Infant Feeding Practices and the Risk of Celiac Disease

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Infant Feeding Practices and the Risk of Celiac Disease

Carin Andrén Aronsson

DOCTORAL DISSERTATION

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Faculty opponent
Luisa Mearin (MD, PhD)
Leiden University Medical Center, the Netherlands
Abstract

Aims: Celiac disease is an autoimmune disorder that occurs in genetically predisposed individuals, and it has been suggested that dietary gluten plays a role in the underlying pathogenesis. The overall objective of the present research was to determine whether infant feeding practices are associated with celiac disease in genetically susceptible children. Specific aims were as follows: to study the effects of HLA genotype on the risk of celiac disease; to describe the characteristics of infant feeding practices in a multinational birth cohort; to ascertain whether timing of gluten introduction and the level of gluten intake during the first 2 years of life are associated with risk for celiac disease.

Methods: Children enrolled in the prospective multinational birth cohort entitled The Environmental Determinants of Diabetes in the Young (TEDDY), were followed at quarterly clinic visits from the age of 3 months. Children screened for high-risk HLA-genotypes (DR-DQ2 or DR4-DQ8) associated with type 1 diabetes and celiac disease were followed up in the United States, Finland, Germany and Sweden. Information about infant feeding practices was collected at each clinic visit. Children were screened annually for tissue transglutaminase autoantibodies (tTGA), using radiobinding assays (RBAs). Primary endpoint was the development of celiac disease autoimmunity (CDA), defined as presence of ITGA in two consecutive tests conducted at least 3 months apart. Secondary endpoint was celiac disease, which was diagnosed by intestinal biopsy (Marsh score >1) or as persistently high levels of ITGA (≥ 100 units).

Results: Carrying any of the HLA-DQ2 genotypes and especially homozygosity for HLA-DQ2 were found to be associated with a six-fold increased risk of celiac disease. After adjusting for HLA-genotype, female gender, and first-degree relative (mother, father or sibling) with celiac disease, the children investigated in Sweden were still at higher risk of both CDA and celiac disease compared to children in other study countries, indicating that environmental factors may be important determinants of celiac disease (Paper I). Significant between-country differences were seen in breastfeeding duration and timing of first introduction to complementary foods. Young maternal age, low education level and smoking during pregnancy were associated with non-adherence to infant feeding recommendations (Paper II). Although the children in Sweden were introduced to gluten earlier than those in other countries, the timing of gluten introduction was not associated with increased risk for CDA and celiac disease (Paper III). Also, in Sweden, the amount of gluten consumed by children up to the age of the age of 2 years of age was associated with at least a twofold increase in the risk of celiac disease, and those that developed celiac disease were reported to have higher intake of gluten at all ages (beginning at 12 months) compared to their matched controls (Paper IV).

Conclusions: HLA was the strongest risk factor for CDA and celiac disease and children homozygous for HLA-DQ2 were at six-fold increased risk of celiac disease compared to those with other risk genotypes. There were between-country differences in duration of breastfeeding and first introduction to complementary foods. Neither duration of breastfeeding nor time to first introduction of gluten was associated with increased risk for celiac disease. A high intake of gluten before 2 years of age increased the risk of celiac disease, which suggests that the amount of gluten consumed can be a trigger for development of celiac disease in genetically at-risk children.

Key words: Celiac disease, children, HLA, DQ2, DQ8, gluten, infant feeding, TEDDY

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Carin Andrén Aronsson

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"If the patient can be cured at all, it must be by the means of diet."
Samuel Gee (1888)
# Table of Contents

**Dedication** 7  
**Abbreviations** 12  
**List of publications** 13  
**Abstract** 15  
**Background** 17  
- Brief historical overview of celiac disease 17  
- Definition of celiac disease 18  
- Clinical presentation 19  
- The occurrence of celiac disease 20  
- Pathogenesis 20  
- Genetics 21  
- Environmental and lifestyle factors 24  
- Infant feeding recommendations 25  
- Infant feeding recommendations for gluten 26  
- Breast milk 27  
- Breastfeeding 27  
- Gluten 28  
- Sources of gluten in the infant diet 29  
- Gluten and risk of celiac disease 30  
**Aims** 33  
**Subjects and methods** 35  
- Overall study design 35  
- Study population 37  
- Dietary assessment 41  
- Tissue transglutaminase autoantibody (tTGA) immunoassays 45  
- Definition of celiac disease autoimmunity (CDA) and celiac disease in TEDDY 45  
- Statistical methods 46  
- Ethical approval 47  
**Results** 49  
- Risk of pediatric celiac disease according to HLA haplotype and country (Paper I) 49  
- Age at first introduction to complementary foods is associated with sociodemographic factors in children with increased risk of developing type 1 diabetes (Paper II) 50  
- Age at gluten introduction and risk of celiac disease (Paper III) 53
Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish birth cohort (Paper IV)

Discussion

Conclusions

Populärvetenskaplig sammanfattning
- Vad är celiaki?
- Hur gick forskningen till?
- Vilka är resultaten och slutsatserna?

