is estimated that as many as 95% of patients with CD remains undiagnosed (48). In Sweden and Italy, screening studies detect about 2/3 of children with CD (37, 49, 50).

CD can develop at any age (51), but the peak incidence seems to occur during childhood (36, 48). Moreover, there is a female predominance in CD (48), which has

been confirmed in longitudinal birth cohort studies (38, 52). Additionally, patients with T1D, autoimmune thyroiditis, Down syndrome, Turner syndrome, and selective IgA deficiency, are at an increased risk to develop CD (28).

Differences in prevalence of CD between countries are partly attributed to genetics and if screenings have been performed in the general population. However, there are still reported differences despite shared proportions of HLA risk genotype distributions (38, 44, 53). In The Environmental Determinants of Diabetes in the Young (TEDDY) study, Swedish children are at an almost two-fold risk of developing CD at a young age compared with children from the United States (US) (38). These differences could be attributed to environmental factors as demonstrated in the Russian/Finnish Karelia area (53).

The incidence of CD has increased on average by 7.5%/year in industrialized countries during the past decades but has stabilized more recently in areas in the UK and Finland (54). Part of the increase may be the result of greater clinical awareness and diagnostic improvements. However, it may partly also be attributed to changes in environmental exposures (51).

Environmental risk factors in celiac disease

Repeated early life infections has been found to associate with increased risk of later CD (55-57). In TEDDY, reported gastrointestinal infections three months prior to seroconversion of tTGA increased the risk of CDA (58). In a nested case control study in the same cohort, children that developed CDA had higher frequencies of enterovirus exposures in early life (59, 60). Infection by rotavirus has also been associated with higher risk of CD (61) and there are some indications that rotavirus vaccination is inversely associated with CD (58). However, no effect on population level by general rotavirus vaccination has been observed in Finland (62).

In several studies, differences in the microbiota have been found in children with CD as compared with healthy children (63). In studies prospectively following high risk children before development of CD, differences in the microbiota have confirmed these previous observations (64, 65). Antibiotics may affect the composition of the gut microbiota, and an association with CD has been found in some (57, 66), but not all, studies (56, 67). Moreover, potential beneficial effects of probiotics have been investigated, however, evidence of an effect on the risk of CD has not been found (68-70).

There are conflicting results on mode of delivery and risk of CD in population-based studies. Elective caesarean section was observed to increase the risk of CD in Swedish children (71). However, an association between mode of delivery and CD could not be confirmed in other cohort studies (57, 72, 73).

Vitamin D levels have been hypothesized to modify the risk of CD after observations on differences in CD prevalence by a north-south gradient (74, 75). However, Vitamin D status during pregnancy or in neonates were not associated with risk of CD in Norwegian children (76).

In contrast, both low (<30 nmol/L) as well as high (>75nmol/L) Vitamin D levels by age one year were associated with increased risk of CDA in TEDDY (77).

Differences in prevalence of CD in Finland and Russian Karelia despite similar genetic predisposition, indicated that sociodemographic factors may influence the risk of childhood CD (53). Children born by Italian mothers with university degree, or older than 30 years at delivery, were at higher risk of CD (57). However, in other studies, associations between socioeconomic parental factors and the risk of CD diagnosis in the child were not found (78, 79).

An interaction between infections and early life dietary factors and risk of CD has been found in some (56, 59, 60), but not all, previous studies (80). In a Swedish retrospective study, children with frequent infections before age six months were at higher risk of CD if the child had stopped breastfeeding before gluten introduction and had a high gluten intake after gluten introduction (56). In a Norwegian study, children exposed to enteroviruses after gluten introduction and cessation of breastfeeding were at increased risk of CD (59). In another birth cohort, there was an interaction between enterovirus infections, higher gluten intake and risk of CDA (60). In contrast, another observational study from Sweden did not find an interaction between infections at the time of gluten introduction and subsequent risk of CD (80). Moreover, children born in spring or summer were at higher risk of CD in register-based studies in Sweden (74, 81). In TEDDY, the association of gastrointestinal infections and CDA was further increased if the child was instead born in winter, with short breastfeeding duration and early gluten introduction (58).

Role of gluten in celiac disease

Wheat and gluten proteins

Wheat has been used as a staple food since domestication about 10,000 years ago (82). During the 20th century, the use of wheat increased globally and has been particularly important in the developing world for food security to overcome famine (83). Grains are grown in about 60% of all agricultural land globally today, of which wheat is the second most common (14). About 95% of grown wheat is bread wheat, and the remaining 5% is durum wheat used for making pasta (82). Wheat is an important source of nutrients and wholegrains and globally provides 20% of the required intake of energy by carbohydrates, protein, fiber, and micronutrients (82). However, in several developing countries with a high proportion of the population living in poverty, wheat provides more than 40% of the daily calories (84). Wheat constitutes 30% of the daily intake of iron in adults, and 35% in children and adolescents (85, 86).

Gluten is traditionally defined as the remaining protein mass after wheat dough has been washed to remove the starch and soluble proteins. This mass consists of the prolamins gliadin and glutenin (87). By the classical definition, gluten is the term used for wheat, however it is also used for prolamins in other wheat varieties such as spelt and kamut and related storage proteins in rye (secalins) and barley (hordeins) (87). Prolamins are found in the endosperm where they serve as storage proteins used when the grain grows (87).

There is a vast number of different gluten proteins. In a single wheat cultivar between 50 and 100 types have been identified and over 600 gluten proteins have been described. The high number of gluten proteins is the result of genetic polymorphism and of growing conditions for the grain (14, 87, 88). Wheat consists of about 10-15% proteins of which 80% is gluten (82), compared with 65% in rye and 50% in barley (89). Wheat proteins contain all essential amino acids, except for adequate amounts of lysine (82). However, both gliadins and glutenins are rich in long repeated sequences of prolines and glutamines linked by disulphide bonds (14). These features make them partly resistant to gastric and pancreatic proteolytic digestion in the human gut, leaving peptides with proline-glutamine bonds of different length (14).

The unique property to make baked goods, including leavened bread, pasta and noodles stems from gluten and has made wheat essential in the global food chain (90). Gliadin give wheat viscosity, whereas glutenin gives elasticity (87). In breadmaking, gluten proteins have the unique ability to form a gliadin-glutenin network that incorporates starch and captures gas bubbles produced when bread rise (90).

Moreover, gluten has the ability to absorb about twice its weight of water. In the food industry, gluten is a cheap protein with a versatile area of use, and several types of modifications can be made to the gluten network. Gluten proteins transform depending on the cooking technique being used, such as kneading of dough, heating, the level of hydration, as well as the presence of other components such as dietary fibers, fruits, and vegetables (91-94). The digestion of gluten interacts with starch and lipid digestion within the gluten network, enabling proteolytic enzymes to access and react with gluten proteins. Gluten in bread and pasta are more resistant to digestion compared with gluten in wheat flour (95-97). Thus, the food matrix has an impact on the gluten peptides remaining after digestion.

Gluten in the infant diet

European texts from as early as the 15th century describe breastmilk substitutes, supplements and early weaning foods made from milk (cow, goat), wheat (flour, dough or rusk), and sometimes sugar, broth or other ingredients (98). These blends came in liquid forms, called gruel, or thicker like a porridge, called pap or panada. During the industrialization of Europe, breastfeeding declined, and the use of homemade milk and wheat mixtures increased (98).

During the 1860's, the first commercial infant formula based on cow's milk, wheat and malt flour became available (99). The tradition of adding grains or rusk in home-made infant formula continued into the 20th century and is still present in some parts of the world today (100, 101).

In Sweden, traditionally commercial infant (or baby) cereals are foods common in the diet of infants and young children. There are mainly two types of infant cereals; *instant porridge* (spoon-fed) and *milk cereal drink* (liquid form served in bottle or cup). These powdered products are nutritionally complete and differ only in the content of water added to the powder. The powders are based on powdered milk and flour from different grains, such as wheat or oats, and have added vitamins and minerals.

At age six months, almost all Swedish children eat instant porridge on a daily basis and half of Swedish children consumes milk cereal drink (102). At age 12 months, 85% are consuming milk cereal drink, which decrease to 10% at age five years (103).

Infant cereals are a commonly introduced food during weaning also in other countries. In the US, about 50% of infants consume iron-fortified infant cereals (104). However, the consumption of infant cereals has declined over time, parallel with increased inadequate iron intake after infancy (105, 106).

In a typical western diet, average daily gluten intake in children is estimated to 15 g (107) with reported country differences. In the early 1990's, the protein intake from wheat, rye, barley, and oats were three times higher in Swedish children at age nine months, and twice as high at age 12 months compared with children in Finland (108). In the PreventCD study, gluten intake in children from five European countries increased with age and ranged from 2.6-5.3 g/day at age 11 months up to 4.4-12.1 g/day at age 36 months (109). Children in Spain had consistently lower gluten intake (2.6-4.4 g/day) as compared with children in Hungary and Italy, who consumed larger amounts (5.2-11.5 g/day, 4.9-12.1 g/day respectively from age 11 to 36 months).

A study from the Netherlands demonstrated that CD family members consumed equal amounts of gluten compared with the general Dutch population (110), while another study from Spain showed that children reported lower gluten intake if they had a family member with CD (111).

The Swedish epidemic

During the 1980's, Swedish pediatricians observed a steep increase in incidence of CD in very young children (112). The incidence rate in children aged two years or younger increased from 200-240 cases/100,000 person years. A decade later, the incidence again decreased to 50-60 cases per 100,0000 person years (**Figure 3**). (113). These changes in incidences coincided with revised infant dietary guidelines on gluten introduction, variation in national breastfeeding rates and gluten content in baby foods (114). In retrospect, this phenomenon, unique for Sweden, is commonly referred to as the "Swedish epidemic" in CD research (113). It was regarded as a natural experiment highlighting the role of environmental factors on the risk of CD (115). Whether attributed to diet or other environmental factors, a Swedish study indicated that introduction of large amounts of gluten conferred an increased risk of CD and that breastfeeding during introduction of gluten had protective effects (116). However, the Swedish study also found that infants being introduced to gluten in solid foods or liquid had similar risk of CD. These findings were later examined in a systematic review which concluded that breastfeeding was protective, both with a longer duration and at the time of gluten introduction (117).

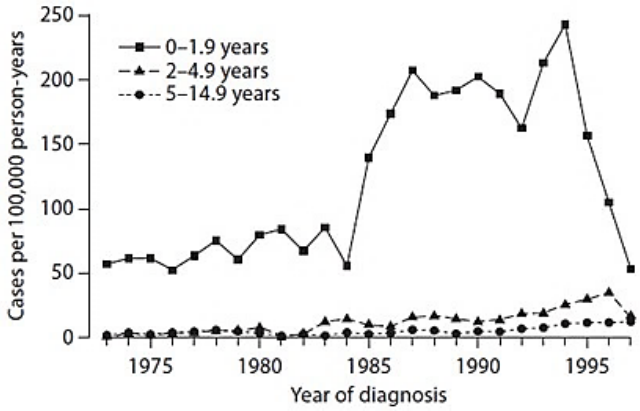


Figure 3 Incidence of CD in Swedish children between 1973-1997; a period aslo referred to as the "Swedish epidemic". Figure from Ivarsson et al, Acta Paediatrica, 2000 (113).

Infant feeding and celiac disease

The Italian multicenter intervention study CELIPREV recruited infants from high-risk families and randomized them to either introduce gluten at age six months or at age 12 months (118). At follow-up after five years, there was no difference in the number of children who developed CD between the two groups, except for a delay in diagnosis in the group with later gluten introduction.

Another randomized intervention trial, PreventCD, included infants with a first-degree relative with CD from eight European countries, and investigated if slow introduction of gluten would prevent CD (52). From age four to six months, one group of infants were given placebo while another group were given 200 mg gluten daily. By the age of three years, no difference in CD diagnosis was found between the two groups.

Since the two randomized controlled trials, results from several prospective longitudinal cohort studies have been published. In 2015, the conclusion of a systematic meta-analysis was that neither breastfeeding, breastfeeding at the time of gluten introduction nor age at gluten introduction during infancy, were associated with CD (119). Another systematic review found inconclusive evidence of an effect of breastfeeding in CD and that an optimal time point of gluten introduction could not be determined (120).

In 2016, ESPGHAN published a position paper on infant feeding on the risk of CD (121) stating that breastfeeding should not be modified to prevent CD and introducing gluten while the infant is still breastfed should not be recommended to reduce the risk of CD.

However, it was recommended to introduce gluten between age four to 12 months, and large amounts of gluten should be discouraged during the first months after gluten introduction.

Although the "Swedish epidemic" indicated that incidence of CD is influenced by the means of infant feeding (115, 122-124), there was no evidence of causality. Moreover, whether the amount of gluten intake influenced the risk of CD could not be concluded due to lack of prospective data. In a nested case-control study within the Swedish TEDDY cohort, children consuming gluten amounts in the upper tertile before age two years were at a higher risk of CD compared with matched controls (125). However, when studying the quantity of gluten intake from age eleven months to three years and risk of CD by age six years, an association with gluten amounts was not found in the PreventCD cohort, (109).

Another hypothesis was that high consumption of milk cereal drink in Swedish toddlers may influence the risk of CD. Indeed, the consumption of milk cereal drink and infant formula may compete with breastfeeding by shortening the duration of breastfeeding (126). Moreover, infant cereals, as well as infant formula, are based on milk powder.

Milk powder is produced by heating and evaporating milk. In this process, proteins and lipids react with sugars in the Maillard reaction, leaving advanced glycation end products (AGE) (127). AGEs have been found both in infant formula and infant cereals (128, 129), but potential negative health effects have not been well studied in infants and children. In adults, dietary intake of AGEs increased the levels of inflammatory markers (130, 131). Furthermore, in an interventional study, higher levels of antibodies to milk proteins were found in children that later developed CD compared with healthy children (132).

Studying dietary exposures is complex, with high intercorrelation between foods as well as interactions between nutrient and other bioactive substances (133). Changes in intake of one dietary component typically leads to changes in other by substitution effect. Therefore, investigating dietary patterns in relation to disease have advantages compared with studies on single foods or nutrients (133). In a recent Dutch study, children adherent to a dietary pattern with high intakes of vegetables, potatoes, pasta and grains, and low intake of baby foods at age one year had 30% lower risk of developing tTGA at age six years (134). These findings indicate that dietary patterns in early childhood may play a role in CD. However, there is a need for extended studies on repeatedly assessed dietary patterns in targeted populations at genetic risk for CD.

Aims

The overall aim is to investigate associations of dietary factors in early childhood with incidence of CDA and CD in children at genetic risk.

Specific aims

- To investigate if the amount of gluten intake is associated with CDA and CD in genetically at-risk children (Paper I).
- To investigate if different gluten-containing foods up to age two years confer different risks of CDA and CD in children at genetic risk (Paper II)
- To investigate if intake of milk powder was associated with CD in Swedish genetically predisposed children (Paper III).
- To identify and explore associations of dietary patterns up to age two years with the risk of CDA and CD in genetically at-risk children (Paper IV).

Subjects and methods

TEDDY Study

TEDDY is an observational, longitudinal birth cohort study aiming to investigate both genetic and environmental factors associated with risk of islet autoimmunity and T1D in children at genetic risk. TEDDY follows children from birth at six clinical sites in Sweden, Finland, Germany in Europe and Colorado, Georgia, and Washington in the United States. The study is managed at the data coordination center (DCC) at University of South Florida in Tampa, Florida. (135-137)

Between September 2004 and February 2010, 424,788 infants were screened for HLA risk-genotypes associated with T1D. Among the screened infants, 21,589 (5%) had eligible HLA genotypes (**Table 2**) and were invited to a 15-year follow-up (**Table 3**) (138).

Table 2 Eligible HLA genotypes for enrollment in the TEDDY study. Adopted from Hagopian et al, *Pediatric Diabetes*, 2011 (138) and Liu et al, *NEJM*, 2014 (38).

HLA genotype	Abbreviation
General population	
DR4-DQA1*03:0X-DQB1*03:02/DR3-DQA1*05:01-DQB1*02:01	**DR3-DQ2/DR4-DQ8***
DR4-DQA1*03:0X-DQB1*03:02/DR8-DQA1*03:0X-DQB1*03:02	**DR4-DQ8/DR4-DQ8***
DR4-DQA1*03-0X-DQB1*03:02/DR8-DQA1*04:01-DQB1*04:02	**DR4-DQ8/DR8-DQ4***
DR3-DQA1*05:01-DQB1*02:01/DR3-DQA1*05:01-DQB1*02:01	**DR3-DQ2/DR3-DQ2***
First degree relative with type 1 diabetes	
DR4-DQA1*03:0X-DQB1*03:02/DR4-DQB1*02:0X	DR4/DR4b
DR4-DQA1*03:02/DR1-DQA1*01:01-DQB1*05:01	DR4/DR1
DR4-DQA1*03:02/DR13-DQB1*06:04	DR4/DR13
DR4-DQA1*03:0X-DQDQB1*03:02/DR9-DQA1*03:0X-DQB1*03:03	DR4/DR9
DR3-DQA1*05:01-DQB1/DR9-DQA1*03:0X-DQB1*03:03	DR3/DR9

*Associated with CD

Table 3 Enrolled study participants with eligible HLA genotypes in the TEDDY study.

	Overall	U.S	Sweden	Finland	Germany
	n (%)	n (%)	n (%)	n (%)	n (%)
Eligible	21,589	12,512 (58.0)	3,725 (17.3)	3,681 (17.1)	1,671 (7.7)
Enrolled	8,676 (40.2)	3,724 (29.8)	2,526 (67.8)	1,832 (49.8)	594 (35.5)

Children were excluded if the natural history of T1D could be influenced by a medical treatment or congenital condition (136). Overall, 8,676 (40.2%) agreed to participate and were enrolled before age four months. The most common reason for exclusion was not being able to contact the families after HLA screening (139).

Study participants and their parents visits a clinical center starting at age three months, every three months up to age four years, and biannually thereafter or to diagnosis of T1D according to a standard protocol common for all sites (**Table 4**) (136). At each visit, parents are interviewed and completes questionnaires on illnesses, infectious episodes, vaccinations, medications, use of supplements and probiotics, as well as information on life events in their child. Biospecimens are collected according to the study protocol. Weight in kg is measured by regularly calibrated scales, most commonly Tanita (Tanita corp. Tokyo, Japan). Height to the nearest 0.1 centimeters is measured laying down in children up to age two years, and by a wall-mounted stadiometer thereafter. Extensive measures are taken to ensure that reliable and valid data are collected (137).

Table 4 Study protocol for TEDDY, adopted from TEDDY study group, *Pediatric Diabetes,* 2007 (136).

	Sampling frequency at age
Blood	Every 3 month up to age 48 months, every 6 month thereafter
Stool	Monthly to age 48 months, biannually therafter
Tap water	Age 9 months, every two years from age 36 months
Toenail clippings	Age 24 months
Weight and height	Every clinic visit
Maternal pregnancy diet	First clinic visit (age 3 months)
Three-day food record	Every 3 months age 6-12 months, biannually therafter
Maternal pregnancy/birth questionnarie	First clinic visit (age 3 months)
Parent questionnaire	Age 3, 6, 15, 27 moths, annually thereafter
Child questionnaire	Annually, starting at age 48 months
Demographic/family questionnaire	Age 9 months
TEDDY book extraction*	Every clinic visit

*Including foods introduced in the diet, use of dietary supplements and medications, vaccinations, illnesses, and symptoms etc.

TEDDY repeatedly assess dietary habits using multiple methods to collect information on dietary factors of interest (**Table 5**), but do not give dietary advice or recommendations to participating families.

Table 5 Main dietary factors of interest in the TEDDY study. Reproduced from TEDDY study group, *Pediatric Diabetes*, 2007 (136).

Foods	Nutrients	Other nutritional factors
Cow's milk	Caloric intake	Nitrates, nitrites N-nitroso compounds
Cereals, wheat,gluten	Proteins	Patulin
Soy	Vitamins C, D and E	Bafilomycin
Meat	Nicotinamide (Vitamin B3)	Increased weight, andr/or heigt gain
Coffea and tea	n-3 fatty acids	
Breastmilk	Zink	
Cod liver oil	Carotenoids	
	Selenium	

Screening for CD

Serum samples are measured for tTGA annually from age 24 months using radio-binding assays (RBA) (140). Samples collected from US participants are analyzed at the Barbara Davis Centre (BDC), Aurora, Colorado and samples collected in Europe by University of Bristol Laboratory, United Kingdom (137). The RBA at the BDC analyzed IgA-tTG and the RBA at University of Bristol Laboratory, a combination of IgA-tTG and IgG-tTG (38).

IgA-tTG levels $\geq$0.01 Units at the BDC are shipped to Bristol (reference laboratory) for reassessment and samples with tTGA levels $\geq$1.3 Units are defined as being positive. In children confirmed positive for tTGA at the University of Bristol Laboratory, all previously collected study samples are analyzed to find the closest time of seroconversion to tTGA positivity. tTGA positive children are retested for tTGA every three months up to age 48 months, and every six months thereafter. Persistently tTGA positive children are referred to a local health provider for clinical evaluation of CD, which is outside the TEDDY protocol (38). However, an intestinal biopsy is recommended in children with persistent tTGA levels >30 Units and in children with clinical suspicion of CD regardless of tTGA level.

Study populations in Paper I-IV

Paper I was a cohort study that included 6,605 study participants from the full TEDDY cohort with at least one three-food-record up to age of five years, and at least one measurement of tTGA (**Figure 4**).

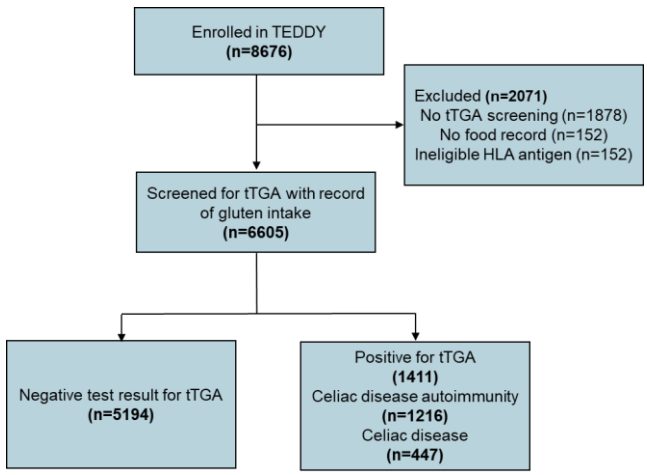


Figure 4 Flow chart of the study population in Paper I.

Paper II was a cohort study that included 2,088 Swedish TEDDY participants with at least one three-day food-record collected up to age 24 months, and at least one tTGA measurement (**Figure 5**).

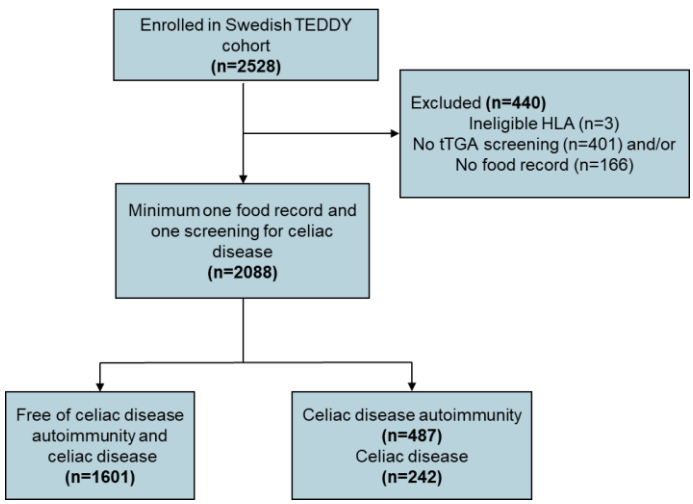


Figure 5 Flow chart of the study population in Paper II.

Paper III was a 1:3 nested case-control study that included Swedish TEDDY participants with at least one three-day food-record collected up to age 24 months. A total of 207 cases and 621 controls matched for HLA genotype, sex, and birth year were included (**Figure 6**).

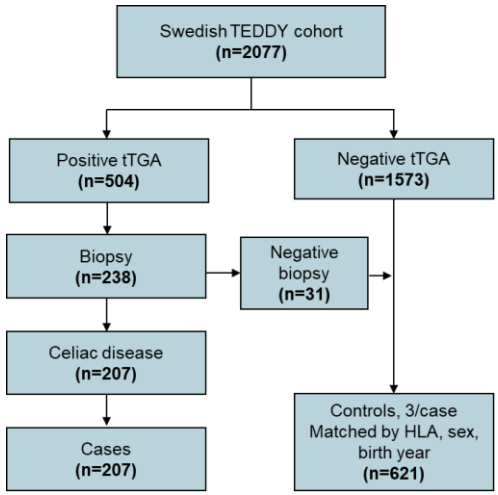


Figure 6 Flow chart of the study population in Paper III.

Paper IV was a cohort study that included 6,677 study participants from the full TEDDY cohort with at least one three-day food record collected up to age two years, and at least one tTGA measurement (**Figure 7**).

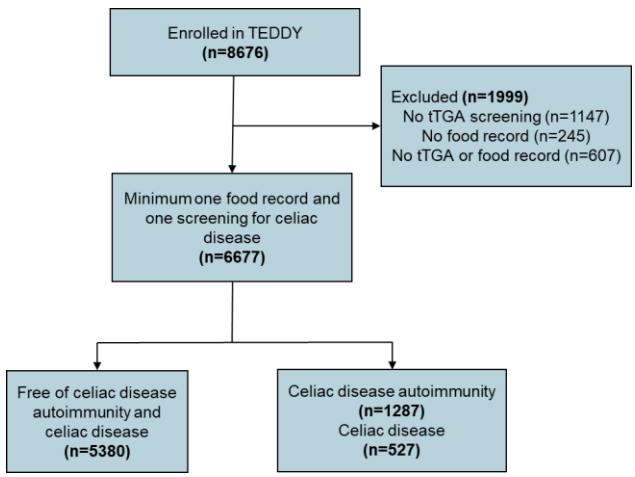


Figure 7 Flow chart of the study population in Paper IV.

Study outcomes

The primary outcome was CDA, which was defined as being tTGA positive in two consecutive samples collected at least three months apart. The secondary outcome was CD, which was defined as having an intestinal biopsy showing a Marsh score >1, or an average level of tTGA ≥100 Units in two consecutive samples if a biopsy was not performed (38).

Dietary assessment methods

Diet related questionnaires

Repeated questionnaires are used to collect information on early life feeding practices including breastfeeding (duration, exclusive or partial) and intake of infant formula (type) (136). Parents are provided with a logbook to record the introduction of new foods (age of the child, type of food), use of dietary supplements and probiotics up to age two years.

24-hour diet recall

At the three-month clinic visit, a study nurse performs a 24-hour recall with the participants caregiver (136). The structured interview gathers information on the infant's dietary intake for the past 24 hours. It also served as training for parents in completing food records. The 24-hour diet recall allows for the interviewer to pose follow-up questions on the received information, to get a detailed and comprehensive report on the dietary intake (141).

Food record

From age six months, three-day food records are collected every three months up to 12 months of age, and semi-annually thereafter. Parents are asked to record all foods and drinks consumed on two weekdays and one weekend withing 10 days of the clinic visit. Parents are encouraged to maintain the child's habitual dietary intake during the dietary assessment. Detailed information of foods and drinks are asked for, including brand names, types of baby foods, and ingredients in home-made foods. Research staff provides written and oral instruction on how to complete the food records.

A booklet developed for TEDDY with pictures of sizes and shapes of different foods (such as bread, fruit, potatoes, meat) and portion sizes (pasta, rice, stew) help caregivers to estimate the amounts of foods eaten. In Germany, parents were instead informed to keep a weighted food record. At the clinic visit, the research staff revise the food record, probing for missing information and unclear reports.

Food and nutrition databases

The 24-hour diet recalls, and food records are entered by trained dietitians and nutritionists in in-house software separate for each country, that are based on the respective national food composition database (142, 143). Quality control is executed, and the food records probed for errors. If needed, the dietitian/nutritionist contacts the caregiver to collect missing or possible erroneous information. Foods dissimilar from standard foods already in the database are added. Reported home-made foods are also added if they differed from standard receipts, and yield factors applied to account for decrease in specific nutrients. All collected data from each country are reported to the TEDDY food database.

In the TEDDY food database, composite foods and dishes are broken down and harmonized on the ingredient level according to the epidemiological approach (143, 144). Every reported ingredient is accounted for, no matter how small the amount, which allows for comparisons on several levels (**Figure 8**). Nutrient intakes, including gluten, are also harmonized across participating countries to ensure comparable data (143). The dietary intake data are averaged by the number of reported days and given in g/day. Nutrient and food data are separated in their respective databases.

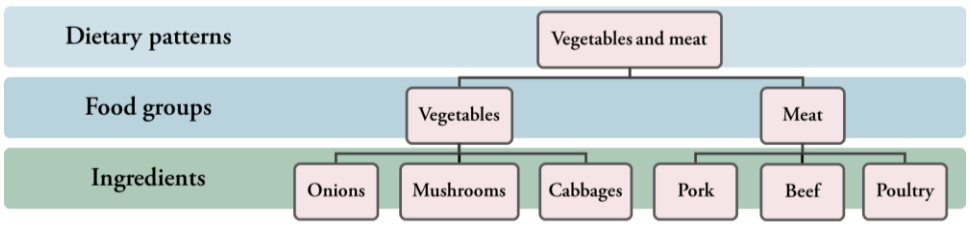


Figure 8 Levels of food intake data used in the thesis, with examples on each level.

Assessment of gluten intake

In TEDDY, gluten intake is estimated based on the intake of proteins from wheat, rye, and barley. A conversion factor of 0.8 is applied, that reflects the gluten content in wheat (14). In Paper III, individual conversion factors were instead used to reflect the varying gluten content in wheat, rye and barley (89).

Assessment of dietary sources of gluten

In Paper II, the food grouping in the Swedish food database was used as a base to aggregate composite, gluten-containing food groups. Food intake data on this level was not possible to extract from the harmonized TEDDY food database. Foods were combined according to similarities in type and proportion of grain or flour included, and cooking techniques used (**Table 6**). Moreover, daily intakes of wheat, rye and barley from the TEDDY food data base were also included.

Table 6 Gluten-containing food groups in Paper II.

Original gluten-containing food groop	Food group	Gluten/portion
Bread	**Rationale for aggregation**	
Soft white bread Mainly wheat, may contain other grains *Rye bread* Mainly rye, may contain wheat *Soft wholemeal bread* Wheat and rye	***Bread*** Similar proportions of flour, and cooking techniques applied	2 g/slice 35 g
Crisp bread Various grains *Crispy flatbread* Various grains	***Biscuit and crackers*** Similar type of grains, proportion of flour, and cooking techniques applied	0.9 g/piece 10 g
Dishes based on bread Filled sandwiches, wraps, breakfast muffins etc.	Excluded. Varied content of flour/dough. Appeared in <10 food records	
Porridge, milk cereal drink		
Porridge Infant cereals and homemade porridge	***Porridge***	0.2-0.9 g/portion 120/g
Milk cereal drink Follow-on formula	***Milk cereal drink***	0.4-1.5 g/bottle 200 ml
Pudding Commercial wheat or rice pudding with jam	Excluded Large variation in gluten content In <50 food records	
Cereals, muesli		
Cereals, muesli, low/unsweetened Mix of grains with/without gluten *Cereals, muesli, sweetened* Mix of grains with/without gluten	***Breakfast cereals*** Different sugar content	0-2 g/portion 1 deciliter
Commercial baby fruit cereal Mainly fruit purée, ≤ 5 % grains (with/without gluten)	Excluded Very low/no content of gluten	
Pancakes, waffles, crêpes		
Pancakes, waffles, crêpes	***Pancakes***	1.8 g/pancake 60 g
Dishes with meat, sausage, poultry Oven baked pancake with/without meat	***Pancakes*** Similar amount flour as in standard pancakes	
Dishes with fish, seafood Filled pancakes/crêpes *Dishes with vegetables* Filled pancakes/crêpes	***Pancakes*** Conversion factor 0.7 to account for pancake/crêpes in the dish	
Pizza, pie, pirogue		
Pizza dough, butter dough	***Bread***	1.9 g/slice 35 g
Filled pizza, pie, pirogue Filling with for ex meat, fish, egg	***Bread*** Similar proportion of flour and cooking techniques. Conversion factor 0.3 to account for dough in the dish	
Pasta		
Pasta cooked Cooked, fresh/dry pasta, pasta in dishes	***Pasta***	0.7g/port 60 g
Bakery sweet		
Biscuits, cookies, crackers Wheat-based, unsweetened/sweetened.	***Biscuits and crackers*** Wheat-based, similar proportions of flour and cooking techniques applied.	0.9 g/piece 10 g
Buns, crusts Based on wheat *Cake, pastry, Swiss roll* Based on wheat *Sponge cake* Based on wheat, without filling	***Sweet baked goods*** Wheat-based, similar proportions of flour and cooking techniques applied.	0.8g/piece 20 g

Assessment of milk powder intake

In Paper III, milk powder intake was assessed from several food groups and individual food items. Milk powder is not an ingredient in the TEDDY food database and is only partly available in the Swedish national food database. All types of milk powders were included, regardless of fat content. (**Table 7**)

Table 7 Estimation of milk powder intake in the Swedish TEDDY food database.

Food	Milk powder in food	Conversion factor
Ingredients		
Skim milk powder		Included as is
Cream powder		Included as is
Whey powder		Included as is
Milk powder		Included as is
Foods		
Infant formula	20% in infant formula powder 2.6% in 100 ml prepared infant formula (13% powder)	0.0026 (prepared)
Milk cereal drink	25 % of milk cereal drink powder 3.8% in 100 g prepared milk cereal drink (15% powder)	0.038 (prepared)
Porrige	25 % of powdered instant porrige 7.5 % in 100 gprepared porrigde	0.075 (prepared)
Yoghurts	9 % in Brand 1 8.5 % in Brand 2 7.5 % in Brand 3 Other brands no milk powder content/no information on %	0.09 Brand 1 0.085 Brand 2 0.075 Brand 3
Instant mashed potatoes	20% milk powder in powdered mashed potatoes.	0.2 (powder)
Milk chocolate	20% milk powder in most milk chocolate	0.2
Ice cream	Large variation in different brands	Excluded
Sauses/condiments	Large variation in different brands	Excluded

Food grouping for dietary patterns

In Paper IV, ingredients in the harmonized TEDDY food database were aggregated to food groups to reduce the number of variables and prepare the data for dietary pattern analysis (**Table 8**).

Table 8 Food groups used to derive a posteriori dietary patterns by principal components analysis in Paper IV.

Food group	Included ingredients
Wheat	Various types of wheat (e.g., flour, flakes)
Rye and barley	Various types of rye and barley (e.g., flour, flakes)
Oats	Dry oats, oat milk converted to dry oats
Rice and GF grains	Rice (cooked, flour, milk converted to dry weight), corn (flakes, meal, polenta, popcorn), gluten free flours and starches (e.g., millet, buckwheat, quinoa, potato flour starch, wheat starch)
Fruits and berries	All fruits and berries (e.g., apple, banana, pear, citrus, strawberries), fresh, canned, and dried.
Juices	All fruit, berry, and vegetable juices.
Vegetables	All vegetables (e.g., leafy vegetables, onions, cabbages, mushrooms, fruit vegetables), fresh, canned, and dried.
Root vegetables	All root vegetables (e.g., sweet potato, carrot, turnip, rutabaga).
Potatoes	Including potatoes cooked with different methods (e.g., cooked, fried, chips, baked)
Legumes	All legumes (e.g., beans, peas, soybeans), fresh, canned. Dried converted to fresh (conversion factor 2). Soy milk converted to raw soybeans (conversion factor 0.2)
Nuts and seeds	All nuts and seeds, included raw and roasted, spreads. Nut seed milks converted to solid nuts.
Unsaturated fats	Oils of vegetable origin (e.g., canola, olive, corn), margarine and margarine-butter spreads with various fat content, fish oil.
Saturated fats	Butter and animal fats (lard)
Breastmilk	Breastmilk
Infant formula	Infant formula from cow/other animal, soy, partially and fully hydrolyzed. Converted to liquid.
Milk	All animal milk, creams with different fat content. Milk powders converted to liquids.
Fermented milk	All fermented dairy (e.g., sour milk, yoghurt, sour cream, crème fraiche). Powders converted to liquids.
Cheese	Fresh and aged cheese
Ice cream	All dairy ice creams
Non-dairy products	Yoghurts, ice cream, kefir etc. from non-dairy origin such as soy, rice, oats and nuts.
Meat	Meat and organ meats of various sources (e.g., pork, beef, lamb, poultry).
Processed meat	Meat and sausages of various type and source (e.g., sausages, cold-cuts, bacon, canned meat).
Eggs	All types from various poultry. Powder converted to raw.
Fish and seafood	All types from various sources, fresh, frozen, processed, and canned.
Sweet beverages	Sugar sweetened beverages (e.g., soft drinks, fruit and berry drinks, nectar).
Lite beverages	Unsweetened, artificially sweetened, low-calorie sweetened (e.g., Stevia) drinks(e.g., soft drink, fruit and berry drinks, nectar), coffee and tea.
Sugar, confectionary	Including sugar, candy, energy bars, chocolate, sugar, syrup, honey, jams, and jellies.

Energy adjustment

To account for variation in dietary intake relative to energy intake (145), several methods for energy adjustment were used in this thesis.

In Paper I, gluten intake was energy and age adjusted according to the nutrient residual method (146). Total daily gluten intake was regressed on total daily energy intake assessed in the corresponding food record, and the residuals (representing the difference compared with the mean intake at the respective energy intake) were modelled in survival analyses. Nutrient residuals are independent of energy intake and the regression analysis may be adjusted for relevant factors related to energy intake, such as age.

In Paper II and IV, the nutrient density method was instead used (146) as the assumptions for the nutrient residual method were not met because of zero-inflated distributions of food intakes. Intakes were standardized to per 1000 kcal (food intake/total daily energy intake*1000 kcal), as this was a representative energy intake for the cohort.

In Paper I, and III, intake variables relative to bodyweight (per kg/day) were included in the analyses. This method resembles the nutrient density method but takes weight, which is independent of assessed dietary intake, into account.

Evaluation of misreporting

To evaluate the level of misreporting, (e.g., under- and over-reporting) of dietary data in the TEDDY cohort, a simplified method developed in a longitudinal, European cohort was used (147). At age 12 and 24 months, estimated energy requirement (EER) was computed individually by calculating the total energy expenditure (separate equations for boys and girls), and adding the amount for energy deposition needed for growth at 12 and 24 months respectively. To the ratio of reported mean energy intake and EER (EER/energy intake), fixed cut-offs determined possible over- and under-reporters. At age 12 months, the cut-offs were 0.80 and 1.20, and at 24 months 0.75 and 1.25, respectively (147).

Statistical methods

The main statistical analysis pre-defined to use in TEDDY is the Cox Proportional hazards regression for analyses on outcomes in the entire cohort (136), and the Conditional logistic regression for outcomes in nested case-control studies. The TEDDY study was designed to have at least 80% power to detect hazard ratios (HRs) of $\geq$2, for exposures prevalent in $\geq$10% of the cohort and therefore dimensioned to enroll 7013 children from the general population and 788 children with a first-degree relative with T1D.

For all analyses in this thesis, two-sided p-values were reported and values <0.05 were considered as statistically significant. The analyses in Papers I and III were performed using SAS version 9.4 (SAS Institute Inc.), while SPSS version 27.0 (IBM Corp) was used for Papers II and IV. Mean values were presented with standard deviations (SD) and median values with quartiles (Q) or inter-quartile ranges (IQR). Spearman correlation coefficients were used in Paper II to investigate the correlation between intakes of gluten-containing foods and total gluten intake.

Survival analyses – time to event and censoring

Time to event (dependent variable) in the survival analyses was pre-defined to be the age at which the event occurred, and the right-censoring time was the age at the last study visit when the child was free of the event (136).

Time to CDA was the age at the first of two positive tTGA samples. The right-censoring time was the age at the last negative tTGA measured.

Time to CD in Paper I was the age of the first of two positive tTGA leading to CDA in children later diagnosed with CD. In papers II and IV, time to CD was the age when CD was diagnosed. The right-censoring time was the age at the last clinic visit with no diagnosis of CD.

Proposed causal pathways between investigated dietary factors and CD

A directed acyclic graph was drawn to illustrate potential causal pathways between dietary exposures and CDA as well as CD (148) (**Figure 9**). In Paper I, II and IV, factors potentially associated with both the exposure and outcome were adjusted for in the survival analyses (38). In Paper III, matching of cases and controls was done instead.

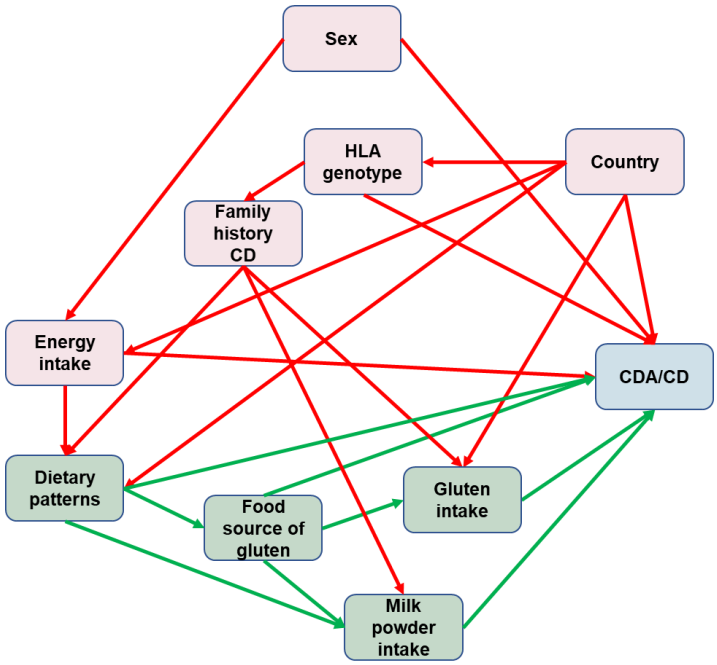


Figure 9 Directed acyclic diagram (148) illustrating the proposed causal pathways for the investigated dietary exposures and studied outcomes of CDA as well as CD. Blue denotes the study outcomes, green are the investigated exposures, while red are confounders.

In papers II-IV, total gluten intake was considered to be a *mediator* (factor on the pathway between the exposure and outcome) to estimate the direct effect of the exposure on the study outcomes, without the effect of gluten.

Mixed effects model

Mixed effects models were used to investigate differences in grain intakes from age six to 60 months between the participating countries. It is a longitudinal model for repeated dependent variables (e.g., grain intake). The output estimated change in intake over time with 95% confidence intervals (CIs). Random effect was subject intercept, fixed effect was country. Early life feeding and demographic factors were included as interaction variables. Visits where children had CDA, or CD were excluded to minimize the effect of possible changes in grain intake.

Joint modelling

In Paper I, joint modelling was used to investigate the association between total gluten intake and CDA as well as CD. The joint model combines longitudinal data, with dependent variables measured over time, with time-to event data like in Cox regression (149). HRs expressed the estimate effect size and were presented with their related 95% CIs.

Cox proportional hazards regression

Cox regression was used in Paper I, II and IV. It is a semi-parametric, robust method commonly used in survival analysis (150). Information on time of event and censoring time must be available for all participants. The effect of the exposure should be proportional over time. To verify this assumption, the Schoenfeld residuals for each model were investigated. The effect size of the association between an exposure and outcome was calculated as HR with 95% CI.

In papers III and IV, Cox models were performed at each visit separately because in Paper II, there were large differences in the proportion of children consuming each food group over time and intake variables were modelled accordingly. Instead in Paper IV, dietary patterns varied over time. No correction for multiple testing was done, as they were not confirmatory studies (151).

Conditional logistic regression

In Paper III, Conditional logistic regression was used to cross-sectionally investigate the association of milk powder intake with CD. This method is used specifically in nested case-control studies and conditions on the matching factors. Odds ratios (ORs) estimated the effect size and were presented with their associated 95% CI. Milk powder intake was modelled at each clinic visit (age three to 24 months), as well as the last reported intake before seroconversion to tTGA positivity and total intake (sum of all reported intakes).

Dietary pattern analysis

In Paper IV, Principal Components Analysis (PCA) with Varimax rotation was used to a posteriori derive dietary patterns cross-sectionally. This is a data dimension reduction technique based on Spearman correlations, that finds underlying, uncorrelated components (dietary patterns) of variables (e.g., foods often eaten together), explaining as much of the variance in the data as possible (152, 153). The outputs of PCA are

factor loadings for each dietary pattern, e.g., how well each food variable correlates with the respective pattern (153). Positive loadings indicate positive correlation, while negative loadings indicate negative correlation with the dietary pattern. The larger the loading, the stronger the impact of the respective food in the dietary pattern. Simplified dietary patterns (SPDs) based on the result of the PCA were analyzed in relation to CDA and CD (154). The reason was to increase interpretability and comparability across the ages investigated, as well as with other studies. Only food groups that contribute the most (absolute factor loading $\geq$0.2) to the pattern were retained. Adherence to SDP's were calculated by summing the standardized intake (mean 0 and SD 1) of food groups with a positive loading, and subtracting the standardized intake of food groups with a negative loading. (154)

Ethical approvals

Written consent for participation, both for the screening study as well as the follow-up study, was collected from a parent or primary caregiver for all participants of the TEDDY study (136). Informed consent was also collected from participating children, between 7-10 years depending on applicable legislation in each country. The TEDDY study was approved in agreement with the Declaration of Helsinki by local ethic boards in each respective country. It was also monitored by the National Institutes of Health (NIH) in the US. In Sweden, the TEDDY study was approved by the Regional Ethical Review Board, Lund after initial application (EPN 217/2004), annual reviews, and later approved additional amendments and additional applications (2012/7, 2013/455, 2014/890, 2016/280, 2016/870, 2017/443, 2017/667, 2018/171). The Clinical Trial Identification number for TEDDY is NCT00279318.

Results

Study outcomes in TEDDY

After a follow-up to mean age 11.0 (3.6 SD) years, 19.3% of the TEDDY cohort had developed CDA and 7.9% were diagnosed with CD. Mean age of CDA was 4.2 years (2.5 SD) and mean age of CD was 5.4 years (2.8 SD), respectively. Time from CDA to diagnosis in children with CD was median 1.1 years (Q1 0.7, Q3 1.5). Diagnosis of CD was biopsy-confirmed in the majority (98.0%) of European participants, whereas diagnosis of CD based on serology was more common among US participants (**Table 9**).

Table 9 Diagnosis of CD by method and country in TEDDY as of 30th November 2020.

	TEDDY	US	Sweden	Finland	Germany
Biopsy-proven, n (%)	480 (90.9)	136 (76.4)	239 (98.8)	88 (98.9)	17 (85.0)
High levels of tTGA	49 (9.1)	42 (23.6)	3 (1.2)	1 (0.1)	3 (15.0)
Total	529	178	242	89	20

Mothers in the US and Finland had more often completed a higher education compared with mothers in Sweden and Germany. The age when participating children started daycare was comparable across study outcomes but differed between countries. Children in Finland and Germany started daycare at a later age compared to in Sweden and the US (**Table 10**).

Table 10 Demographics of children particpating in TEDDY by study outcome and country as of 30th November 2020. All children had been screened for tTGA at least once and with at least one food record up to age 60 months.

	TEDDY (n=6,726)	Free of CDA (n=5,419, 80.6%)	CDA (n=1,296, 19.3%)	CD (n=529, 7.9%)	US (n=2,697)	Sweden (n=2,094)	Finland (n=1,529)	Germany (n=406)
Family history								
CD, n (%)	329 (4.9)	173 (3.2)	155 (12.0)	94 (17.8)	92 (3.4)	133 (6.4)	99 (6.5)	5 (1.2)
T1D, n (%)	800 (11.6)	650 (12.0)	148 (11.4)	57 (10.8)	328 (12.2)	166 (7.9)	140 (9.2)	166 (40.9)
Sociodemographic								
Maternal education level								
Primary education, n (%)	1239 (18.4)	1039 (19.2)	198 (15.3)	95 (18.0)	373 (13.8)	688 (32.9)	140 (9.2)	38 (9.4)
Trade school, n (%)	1655 (24.6)	1363 (25.2)	287 (22.1)	97 (18.3)	652 (24.2)	344 (16.4)	459 (30.0)	200 (49.3)
Higher education, n (%)	3712 (55.2)	2915 (53.8)	795 (61.3)	330 (62.4)	1629 (60.4)	1036 (49.5)	895 (58.5)	152 (37.4)
Mothers first child, n (%)	2647 (43.8)	2362 (43.6)	576 (44.4)	234 (44.2)	1106 (41.0)	973 (46.5)	672 (44.0)	196 (48.3)
Age starting daycare, month (SD)	17.9 (12.9)	17.7 (12.9)	18.5 (12.9)	17.8 (12.0)	16.1 (16.6)	16.5 (5.1)	22.2 (12.6)	19.6 (11.0)
Parental smoking*, n (%)	1225 (19)	1042 (20.1)	183 (14.5)	72 (13.9)	345 (13.5)	320 (15.6)	447 (30.6)	113 (29.5)

*At child's age nine months.
All numbers may not round up to 100% because of rounding or missing information in some categories.

Dietary intake in TEDDY

Infant feeding habits were similar in healthy children compared with children with CDA and CD but differed between children from the participating countries. Children in Germany were introduced to solid foods later and Swedish children were introduced to gluten earlier than children form the other countries. (**Table 11**).

A total of 55,649 food records were collected from age six to 60 months. Participants completed a median of eight food records (Q1 4, Q3 11) out of the 11 requested. With increasing age, the proportion of missing food records (not completing an expected food record) increased, but with country differences (**Table 12**).

Table 12 Missing food records per clinic visit in TEDDY by study outcome and country. "Missing" is defined as an expected food record not collected in a child who attended a clinic visit.

		Missing food records, %						
Age, months	**Cohort**	**Free of CDA**	**CDA**	**CD**	**US**	**Sweden**	**Finland**	**Germany**
6	4.4	4.7	2.9	2.3	5.8	3.1	3.1	6.7
12	8.9	9.4	6.9	7.5	9.5	6.1	9.2	18.8
24	16.9	18.1	12.1	17.3	15.9	12.3	20.2	36.6
36	20.0	20.8	17.0	24.5	17.3	14.2	26.0	41.5
48	27.6	28.6	24.3	27.0	24.5	22.4	34.9	49.0
60	28.4	30.1	22.8	30.5	25.4	21.8	38.8	46.5

At age 12 months, 75.2% of the TEDDY cohort were estimated to be plausible energy reporters, and 77.9% at age 24 months. Children from Finland were more likely to be plausible reporters at both ages. Estimated underreporting was more frequent than overreporting, but children from the US were more often estimated to be over-reporters compared with children in the European countries (**Table 13**).

Table 13 Estimated proportion of misreporting of energy intake at age 12 and 24 months in TEDDY and by country. Misreporting was estimated using the method suggested by Gomes et al (147).

	Age 12 months			**Age 24 months**		
	Under-reporters	**Over-reporters**	**Plausible reporters**	**Under-reporters**	**Over-reporters**	**Plausible reporters**
TEDDY %	16.3	8.5	75.2	9.1	13.0	77.9
US, %	15.0	13.3	71.7	8.0	21.2	70.9
Sweden, %	18.3	5.8	75.9	11.5	6.5	82.0
Finland, %	12.9	5.5	81.6	6.1	9.9	84.0
Germany, %	28.2	2.0	69.9	17.0	4.8	78.3

Table 11 Infant dietary factors by study outcome and country.

	TEDDY (n=6726)	Free of CDA (n=5419, 80.6%)	CDA (n=1296, 19.3%)	CD (n=529, 7.9%)	US (n=2697)	Sweden (n=2094)	Finland (n=1529)	Germany (n=406)
Breastfeeding								
Ever breastfed, n (%)	6,571 (97.7)	5,267 (97.2)	1,271 (98.1)	518 (97.9)	2,554 (94.7)	2,075 (99.1)	1529 (100)	390 (96.1)
Breastfed at gluten introduction, n (%)	4,092 (60.8)	3,222 (59.5)	862 (66.5)	368 (69.6)	1,402 (52.0)	1,454 (69.4)	1,033 (67.6)	203 (50.0)
Breastfeeding to age (months), median (Q1, Q3)	8.0 (3.7, 12.0)	7.7 (3.2, 12.0)	8.3 (5.0, 12.3)	8.1 (5.0, 12.0)	8.0 (2.8, 13.0)	7.3 (4.0, 10.0)	8.9 (4.9, 12.4)	7.7 (3.0, 11.1)
Age (months) introduced to								
Solids (any), mean (SD)	4.0 (1.3)	4.0 (1.3)	4.0 (1.3)	3.9 (1.2)	3.9 (1.5)	3.9 (0.8)	3.8 (1.4)	5.0 (1.3)
Gluten, median (Q1, Q3)	6.0 (5.0, 7.0)	6.0 (5.0, 7.0)	6.0 (5.0, 7.0)	6.0 (4.9, 7.0)	7.0 (6.0, 8.0)	5.0 (4.0, 5.5)	6.0 (5.5, 7.0)	7.0 (6.0, 8.5)

All numbers may not round up to 100% because of rounding or missing information.

Mean energy intake increased with age and differed between participating countries. German children had overall a lower energy intake, whereas children from the US had higher energy intakes (**Figure 10**).

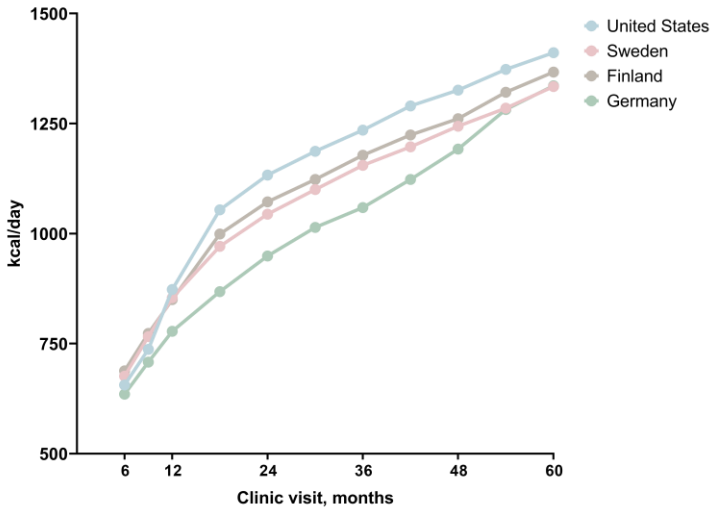


Figure 10 Mean energy intake estimated by three-day food records in TEDDY children by country age to 60 months.

The mean daily intake of grains, as well as of individual grains, varied between children depending on country of residence. Highest intakes of grains were observed in Swedish children, and the lowest in children from the US. Intake of wheat was highest in Swedish children, whereas rye and barley were consumed more frequently in Finland and Germany, respectively (**Table 14, Figure 11**).

Table 14 Proportion of consumers (intake >0g/day) of different grains by country and age in TEDDY.

	US			Sweden			Finland			Germany		
Age (months)	**6**	**9**	**12**	**6**	**9**	**12**	**6**	**9**	**12**	**6**	**9**	**12**
Grain												
Wheat, %	20.2	81.7	97.5	72.0	98.8	99.8	43.2	86.4	95.3	41.4	78.6	94.6
Rye, %	1.3	4.5	4.7	6.3	46.8	84.4	28.0	61.0	81.2	8.2	36.2	73.2
Barley, %	4.4	18.9	22.6	0.0	1.9	2.0	25.6	50.6	57.2	4.8	18.1	24.5
Oats, %	37.6	85.4	85.9	68.2	95.6	96.1	67.5	92.5	94.1	27.1	55.0	61.8
Rice, %	65.5	82.1	73.7	83.1	95.6	93.9	50.7	74.8	78.1	69.5	92.6	61.8
Corn, %	5.3	25.7	51.8	52.6	68.4	65.4	24.7	40.6	44.0	0.0	0.7	1.7
Other GF grains, %	11.5	66.0	85.8	30.7	30.6	24.8	88.3	97.8	98.2	55.7	91.0	88.3

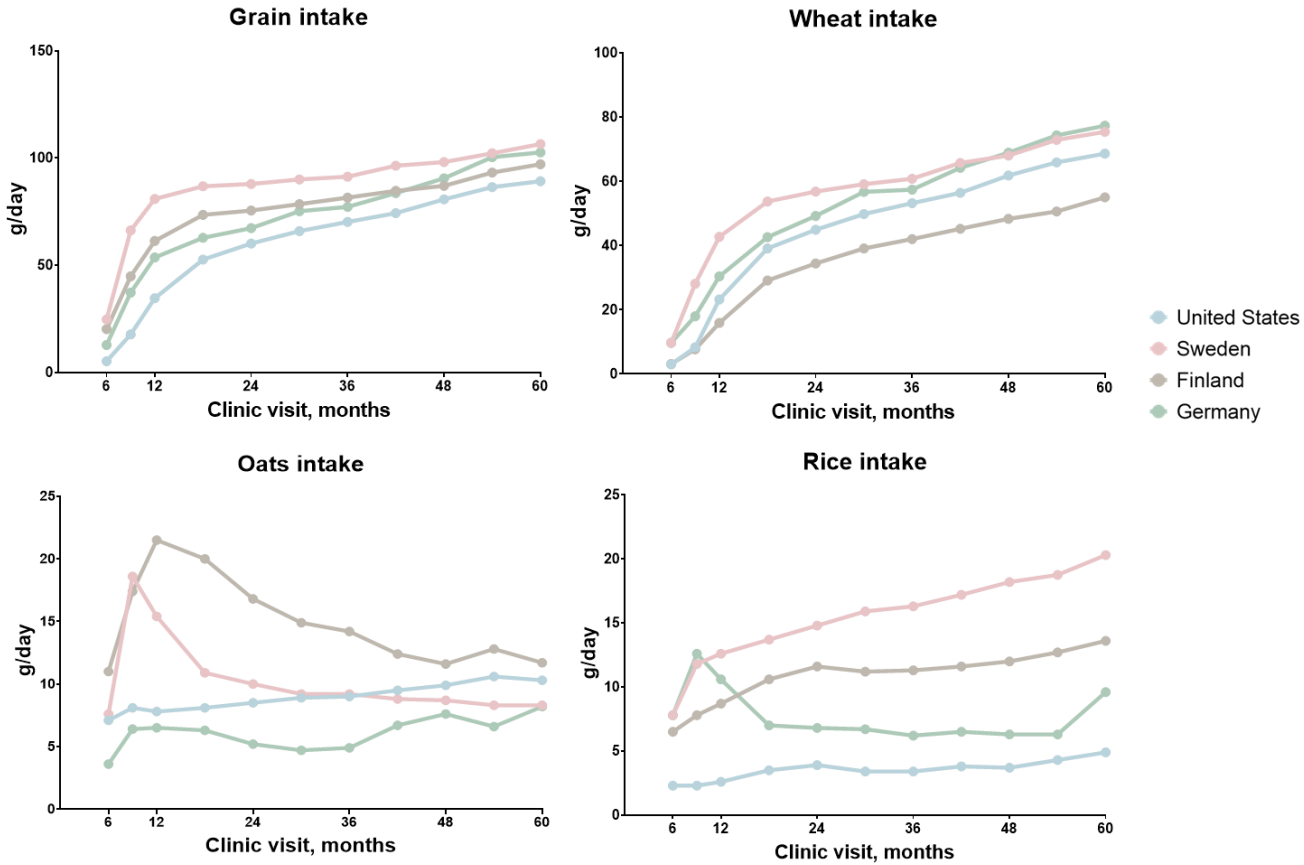


Figure 11 Mean daily intake of total amount of grains, wheat, oats and rice assessed by three-day food records by country. Reported only in consumers (intake >0 g/day).

The intake of grains overall increased with age and energy intake. Swedish children consumed about 55 g/day (95% CI 48.9, 61.5) more over time relative to US children. US children with a family member with CD consumed 11 g/d (95% CI -17.6, -4.3) less grains compared with US children without a family member with CD. In the other countries, there was no difference in grain intake over time in children with a family member with CD compared with children without a family member with CD. The grain intake increased in children in the US and Finland with increasing maternal education. The duration of breastfeeding and age at gluten introduction had a minor or no effect on grain intake over time (**Table 15**).

Table 15 Estimates from mixed effects model and related 95% confidence intervals for change in daily intake of grains from age six to 60 months by country. Socioeconomic factors were included as interaction covariates. Children with CDA or CD at the visit were excluded to minimize the effect of possible dietary changes.

	Change g/day (95% CI), *p*-value			
	US	**Sweden**	**Finland**	**Germany**
Intake over time	54.5 (ref) (53.8, 55.3)	+55.2 (48.9, 61.5), <0.001	+22.1 (15.3, 29.9), <0.001	+12.6 (-0.8, 26.0), 0.066
Per 100 kcal/day	+7.3 (7.2, 7.4), <0.001	+6.2 (6.0, 6.4), <0.001	+7.0 (6.8, 7.2), <0.001	+7.3 (6.8, 7.9), <0.001
Having a family member with CD	-11.0 (-17.6, -4.3), 0.001	-2.5 (-7.3, 2.3), 0.311	+0.0 (-4.4, 4.5), 0.990	-9.7 (-26.7, 7.4), 0.266
Higher maternal educational level	+3.6 (1.6, 5.7), 0.001	+2.5 (1.0, 6.3), 0.001	+2.7 (-0.5, 5.9), 0.102	+0.0 (-6.3, 6.3), 0.990
Child is in daycare	+7.7 (6.6, 8.8), <0.001	+0.6 (-0.5, 1.8), 0.259	+4.2 (2.8, 5.5), <0.001	+7.9 (4.9, 10.9), <0.001
Crowding*, per person	-1.2 (-3.1, 0.6), 0.192	+3.0 (0.6, 5.4), 0.013	-1.5 (-4.3, 1.3), 0.308	+0.9 (-4.3, 6.0), 0.737
Breastfeeding duration, per month	+0.1 (0.0, 0.2), 0.003	-0.2 (-0.3, -0.1), 0.001	+0.2 (0.0, 0.4), 0.016	-0.1 (-0.4, 0.2), 0.565
Age at gluten introduction, per month	-0.1 (-0.4, 0.3), 0.690	-0.1 (-0.8, 0.6), 0.741	+0.5 (0.0, 1.0), 0.036	-0.4 (-1.3, 0.4), 0.334

*crowding is calculated as number of househod members divided by number of rooms in the house.

Paper I

The amount of protein in wheat, rye and barley differed slightly between the national food databases, and therefore also the amount of gluten. In the German database, the gluten content of wheat and barley was the highest, as well as the lowest for rye, compared with in the other food databases (**Table 1**).

Table 16 Amount of protein and gluten in wheat, rye and barley in the national food databases in the TEDDY countries.. Gluten content was estimated by applying a conversion factor of 0.8 x protein intake from each respective grain.

Grain	National food database	Protein/100 g	Calculated gluten/100g
Wheat	US	11.0	8.8
	Sweden	11.0	8.8
	Finland	10.5	8.4
	Germany	11.5	9.2
Rye	US	10.6	8.5
	Sweden	9.2	7.4
	Finland	9.3	7.4
	Germany	8.5	6.8
Barley	US	10.2	8.2
	Sweden	10.0	8.0
	Finland	8.25	6.6
	Germany	10.6	8.5

The amount of gluten intake increased with age in children in all countries. Swedish children consumed higher amounts of gluten during the first two years of life compared with children from the other countries (**Figure 12**).

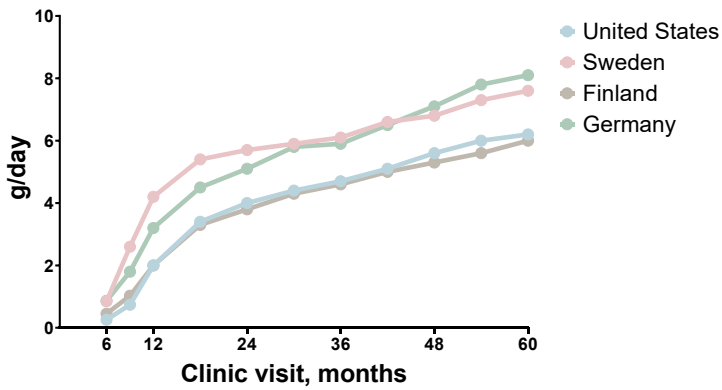


Figure 12 Daily gluten intake in consumers in TEDDY (intake >0g) by country and age. Children with CDA or CD at the visit were excluded.

When adjusted for other risk factors, the amount of gluten intake up to age five years was associated with an increased risk of CDA (HR1.30, 95% CI 1.22, 1.38, p <0.001) and CD (HR 1.50, 95% CI 1.35, 1.66, p <0.001) for every g/day increase in intake. Every g/day above the mean gluten intake at age 24 months was associated with 6.1 % (4.5, 7.7) and 7.2 % (6.1, 8.3) higher absolute risks of CDA and CD, respectively, by the age of three years. When gluten intake was energy and age adjusted, as well as relative to body weight, the findings remained.

Sensitivity analyses using Cox regression models overall confirmed the results. Additionally, the results were supported when investigating gluten intake per country as well as when only analyzing children with a reported gluten intake within a year of the study outcomes.

In post hoc analyses, gluten intake at age 24 months was found to be independently associated with both CDA and CD. At this age, a gluten intake of more than 2 g/day was associated with an increased risk of CDA (HR 1.49, 95% CI, 1.16, 1.91, p=0.002) and CD (HR 1.75, 95% CI, 1.10, 2.81, p=0.02) compared with an intake of less than 2 g/day.

Paper II

Eight food groups were identified as sources of gluten in Swedish participants. The intake of these gluten-containing food groups varied over time, and milk cereal drink was common across all ages (**Figure 13**).

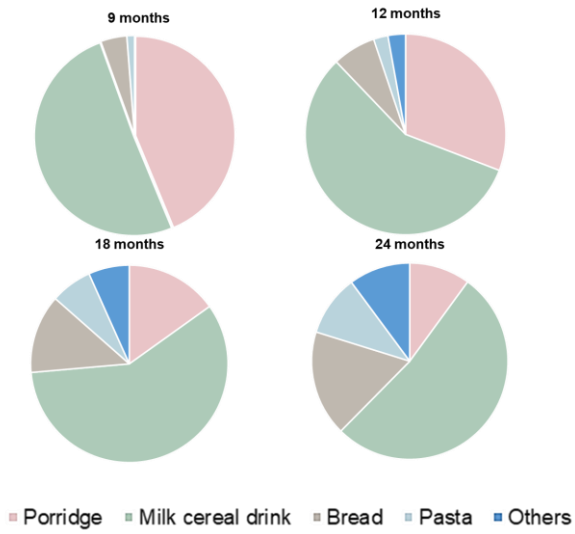


Figure 13 Intake of gluten-containing food groups in Swedish children in relation to the total daily intake of gluten-containing foods.

Intake of bread and pasta were the food groups most strongly correlated with daily gluten intake at all ages (bread ρ 0.44 to 0.34, $p<0.001$, pasta ρ 0.72 to 0.63, $p<0.001$). Intake of milk cereal drink correlated weakly with daily gluten intake (ρ 0.17 to 0.10, $p<0.05$) (**Table 17**).

Table 17 Spearmans correlation between daily intakes of gluten-containing food groups and total daily gluten intake at age 6 to 24 months in Swedish TEDDY children. Only consumers (intake >0g/day) of each food group were considered, and children with CDA or CD at the visit were excluded.

	Correlation with total gluten intake, ρ (*p*-value)				
Age in months	**6**	**9**	**12**	**18**	**24**
Food group					
Porrigde	0.514 (<0.001)	0.269 (<0.001)	0.053 (0.037)	-0.008 (0.813)	-0.043 (0.325)
Milk cereal drink	0.170 (<0.001)	-0.133 (<0.001)	0.086 (<0.001)	0.071 (0.011)	0.098 (0.002)
Bread	0.444 (<0.001)	0.451 (<0.001)	0.439 (<0.001)	0.316 (<0.001)	0.339 (<0.001)
Pasta	0.723 (<0.001)	0.671 (<0.001)	0.549 (<0.001)	0.643 (<0.001)	0.630 (<0.001)
Biscuit and crackers	0.179 (0.003)	0.119 (<0.001)	0.142 (<0.001)	0.090 (<0.001)	0.112 (<0.001)
Pancakes	0.224 (0.527)	0.393 (<0.001)	0.198 (0.004)	0.196 (<0.001)	0.155 (0.004)
Sweet baked goods	0.419 (0.058)	0.174 (0.025)	0.157 (<0.001)	0.222 (<0.001)	0.134 (<0.001)
Breakfast cereals	n.a.	0.261 (0.130)	0.206 (0.003)	0.124 (0.002)	0.058 (0.109)

At age nine months, daily intake up to the equivalent of 1.3 portions (158 g) of porridge, compared with no intake, was associated with a 50% increased risk of CDA (HR 1.53, 95% CI 1.05, 2.23, p=0.026), but not with CD (HR 1.51, 95% CI 0.87 2.62, p=0.144). At age 12 months, daily bread intake of >18.3 g/day was associated with increased risk of CDA (HR 1.47, 95% CI 1.05, 2.05, p=0.023) and CD (HR 1.79, 95% CI 1.10, 2.91, p=0.019) as compared with no intake of bread. Intake of milk cereal drink at age 18 months was associated with increased risk of CD (HR 1.16, 95% CI 1.00, 1.33, p=0.047), but not with CDA (HR 1.03, 95% CI 0.93, 1.14, p=0.606) for every additional bottle intake per day. Intake of wheat, rye and barley were not associated with CDA or CD at any of age. Modelling intake variables as energy-adjusted to g/1000 kcal/day had overall minor effects on risk estimates. (**Table 18**)

Table 18 Estimated HR and their related 95% CI of the association between intake of gluten-containing foods and either time to CDA or to CD in Swedish children. Depending on the percent of consumers (having an intake >0 g/day) at each age, intake variables were modelled as binary (if <50% consumers; no intake, intake), categorical (if ≥50% consumers; no intake (reference), <median intake, ≥median intake) and continuous variables (if >75% consumers). Included covariates in the analyses were HLA risk group, sex, having a parent or sibling with CD, and energy as well as total gluten intake assessed by the respective food record. Statistically significant *p*-values are highlighted.

Age 6 months					
		CDA		**Celiac disease**	
Food group	**Intake modelled**	**HR (95% CI)**	***p-value***	**HR (95% CI)**	***p-value***
Porridge	*No intake*	1		1	
	≤118 g/day	1.28 (0.96, 1.69)	0.089	1.07 (0.72, 1.58)	0.737
	>118 g/day	1.28 (0.95, 1.72)	0.102	1.31 (0.87, 1.97)	0.198
	Per 120 g (portion)	1.04 (0.91, 1.20)	0.561	1.16 (0.96, 1.40)	0.118
Milk cereal drink	*Yes (no reference)*	1.11 (0.91, 1.35)	0.320	1.25 (0.95, 1.64)	0.117
Bread	*Yes (no reference)*	1.01 (0.75, 1.38)	0.927	1.23 (0.82 1.85)	0.316
Biscuits and crackers	*Yes (no reference)*	0.91 (0.69, 1.21)	0.527	1.20 (0.84, 1.74)	0.320
Age 9 months					
Porridge	*No intake*	1		1	
	≤158 g/day	**1.53 (1.05, 2.23)**	**0.026**	1.51 (0.87, 2.62)	0.144
	>158 g/day	1.41 (0.95, 2.09)	0.088	1.38 (0.78, 2.46)	0.270
	Per 120 g (portion)	0.96 (0.85, 1.08)	0.521	0.94 (0.79, 1.11)	0.467
Milk cereal drink	*Ref no intake*	1		1	
	<400 g/day	0.93 (0.74, 1.18)	0.574	1.00 (0.71, 1.41)	0.989
	>400 g/day	0.98 (0.78, 1.25)	0.889	1.10 (0.77, 1.56)	0.604
Bread	*No intake*	1		1	
	≤10.7 g/day	1.16 (0.93, 1.45)	0.196	1.22 (0.89, 1.66)	0.217
	>10.7 g/day	1.10 (0.86, 1.41)	0.445	0.91 (0.63, 1.30)	0.585
Pasta	*Yes (no reference)*	1.01 (0.80, 1.27)	0.382	0.89 (0.63, 1.26)	0.502
Biscuits and crackers	*Yes (no reference)*	1.09 (0.90, 1.31)	0.958	1.25 (0.96, 1.63)	0.093
Age 12 months					
Porridge	*No intake*	1		1	
	≤133 g/day	1.19 (0.93, 1.53)	0.174	1.26 (0.89, 1.80)	0.198
	>133 g/day	0.97 (0.75, 1.27)	0.843	1.07 (0.73, 1.56)	0.739
	Per 120 g (portion)	0.94 (0.83, 1.06)	0.320	0.94 (0.79, 1.13)	0.500
Milk cereal drink	*No intake*	1		1	
	≤410 g/day	0.96 (0.74, 1.23)	0.726	1.01 (0.70, 1.47)	0.940
	>410 g/day	1.04 (0.80, 1.35)	0.770	1.19 (0.81, 1.73)	0.372
	Per 200 ml (bottle)	1.02 (0.93, 1.11)	0.736	1.04 (0.91, 1.18)	0.558
Bread	*No intake*	1		1	
	≤18.3 g/day	1.24 (0.91, 1.70)	0.176	1.21 (0.76, 1.94)	0.427
	>18.3 g/day	1.47 (1.05, 2.05)	0.023	**1.79 (1.10, 2.91)**	**0.019**
	Per 10 g	1.04 (0.98, 1.10)	0.205	1.07 (0.98, 1.16)	0.131
Pasta	*Yes (no reference)*	0.91 (0.74, 1.12)	0.379	1.24 (0.93, 1.66)	0.137
Biscuits and crackers	*No intake*	1		1	
	≤3.0 g/day	1.18 (0.95, 1.47)	0.144	1.04 (0.76, 1.42)	0.798
	>3.0 g/day	1.10 (0.88, 1.39)	0.404	1.00 (0.72, 1.39)	0.993
Sweet baked goods	*Yes (no reference)*	1.16 (0.94, 1.43)	0.172	0.97 (0.72, 1.32)	0.863
Pancakes	*Yes (no reference)*	0.90 (0.67, 1.22)	0.500	0.96 (0.64, 1.46)	0.858
Breakfast cereals	*Yes (no reference)*	0.86 (0.63, 1.18)	0.354	0.89 (0.57, 1.38)	0.595

Food group	Intake modelled	CDA HR (95% CI)	*p-value*	Celiac disease HR (95% CI)	*p-value*
Age 18 months					
Porridge	*Yes (no reference)*	0.92 (0.75, 1.12)	0.394	0.91 (0.68, 1.20)	0.485
Milk cereal drink	*No intake*	1		1	
	≤383 g/day	0.97 (0.75, 1.25)	0.819	1.14 (0.78, 1.67)	0.511
	>383 g/day	0.99 (0.75, 1.29)	0.922	1.40 (0.95, 2.06)	0.092
	Per 200 ml (bottle)	1.03 (0.93, 1.14)	0.606	**1.16 (1.00, 1.33)**	**0.047**
Bread	*No intake*	1		1	
	≤26.0 g/day	1.20 (0.74, 1.95)	0.468	1.27 (0.64, 2.53)	0.492
	>26.0 g/day	1.18 (0.72, 1.94)	0.520	1.19 (0.59, 2.40)	0.633
	Per 10g	1.01 (0.96, 1.07)	0.648	1.03 (0.95, 1.11)	0.458
Pasta	*No intake*	1		1	
	≤23.0 g/day	1.18 (0.93, 1.51)	0.182	0.94 (0.67, 1.31)	0.717
	>23.0 g/day	0.98 (0.73, 1.33)	0.914	0.76 (0.51, 1.15)	0.199
Biscuits and crackers	*No intake*	1		1	
	≤5.0 g/day	0.95 (0.75, 1.20)	0.662	1.01 (0.73, 1.41)	0.954
	>5.0 g/day	0.96 (0.75, 1.23)	0.747	1.11 (0.80, 1.56)	0.527
Sweet baked goods	*Yes (no reference)*	1.04 (0.85, 1.28)	0.717	0.98 (0.74, 1.30)	0.881
Pancakes	*Yes (no reference)*	0.93 (0.73, 1.19)	0.548	1.06 (0.77, 1.47)	0.722
Breakfast cereals	*Yes (no reference)*	0.88 (0.71, 1.08)	0.205	0.89 (0.67, 1.18)	0.414
Age 24 months					
Porridge	*Yes (no reference)*	1.13 (0.90, 1.43)	0.301	1.13 (0.84, 1.51)	0.412
Milk cereal drink	*No intake*	1		1	
	≤360 g/day	0.82 (0.63, 1.08)	0.159	1.15 (0.81, 1.63)	0.437
	>360 g/day	0.81 (0.60, 1.09)	0.168	1.35 (0.93, 1.96)	0.119
Bread	*No intake*	1		1	
	≤30.3 g/day	1.14 (0.58, 2.24)	0.712	1.67 (0.67, 4.14)	0.268
	>30.3 g/day	1.28 (0.64, 2.54)	0.487	1.91 (0.76, 4.80)	0.170
	Per 10g	1.04 (0.98, 1.10)	0.204	1.05 (0.97, 1.13)	0.210
Pasta	*No intake*	1		1	
	≤25.0 g/day	0.85 (0.64, 1.13)	0.269	0.93 (0.64, 1.34)	0.691
	>25.0 g/day	0.81 (0.55, 1.17)	0.260	1.38 (0.86, 2.21)	0.184
Biscuits and crackers	*No intake*	1		1	
	≤6.0 g/day	1.27 (0.96, 1.68)	0.101	1.12 (0.79, 1.59)	0.517
	>6.0 g/day	1.23 (0.93, 1.64)	0.153	1.13 (0.79, 1.61)	0.501
Sweet baked goods	*Yes (no reference)*	1.09 (0.87, 1.36)	0.473	1.10 (0.83, 1.46)	0.516
Pancakes	*Yes (no reference)*	1.13 (0.87, 1.48)	0.356	1.09 (0.77, 1.53)	0.644
Breakfast cereals	*Yes (no reference)*	0.85 (0.68, 1.06)	0.154	1.01 (0.76, 1.34)	0.967

Paper III

Intake of milk powder increased from age three to nine months after which it declined similarly for both cases and controls.

In the unadjusted model, intake of milk powder at age nine months was associated with increased risk of CD per g/day increase (OR 1.01, 95% CI 1.00, 1.02, p=0.037) and g/kg/day increase (OR 1.10, 95% CI 1.00, 1.20, p=0.044). In the adjusted model, no association between intake of milk powder, either for absolute intake or relative to body weight, was found at any timepoint.

A Kaplan-Meier plot illustrated the incidence of CD over time, when stratifying on the last reported intake of milk powder by high (>23.2 g/day), medium (9.7-23.2 g/day) and low intake tertiles (<9.7 g/day) (**Figure 14**).

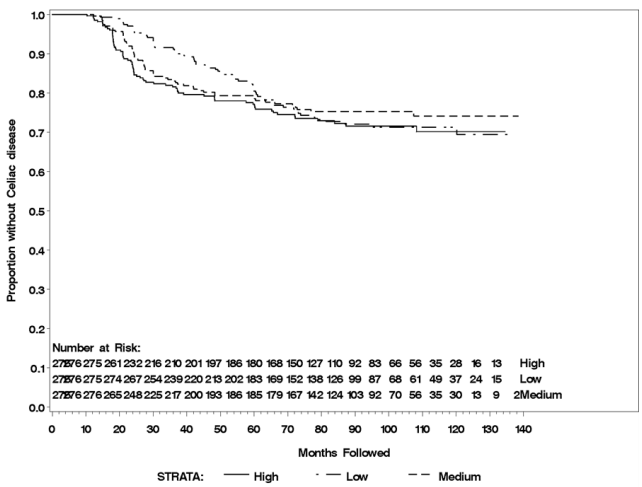


Figure 14 Kaplan-Meier plot stratified by tertiles of milk powder intake (*Low* <9.7 g/day, *medium* 9.7-23.2 g/day, *high* >23.2 g/day) and CD in Swedish TEDDY participants.

Paper IV

Overall, 27 food groups were included in the PCAs to derive dietary patterns. Modelling food group intakes in absolute amounts or energy adjusted to per 1000 kcal/day resulted in different dietary patterns and factor loadings extracted by the PCAs. Therefore energy-adjusted variables were used in the PCAs.

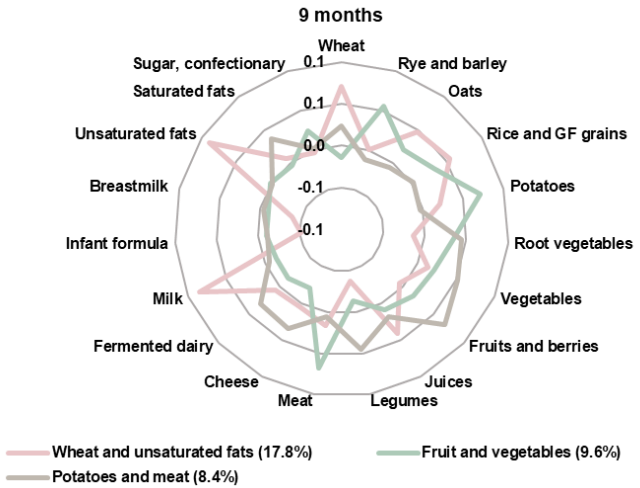
At age nine, 12 and 24 months, three dietary patterns were extracted, explaining 35.8%, 31.3%, 32.3% of the variance in food group intake, respectively. At age 18 months, four patterns were extracted that explained 24.9% of the total variance (**Figures 15-18**). At all ages, the patterns *Unsaturated fats, Fruit and vegetables,* as well as *Potatoes and meat* were identified, although some of the included food groups in the patterns differed.

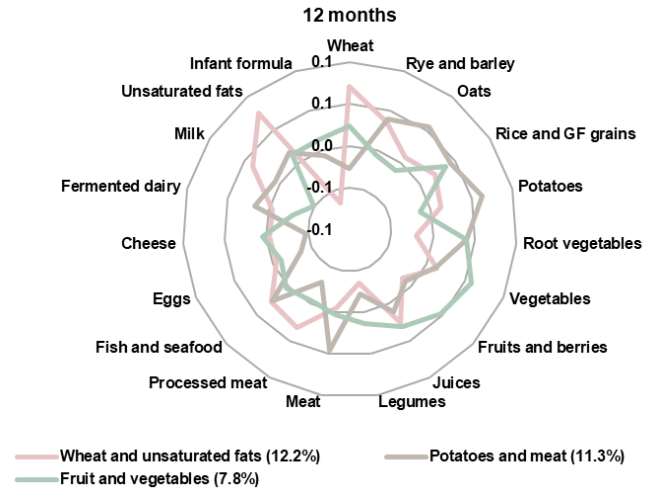
Adherence to the SDP's differed between countries and age (**Figures 19-21**). The *Unsaturated fats and wheat* pattern was most common in Swedish TEDDY children at all ages, whereas the *Potatoes and meat* pattern was most common among Finnish children. The *Fruit and vegetables* pattern was found in all four countries.

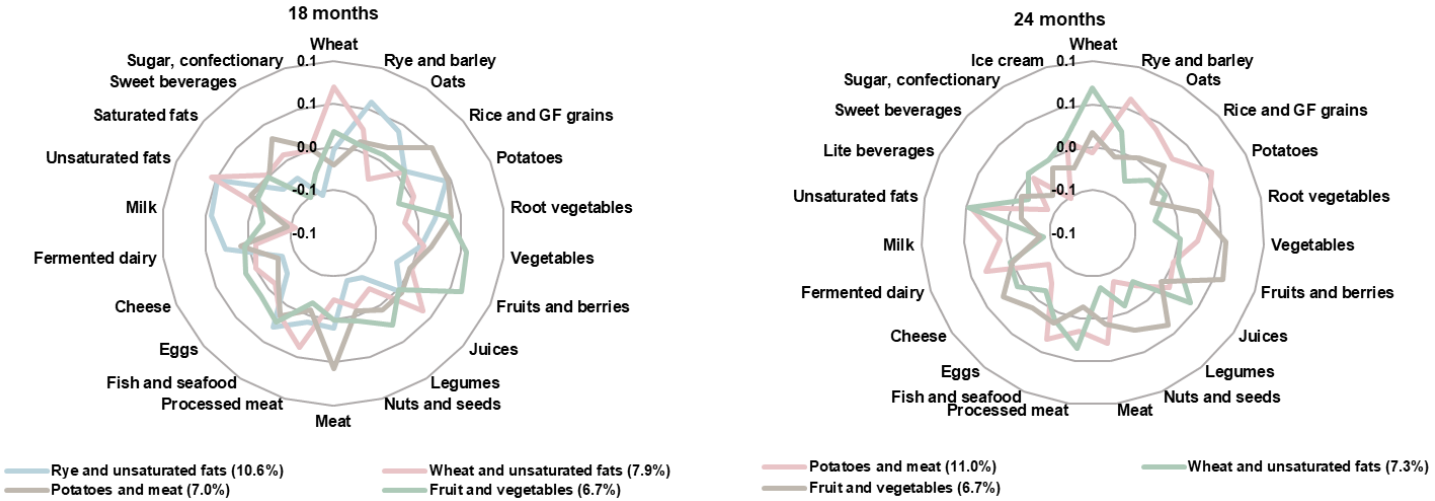
Higher adherence to *Unsaturated fats and wheat* at age nine months was associated with a reduced risk of CDA (HR 0.97, 95% CI 0.95, 1.00, p=0.018) for every SD increase in adherence. Additionally, an adherence in the second quartile was associated with a reduced risk of CD (HR 0.70, 95% CI 0.50, 0.97, p=0.033) as compared with the reference quartile.

Adherence in the third quartile to *Potatoes and meat* at age 18 months was associated with a reduced risk of CDA (HR 0.83, 95% CI 0.69, 0.99, p=0.040) as compared with the reference category. A higher adherence was also associated with reduced risk of CD (HR 0.96, 95% CI 0.93, 1.00, p=0.035) for every SD increase in adherence.

Higher adherence to *Unsaturated fats and wheat* at age 24 months was associated with increased risk of CDA (HR 1.03, 95% CI 1.01, 1.06, p=0.006) and CD (HR 1.04, 95% CI 1.01, 1.08, p=0.028) for every SD increase in adherence.

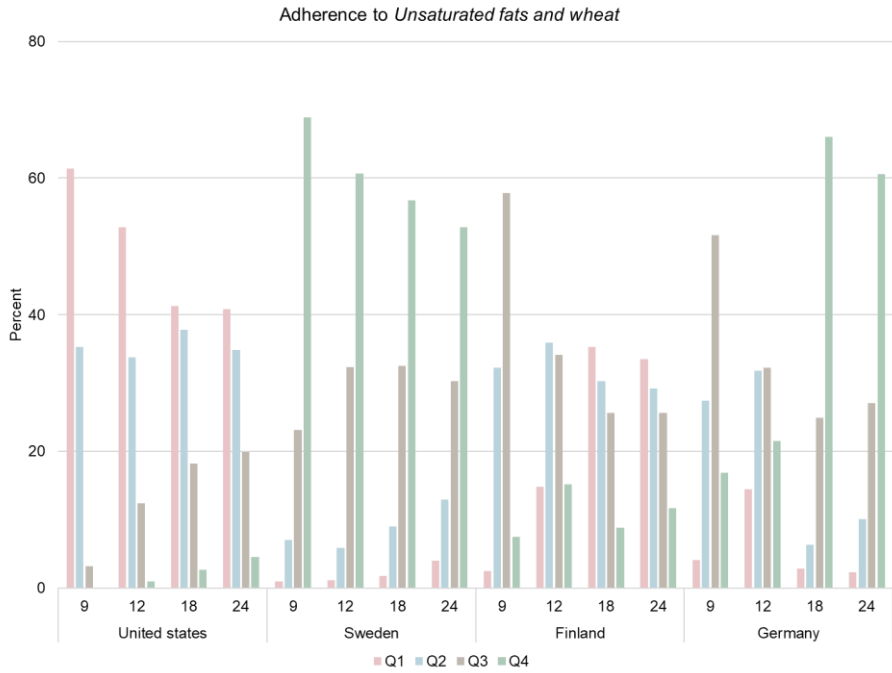

9 months
Wheat
Rye and barley
Oats
Rice and GF grains
Potatoes
Root vegetables
Vegetables
Fruits and berries
Juices
Legumes
Meat
Cheese
Fermented dairy
Milk
Infant formula
Breastmilk
Unsaturated fats
Saturated fats
Sugar, confectionary
0.1
0.1
0.0
-0.1
-0.1
Wheat and unsaturated fats (17.8%)
Fruit and vegetables (9.6%)
Potatoes and meat (8.4%)

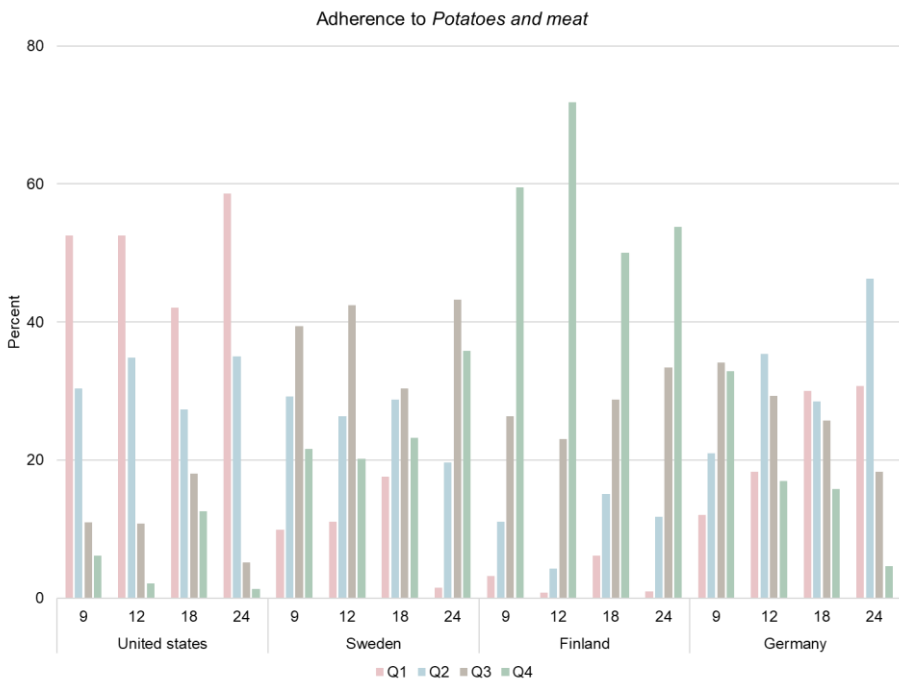

12 months
Wheat
Rye and barley
Oats
Rice and GF grains
Potatoes
Root vegetables
Vegetables
Fruits and berries
Juices
Legumes
Meat
Processed meat
Fish and seafood
Eggs
Cheese
Fermented dairy
Milk
Unsaturated fats
Infant formula
0.1
0.1
0.0
-0.1
-0.1
Wheat and unsaturated fats (12.2%)
Potatoes and meat (11.3%)
Fruit and vegetables (7.8%)

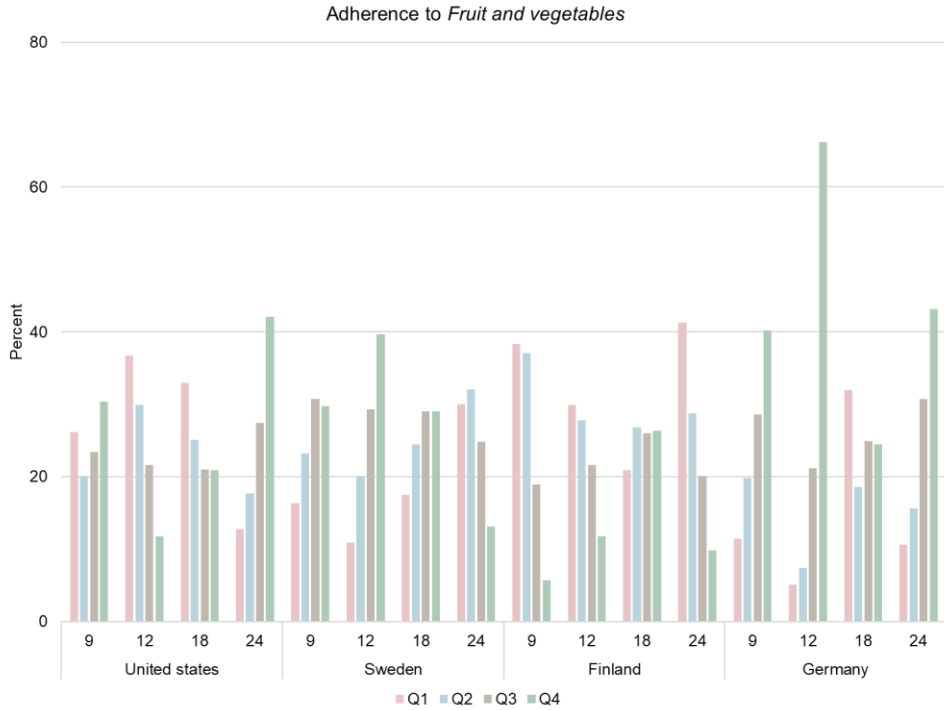


Figures 15-18 Spider chart representing dietary patterns from Principal Components Analysis (PCA) on intakes of 27 food groups at age nine to 24 months.

Variance explained is reported for each pattern (%).


Adherence to *Unsaturated fats and wheat*
80
60
40
20
0
Percent
9 12 18 24 9 12 18 24 9 12 18 24 9 12 18 24
United states
Sweden
Finland
Germany
Q1 Q2 Q3 Q4


Adherence to *Potatoes and meat*
80
60
40
20
0
Percent
9 12 18 24 9 12 18 24 9 12 18 24 9 12 18 24
United states
Sweden
Finland
Germany
Q1 Q2 Q3 Q4



Figures 19-21 Proportion of children adhering to simplified dietary patterns by country and age. Adherence to the patterns is reported in quartiles (Q) by country at age nine to 24 months. Q1 representing low similarity with the pattern and Q4 high similarity.

Discussion

Main findings

The present thesis showed that higher amounts of gluten intake in the first five years of life was associated with increased risk of CDA and CD in genetically predisposed children. Intake of bread as well as infant cereals in the form of milk cereal drink or instant porridge in the first two years of life further increased this risk in Swedish children whereas intake of milk powder did not. There was no difference in risk observed depending on the grain source of gluten. However, specific dietary patterns up to age two years conferred different risk of CDA and CD.

Amount of gluten intake

Paper I concluded that the daily intake of gluten from age six to 60 months was associated with higher risk of both CDA and CD for every g/day increase in intake. The finding remained regardless of whether gluten intake was energy adjusted or relative to body weight, by HLA risk-genotype or country of residence. This is in contrast with findings from the PreventCD study where no association between gluten amount and CD was observed (109). However, there are considerable methodological differences between the two studies that could explain the discrepant results. TEDDY is an observational study enrolling children at genetic risk, assessing dietary intake by repeated three-day food records. PreventCD is an intervention study including children from families with CD, with restricted gluten intake during infancy, and dietary intake assessed by food frequency questionnaires (FFQ) specific to each country (155, 156). However, a sub-analyses of the Italian PreventCD study found that children who developed CD consumed higher amounts of gluten intake from age 12 months compared with healthy children (157).

There are methodological differences in assessing dietary intake by food records compared with FFQ. Food records prospectively assess actual daily intake with a high level of detail that captures day-to-day variation and allows for estimating food and nutrient intake on an individual level (158).

In contrast, FFQs retrospectively captures dietary habits over a predefined time with risk of memory bias, and the less detailed data collected is better suited for ranking individuals according to their food intake (158).

Both TEDDY and PreventCD investigated dietary intake in children from several countries, which requires harmonization of dietary data across national food databases (159). In TEDDY, all reported intakes are harmonized which allowed for comparisons of gluten in the full cohort (144).

In line with the findings of Paper I, two observational birth cohort studies on the amounts of gluten intake showed similar associations. The DAISY study included US children with genetic risk and used FFQ reflecting dietary intake in the second year of life and found that a higher intake of gluten was associated with a higher risk of CDA and CD (160). The Norwegian Mother and Child study also found an association of increased risk of CD with higher gluten intake assessed with a FFQ at age 18 months (161), although children with CD were only identified from the national patient register.

Contradictory to these findings, a British study showed that a high gluten intake in early life could be protective of CD (162). However, this intervention study on early introduction of common food allergens identified few numbers of cases and screening for CD was done once at age three years. Furthermore, information on HLA and family history of CD were not available in the cohort.

Dietary source of gluten

Paper II showed that the main source of gluten from grains in Swedish children under the age of two was wheat, and that infant cereals were common dietary sources of gluten. There was no difference in risk of CDA or CD depending on the grain source of gluten, which is in line with an intervention trial on children with CD (163). However, intake of up to 1.3 portions of porridge at age nine months, more than the equivalent of half a slice of bread at age 12 months as well as per each bottle milk cereal drink at age 18 months were associated with increased risk of CDA and CD, when controlling for risk factors including total daily gluten intake.

The digestibility of bread has been shown to be lower compared with other food matrices (95-97), potentially leaving larger amounts of immunogenic peptides. Moreover extra gluten is often added to commercially baked bread (90).

Although the total intake of gluten was adjusted for in the analyses, this extra gluten may have been underestimated in the food database. It may also be that the bread food group was too heterogenous, and that an association with bread and the study outcomes was driven only by certain types of bread.

Infant cereals and their content of gluten may have played a role in the "Swedish epidemic" (113). This has partly been investigated in a Swedish study that concluded that gluten introduced in solid or liquid form was not associated with later risk of CD (116). This was in contrast with findings in Paper II.

An association was found for low, but not high, intakes of porridge at age nine months and CDA. For high intakes of porridge at this age, the lower bound CI and *p*-value indicated that this may be explained by low statistical power. However, the risk estimates were lower with high intakes for both CDA and CD at ages nine and 12 months. To be biologically plausible, more consistent risk estimates would have been expected, which indicates that the association found for porridge and CDA may be a chance finding.

For milk cereal drink, there was an association for intake of milk cereal drink and CD but not CDA. Similar with bread, the milk cereal drink food group may have been too varied when aggregating products with various grains and amounts of gluten, and that a true association is present only with specific types.

Only Swedish TEDDY children were included in Paper II because of the approach chosen in TEDDY to harmonize food intake on ingredient level (144). This was a limitation as comparisons could not be made in the full cohort. However, considering the diversity of intakes of grains and dietary patterns observed between the participating countries in TEDDY, it could also be considered a strength of Paper II to only include children from Sweden with more homogenous dietary habits.

Wheat was the main cereal consumed in Swedish participants as well as overall in TEDDY. The total intake of grains a well as of individual grains differed between the four countries. These differences were mainly determined by the country of residence, showing the external influence of food culture on dietary intake of grains and in extension also gluten. Interestingly, breastfeeding duration and age at gluten introduction did not predict the intake of cereals later in childhood. Moreover, wheat also has the highest content of gluten compared with rye and barley (89). In Paper II, there was no association found of a direct effect of the grain source of gluten and risk of CDA and CD. Instead in Paper IV, wheat as well as rye and barley were included both in SDP's negatively and positively associated with CDA and CD. A summary of these findings together with the results in Paper I, would suggest that the total amount of gluten intake influences the risk of CD, irrespective of the grain source.

Milk powder intake

Paper III concluded that daily intake of milk powder from age three to 24 months was not associated with CD in Swedish children at genetic risk, either in absolute amounts or relative to their body weight when accounting for other risk factors. These findings are in line with an RCT concluding that intake of cow's-milk based infant formula compared with an extensively hydrolyzed formula, did not confer increased risk of CD (132). In Paper II, infant cereals were found to be associated with higher risk of CDA and CD while the findings of Paper III suggests that this risk increase is not attributed to the content of milk powder in infant cereals.

In the TEDDY food database, intake of milk and other dairy are grouped according to the amount of fat in the products (144) and do not allow for extracting milk powder intake specifically. This structure limited to only include Swedish TEDDY participants in Paper III. Although milk powder intake from some foods could not be accounted for, the main food sources of milk powder in Swedish children to age two years are most likely infant formula and infant cereals based on dietary data examined in papers II and IV.

There is evidence that a high AGE milk-powder based diet results in chronic intestinal inflammation in rats, (164, 165). In humans, AGEs are also associated with inflammation in adults (130). However, Paper III did not assess intake of AGEs, as these are present in a various range of foods besides milk powder, primarily in heat-processed foods (130).

Since milk powder intake was not readily available in the TEDDY food database and only partly in the Swedish food database, estimation of the daily milk powder intake was resource intensive and required additional computations and data preparation. The nested case-control study was therefore designed to only include Swedish TEDDY children. Strengths of this design are that conditioning reduces confounding, even un-measured, and the use of three controls per case increased the statistical precision. However, inherent in the cross-sectional design, a control can later become a case which is a limitation of the study design. Additionally, using only a part of the cohort leads to loss of information in the whole cohort.

Dietary patterns

In Paper IV, several SDPs were associated with CDA and CD. At age nine months, higher adherence to *Unsaturated fats and wheat* was associated with a reduced risk of CDA and partly for CD, whereas this patter increased the risk of CDA and CD at age 24 months.

One explanation for the contradictory findings may be that this pattern differed in included food groups depending on what ages were investigated. At 18 months, higher adherence to *Potatoes and meat*¸ was associated with a reduced risk of CDA and CD.

There were overlaps of some food groups included in the SDPs associated with the study outcomes. When summarizing, the findings suggest that a diet high in potatoes, oats, rice and GF grains, meat, and root vegetables could be attributed the risk reduction observed. This was partly in line with foods found in a *Prudent* dietary pattern associated with lower risk of CDA in healthy Dutch children from a multi-ethnic background, with dietary intake assessed at age one year by a semi-quantitative FFQ and screening for CDA once at age six (134). However, this FFQ captured intake of composite foods, as opposed to ingredients in Paper IV.

Some of the foods in the SPDs associated with reduced risk of CDA and CD in Paper IV are high in fibers. However, all high fiber foods were not consistently associated with lower risk of CDA and CD, as the *Fruits and vegetables* SDPs were weakly inversely associated with CDA at age nine and 12 months. In contrast, in the Norwegian Mother and Child cohort, maternal fiber intake, especially from fruits and berries, reduced the risk of CD in the offspring (166). Moreover, meat and oats, found in SDPs inversely associated with CDA and CD in Paper IV, are important sources of iron. Although the use of iron supplements in pregnant women, as well as in their child at age 18 months, were associated with increased risk of CD in the Norwegian Mother and Child cohort, dietary iron intake in pregnant women was not associated with CD in the offspring (167).

Associations of adherence in the 2nd or 3rd quartile to *Fruit and vegetables* at age nine and 12 months, *Potatoes and meat* at 12 months, as well as *Unsaturated fats and wheat* at age 12 months and CDA, were also found. However, the risk estimates were inconsistent across the adherence quartiles which would not be biologically plausible and indicates that these findings may be the result of multiple testing.

Dietary intake is not stable through early childhood (168), thus repeated assessment of dietary intake is necessary to be able to analyze dietary patterns in different ages. A strength of Paper IV is that similar dietary patterns could be investigated at different ages in relation to CDA and CD, although there were some variations in the food groups included. However, PCA finds the most common dietary patterns in a population, and the results are driven by groups with similar dietary habits. In Paper IV, PCA was applied to a cohort of children from four countries, where infant dietary habits have been found to vary (79, 169). And indeed, the adherence to SDPs from age nine to 24 months differed by country.

Consequently, if the PCA would have been performed separately in each country, other dietary patterns would likely have been found. Nevertheless, it may be considered a strength of Paper IV to investigate dietary patterns and association with CD in a multinational cohort while adjusting for the influence of country of residence.

The findings presented in this thesis are all based on observational data in children at genetic risk. Therefore, inference of causality cannot be determined. The findings of Paper I, as confirmed by others, should be investigated by an RCT to investigate the effects of different amount of gluten intake on the risk of CD. The associations found in Paper II and IV have not previously been extensively investigated and should thus be validated in other populations.

Methodological considerations

Study population of TEDDY

TEDDY allows for investigating numerous hypotheses at different ages, to combine exposures, and investigate interactions, all before disease onset while parents are unaware of their child's disease status. Moreover, as TEDDY includes only children at genetic risk, it has greater statistical power to detect differences in environmental exposure than in a cohort enrolling children from the general population. Although there are several genetical overlaps in CD and T1D, TEDDY is designed for T1D, and children from families with T1D are overrepresented.

Of all eligible infants screened at birth, 40% were enrolled to follow-up in TEDDY. The willingness to participate differed between countries with only about 1/3 of eligible children from the US accepting participation compared with 2/3 of Swedish children. Dietary habits may differ between participating children compared with those who declined participation, based on the fact that higher educational level and socio-economic status were factors related to study retention (170, 171). Although TEDDY is an observational study and dietary recommendations were not given, 30% of mothers at age six months and 43% at age 15 months reported engaging in preventive behaviors (172). German mothers most often reported preventive behaviors, and the most common action was dietary changes. Whether socio-economic factors are related to CD has not been established (2). However, when CD is detected by screening, and thus not influenced by mechanisms of health care seeking behaviors, no effect of socio-economic factors on the risk of CD has been found (78).

Still, it cannot be ruled out that the there is a selection bias in TEDDY based on differences both in the investigated dietary intakes and prevalence of CD between enrolled participants and those declining participation.

In a closed, longitudinal cohort study, it is important that participants remain in the study, and that withdrawal is not biased to a particular subgroup. Early withdrawal from TEDDY was most common in the first year of life and differed between the countries. Children from the US were more prone to withdrawal from the study as compared to children from Finland (173). Blood draws and not having enough time were the most common reasons for withdrawal. Factors related to staying in the study were in-study behavior (attending all clinic visits, father active participation, study satisfaction), if the child had repeated illnesses, and accurate perception of the child's genetic risk (171).

Study outcomes

Prospective screening for CD in TEDDY allows for determining the time close to seroconversion to tTGA positivity, and thus investigate prior exposures. TEDDY uses RBA for measuring tTGA with rigorous standards for quality control (137). The RBA has shown both high sensitivity and specificity for CD in an international tTGA workshop (140). However, there is a risk of systematic bias in TEDDY by starting the screening at age two years. There were almost 1900 children, or 20% of the cohort, who were never screened in TEDDY. A child who left the study before age two years may have been missed to have CDA.

Using CDA as the primary outcome allowed more precise investigation on exposures prior to seroconversion of tTGA. CD was included as a secondary outcome since not all children with CDA progress to CD (34, 36, 37). It cannot be excluded that children with CDA that progress to CD have a different genetic makeup and/or have been exposed to other environmental factors compared with those that do not develop CD. Nevertheless, the time when CD develops is difficult to estimate. In TEDDY, there was on average one year between onset of CDA and diagnosis of CD. This delay is naturally explained by when an intestinal biopsy was performed but is not the true time when CD started. Using high levels of tTGA as a proxy for CD when biopsy was not performed may better determine the time when CD developed. However, when the CD outcomes were decided in TEDDY, high levels of tTGA were not yet accepted in the ESPGHAN diagnostic criteria.

Dietary intake data

Food records prospectively assess dietary intake and are often used to validate other dietary assessment methods (158). Food records provide information on an individual level, of energy and nutrients, as well as intake of foods. Repeated food records capture dietary changes over time and allow for analyzing cumulative intake. However, this method is related to a high burden on the study participant. Dietary intake is a difficult exposure to measure. It changes from day-to-day and over time. All dietary assessment methods are prone to several biases, for example related to social desirability, memory bias and the burden of assessment itself (158).

Validating the dietary assessment method used in nutrition research is crucial (145). Food records covering three-days, including one weekend day, have been shown to be a precise method for estimating food and nutrient intake in children (174, 175). However, a validation of the three-day food record compared with a reference method has not been done in TEDDY. This is in contrast to the extended quality measures taken in other types of data collected (137). TEDDY includes dietary data from four countries and systematic bias related to differences between the countries could be present. Furthermore, the German cohort mainly used weighted food records as compared with estimated in the other countries. This could explain both their lower energy intake observed, as well as the higher frequency of missing food records. However, using only the Swedish cohort in papers II and III would reduce such systematic bias. In Paper I, gluten intake was additionally analyzed separately per country and the results remained, which increases the robustness of the findings.

A large amount of food records has been collected in TEDDY, allowing for exploring diverse exposures in relation to CDA and CD. The number of missing food records increased with age in TEDDY which can be expected because of higher burden of dietary measurement with this method (158). A higher compliance with completing food records in TEDDY has been associated with several factors, including being a single child, older maternal age, higher maternal education, and sharing of the study participation load between parents (176). A lower compliance in children was related to lower socioeconomic status. When examined, Swedish participants completed more of the expected food records. Consequently, using only Swedish dietary data in papers II and III would reduce systematic bias related to potential differences in dietary habits between participants completing the food records and those who did not.

All dietary data in TEDDY were entered by qualified personnel with nutrition background. Quality control was exercised in several steps to ensure the quality of the dietary data. Extreme values of nutrients and foods for every visit were checked against the original food record to correct for data entering errors. Agreement on standard

operating procedures when entering dietary data across several research centers has been shown to increase the quality of dietary data (177). The harmonization of food and gluten data in TEDDY is another strength. This allows for researching dietary exposures across several countries with variation in national food databases. However, the harmonization limited the use of the whole cohort in Paper II and III.

Despite rigorous quality control of collected dietary data in TEDDY, it does not include reporting errors made by the caregiver. Misreporting of dietary intake is common and complex (178). Over- and underreporting can result from changes in dietary intake during the measurement period, leaving out food items consumed, or memory recall bias. Estimating portions sizes, even when being provided guidance, can be difficult, as well as for staff to decode the food record to right type of food/drink and amount consumed. Hence, measuring dietary intake can be considered as measuring dietary behavior. Little is known about diet misreporting in infants and young children, and factors influencing it. Food records in children may underestimate up to 30% of energy intake compared with the method of doubly labeled water (179). However, when parents reported food records in children aged six months to two years, reported energy intake was more accurate (179).

The "Goldberg cut off" is a golden standard used in adults to estimate plausible dietary reporters (180, 181), but has been questioned in young children as it assumes energy balance (147). When using a method suggested for young children in TEDDY, about 75% of children at 12 and 24 months of age were determined to be plausible reporters, e.g., having an energy intake within 80-120 % and 75-125% of their EER at each respective age (147). This was in line with the number of plausible energy reporters in the National Health and Nutrition Examination Survey 2003-2012 in US healthy children (182). TEDDY allows for food records to be completed while the child was ill, as is common in young children. Consequently, the energy intake may truly be below the cut off during acute infections, while it may be above the cut off once the child again recovers from the infection. On a population level, the food records would capture the natural changes in energy intake over time in young children. Furthermore, the measurement period of food records collected at the annual visits in TEDDY were around the child's birthday. This could drive the dietary data to be influenced by temporary changes in dietary intake, not fully reflecting their habitual intake. In summary, it cannot be ruled out that implausible dietary reporters could have impacted the results of this thesis.

A method to overcome some of the difficulties related to measurement errors of diet is to use biomarkers for nutrient or food intakes. Alkylrecorcinols, a phenolic lipid present in whole-grain rye and wheat, have been proposed as a biomarker for gluten intake and may be feasible in future research as an objective measure (183-185).

Statistical methods

Increasing missing data with age was an important reason for including dietary data only up to age 24 months in papers II-IV. In Paper I, the joint model was the primary method chosen for survival analysis. It has advantages compared with Cox regression when dealing with missing data and data collected at different time points (149). Joint modelling accounts for intra-individual variation of time-dependent variables and imputes missing values so that all subjects remain in the analysis. However, as compared with the Cox regression, the joint model uses imputed intake to estimate associations with the outcome.

The nature of the dietary data in Paper II, as well as in Paper IV, with zero-inflated distributions and differences over time, made it unsuitable to model dietary intake as time-dependent variables in outcome analyses. Instead, Cox regressions were performed for each investigated age, hence introducing the risk of type 1 error. However, the same pre-defined hypotheses were tested across all investigated timepoints, and the papers were not aiming to confirm previous findings. In such settings, adjustment for multiple comparisons can result in higher risk for rejecting true associations (151). Instead, another approach is to examine risk estimates with their CIs, search for coherency and biologically plausible associations when interpreting the results.

Dietary intake is often assessed cross-sectionally in epidemiological research and accordingly most methods to derive dietary patterns are cross-sectional, such as PCA in Paper IV. It is a data-driven method with some subjective decisions to be made by the researcher, and it extracts dietary patterns that are present in the studied population but not necessarily the patterns most related to a specified disease. Instead, other methods include response variables related to the disease of interest, and find dietary patterns related to the response (186, 187). There may be other, more uncommon dietary patterns, found in subgroups with similar traits, associated with CDA and CD that were not identified in Paper IV.

Energy intake can confound the relation between diet and disease. Dietary intake is dependent on the energy intake, which in turn is related to the individual's energy requirement based on age, sex, weight, and physical activity (146). As an example, a one-year-old girl, weighing 8.1 kg (20^{th} percentile according to WHO growth charts) would have an EER of about 650 kcal (80 kcal/kg), as compared with one-year old boy, weighing 10.6 kg (80^{th} percentile according to WHO growth charts) with an EER of 860 kcal (81 kcal/kg) – or 30% more than the girl. Thus, both sex and weight clearly determine differences in energy requirements. In CD, factors related to energy intake (such as weight, BMI) have not been extensively researched. In these circumstances,

STROBE-NUT guidelines recommend including energy intake as a confounder when investigating diet in relation to disease (145).

Energy-adjusted gluten intake confirmed the findings in Paper I as well as in Paper II. However, in Paper IV, performing PCA on food group intakes in absolute amounts or energy adjusted had an impact on the components extracted and survival analyses were thus only performed on the energy-adjusted dietary patterns. This was in contrast to a study on healthy children finding no effect of energy adjustment of food variables in PCA (188). It should thus be prudent to verify the effect of energy adjustment in dietary pattern analysis.

Conclusions

The conclusions in the present thesis are:

- Higher gluten intake during the first five years of life is associated with increased risk of CDA and CD among genetically predisposed children (Paper I).
- A high daily intake of bread for the age and milk cereal drink during the second year in life is associated with increased risk of CDA and CD in children at genetic risk (Paper II).
- Intake of milk powder in early childhood is not associated with CD in genetically susceptible children (Paper III).
- Exposure to different dietary patterns during the first two years of life confer different risk of CDA and CD in children at genetic risk (Paper IV).

Future directions

Summarizing the research on dietary exposures after the "Swedish epidemic" with the findings of this thesis, the investigation of dietary factors should not be restricted to infancy. Diet may play a role in the development of childhood CD after gluten introduction and weaning. Although this thesis focused on the first years of life, research on dietary factors later in life is warranted to extend the knowledge on the role of diet. Clinical trials are needed to draw conclusions on causality.

Studies on dietary intake in children after the first years of life are missing. In TEDDY, dietary data on children >age five years will be available for investigating the role of gluten amounts also in older children. Furthermore, the association of gluten intake with CD needs to be confirmed in RCTs.

The Preventing Celiac Disease in Skane (PreCiSe) study is an RCT which enrolls infants homozygous for HLA-DQ2, randomly selected to one of three arms of intervention to the age of three years of either receiving probiotics (two strains of *Lactobacillus*), placebo or strict gluten-free diet. Children are followed to age seven years (Clinical Trials Identifier NCT03562221).

The Gluten Reduction after Infancy (GRaIn) study is an intervention study enrolling infants heterozygous for HLA-DQ2, randomized to either an intervention group on a gluten reduced diet up to age five years, or a control group with regular diet. Children are followed to age 10 years (Clinical Trials Identifier NCT04593888).

In future research on the role of childhood diet in CD, quality of dietary data should be ensured. Using a valid dietary assessment method that captures day-to-day variation and allows for studying diet on several levels, combined with biomarkers are warranted.

Closing remarks

Dietary intervention with gluten intake is currently the only way to modify the risk of CD. Complete avoidance of gluten would theoretically prevent CD however it would not be a feasible strategy for the general population. Caution should be made in restricting intake of gluten-containing foods as they provide energy, nutrients and wholegrains for infants and young children. Lower intake of these foods comes with a high demand on replacing it with nutritionally adequate alternatives. Moreover, only some individuals at genetic risk actually develop CD.

Both international and national organs have dietary guidelines aiming to decrease the incidence of CD in the population. Accordingly, societal impact has been made in the revised Swedish national dietary guidelines for healthy infants in 2020.

It is important that dietary guidelines are evidence based as they can impact the dietary behavior of a population. Especially parents of infants are vigilant to make sure to follow guidance given about their child. Gluten-rich products should not be perceived as unhealthy foods prone to lead to lifelong disease, but instead carefully balanced recommendations should be given to targeted groups in the population.

The focus of the present thesis was to extend previous research on associations of dietary factors with the risk of CD in early childhood. Although preceding assumptions that high amounts of gluten intake seem to increase the risk was found, new findings on gluten-containing foods that attenuate the effect of gluten in young children, and combinations of foods with the potential to reduce the risk despite intake of gluten, were discovered.

This thesis shows that several dietary factors during first years of life may modify the risk of CD in children at genetic risk. The role of diet on disease risk is suggested to be more influential after infancy and the weaning period but needs to be validated in other prospective studies. If confirmed, the findings of this thesis may be used in future dietary guidelines for children.

Acknowledgements

This thesis would not even have been an imaginary object of my mind if not for my supervisor Daniel. You opened a door that I had merely reflected on, but not stopped to consider in the hectic working-full-time-in-clinic and parenting-kids-life. A project with room to be curious and creative in, but with firm and steady supervision to stay on track. Throughout the bumpy PhD-road, you were the engine that kept everything running. With dedication and determinedness, you have supervised me on this emotional rollercoaster. Still, you managed to somehow get this stubborn mule (apparently me, but may it also be applied to whoever said it?) to the end of the road. I am grateful for the opportunities you have paved the paths for, and for all the encouragement you have given. Thank you!

And to my best co-pilot supervisor Carin. You always found the time to discuss the gnarly details in nutrition methodology and dietary data in TEDDY. You make up an excellent counterpart with your relaxed and reflective manner. Thank you for being grounded and for cooling off the stress of it all.

To Åsa and Elin who welcomed me into their world of microbiology at the LTH lab. Thank you Åsa for rooting for us and igniting us with sparks of energy, and to Elin for being an excellent teacher. Although our project did not end up in this thesis, I am thankful for the experience and all the things I learned working with you!

Thanks to my ever-supporting "physio"-bosses Charlotte (thanks for employing me, twice!) and Maria, who always cheered me on, were proud of me and made sure that research could be the number one priority. You both engage in the motto that the main task for a manager is to make sure that the employee can use her full potential and grow with work. I have never been in a workplace with better managers. Without you, this PhD would have been ongoing for decades, if ever finished.

To all my fellow pediatric dietitian colleagues, for your support, understanding and caring for patients when I was not around, thank you. To Lotta and Helena – you are competent, experienced, sharp-minded, and dedicated. I love working with you, and I look forward to be back in the clinic with you again!

Thanks to all the children and families participating in the demanding 15-year follow-up in TEDDY. All those food records and recalls that needed to be entered in the food database by us, who admired the effort that you put in to ensure that you recorded every single detail. You made our day by recording that a child ate half a lip balm - that's the level of detail we're after, hard-working parents!

Thanks to the Swedish TEDDY site. For doing such an impressive job with taking care of the participating families in TEDDY and making this massive project possible. For all the bubbles blown at blood draws and engaging with the children and their parents.

Thanks to all collaborators in TEDDY, both in the celiac as well as the diet committee. So much knowledge and experience present. Especially thanks to Ulla for being engaged in the food grouping process and the littles nudges to help me get things just right.

Thanks to my family and friends for rooting and cheering for me, who believed that I was capable despite only having a slight hint of what I was doing. Thanks to Cornelia for putting in all those hours into making the coolest ever thesis cover! Thank you, Geir-Arne, for writing the code that saved me weeks of work! To my parents who supported me in all ways possible, thank you. Perseverance, creativity, and stubbornness must be characteristics that came from you.

Embarrassingly large amounts of chocolate and big cups of green tea made this thesis possible. I thank you for giving me the energy and strength to continue working, especially through some tremendously hard parts where it all felt like a hopelessly slippery mountain to climb.

Last but not least, Jan-Fredrik, thanks for being the primary parent during this last, intensive period, and for putting up with my process. You carried the load and was the dumpster in which I could throw the garbage when needed. Thank you for explaining the math behind it all, although my frustration sometimes made my mind as susceptible as a non-stick-pan. I love you, forever. And thanks to those two wildlings, for the overflow of hugs and kisses and the energy you give (although, you are never as cute as when you are asleep).

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Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

Early life dietary factors on the risk of celiac disease

This PhD thesis studies associations of early life dietary factors and risk of celiac disease autoimmunity and celiac disease in children at genetic risk. Specifically, exposures to gluten amounts, dietary sources of gluten, milk powder as well as dietary patterns were investigated and explored. The thesis included data from The Environmental Determinants of Diabetes in the Young (TEDDY), a multinational birth cohort following children from the US, Sweden, Finland, and Germany.

Foto av Tove Gilvad
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LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:29
ISBN 978-91-8021-190-1
ISSN 1652-8220