Acknowledgements

References
Abbreviations

CDA  celiac disease autoimmunity
CI   confidence interval
ESPGHAN European Society for Pediatric Gastroenterology, Hepatology and Nutrition
FDR  first degree relative (mother, father or sibling)
FFQ  food frequency questionnaire
GWAS genome-wide association study
HLA  human leukocyte antigen
HR   hazard ratio
IA   islet autoimmunity
IEL  intraepithelial lymphocytes
MHC  major histocompatibility complex
NIH  National Institutes of Health
OR   odds ratio
RCT  randomized controlled trial
SNP  single-nucleotide polymorphism
TEDDY The Environmental Determinants of Diabetes in The Young
rTG  tissue transglutaminase
rTGA tissue transglutaminase autoantibody
T1D  type 1 diabetes
WHO World Health Organization
List of publications

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


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Abstract

**Aims:** Celiac disease is an autoimmune disorder that occurs in genetically predisposed individuals, and it has been suggested that dietary gluten plays a role in the underlying pathogenesis. The overall objective of the present research was to determine whether infant feeding practices are associated with celiac disease in genetically susceptible children. Specific aims were as follows: to study the effects of HLA genotype on the risk of celiac disease; to describe the characteristics of infant feeding practices in a multinational birth cohort; to ascertain whether timing of gluten introduction and the level of gluten intake during the first 2 years of life are associated with risk for celiac disease.

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**Results:** Carrying any of the HLA-DQ2 genotypes and especially homozygosity for HLA-DQ2 were found to be associated with a six-fold increased risk of celiac disease. After adjusting for HLA-genotype, female gender, and first-degree relative (mother, father or sibling) with celiac disease, the children investigated in Sweden were still at higher risk of both CDA and celiac disease compared to children in other study countries, indicating that environmental factors may be important determinants of celiac disease (Paper I). Significant between-country differences were seen in breastfeeding duration and timing of first introduction to complementary foods. Young maternal age, low education level and smoking during pregnancy were associated with non-adherence to infant feeding recommendations (Paper II). Although the children in Sweden were introduced to gluten earlier than those in other countries, the timing of gluten introduction was not
associated with increased risk for CDA and celiac disease (Paper III). Also, in Sweden, the amount of gluten consumed by children up to the age of the age of 2 years of age was associated with at least a twofold increase in the risk of celiac disease, and those that developed celiac disease were reported to have higher intake of gluten at all ages (beginning at 12 months) compared to their matched controls (Paper IV).

**Conclusions:** HLA was the strongest risk factor for CDA and celiac disease and children homozygous for HLA-DQ2 were at six-fold increased risk of celiac disease compared to those with other risk genotypes. There were between-country differences in duration of breastfeeding and first introduction to complementary foods. Neither duration of breastfeeding nor time to first introduction of gluten was associated with increased risk for celiac disease. A high intake of gluten before 2 years of age increased the risk of celiac disease, which suggests that the amount of gluten consumed can be a trigger for development of celiac disease in genetically at-risk children.
Background

Brief historical overview of celiac disease

The condition that we today call gluten intolerance, or celiac disease, was first described by the Greek physician Aretaeus of Cappadocia in the 1st century AD. However, it was not until the late 1800s, that Samuel Gee discovered the connection between dietary factors and malabsorption, and he called it “coeliac affection” based on the conclusion that the colon was the affected organ. Gee described the symptoms of the condition with precision and mentioned that “if the patient can be cured at all it must be by the means of diet” (1). Years later, Dr. Christian Archibald Herter noted that children suffering of retarded growth, at that time called “intestinal infantilism”, tolerated diets with a high fat content more readily than diets rich in carbohydrates. During the 1920s, several researchers focused on different types of low carbohydrate diets in patients to address the inability to digest such compounds. In 1924, the American pediatrician Sidney Haas was successful in using a banana diet to treat eight children diagnosed with celiac disease, which at that time was called Gee-Herter syndrome (2, 3). In short, this diet was limited to intake of milk, pot cheese, bananas (4-8 per day), oranges, vegetables, gelatin, and meat. Notably, even though the banana diet contained carbohydrates, all patients recovered from their symptoms.

The association between wheat and malabsorption was first demonstrated by the Dutch pediatrician Willem-Karel Dicke in the 1940s. When Dicke excluded wheat and rye from the diet of some of his pediatric patients suffering from severe malabsorption syndromes, the symptoms and growth retardation in those individuals clearly improved (4). In the 1950s, Dicke performed experiments together with the Dutch biochemist Kamer, to further investigate on fractions of wheat to identify which fraction of the wheat that were harmful. Experiments were performed on gluten, gluten wash water (water soluble proteins), gliadin and glutenenin (soluble in alcohol and a solution based on 10% sodium chloride), ash, crude fiber, and fat, and the findings led to the conclusion that both gliadin and gluten were harmful but wheat starch was harmless for pediatric patients with celiac disease (5).
Charlotte M Anderson in Great Britain, extended the abovementioned investigations by studying the effect of wheat-containing diets on the intestinal mucosa (6). At the end of the 1950s, she was the first scientist to use intestinal biopsies to demonstrate that gluten induced abnormal histological changes of the duodenal mucosa of children. When Anderson put her pediatric patients (all younger than 10 years of age) on a wheat-free diet, the detrimental changes in the intestinal histology were partially or completely normalized within a year after treatment (7).

Tissue transglutaminase (tTG), the ubiquitously expressed intracellular enzyme, has been described as the “key player” in celiac disease (8). It was not until the late 1980s that the association between tTG and celiac disease was demonstrated by Bruce et al. (9), who found that tTG activity was increased in celiac patients compared to healthy controls, and that gluten (i.e. gliadin) was the ideal substrate for tTG. A decade later, Dieterich and colleagues (10) identified tTG as the predominant autoantigen for celiac disease and autoantibodies to the enzyme has subsequently been used as a diagnostic tool and to facilitate screening for celiac disease (11-14).

**Definition of celiac disease**

The European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has been responsible for defining the diagnostic criteria of celiac disease since 1970 (15). Over the years, the criteria have been revised due to improvement of diagnostic tools and discovery of new serological markers for the disorder (16). Celiac disease is defined as an autoimmune-mediated enteropathy that is triggered by ingestion of gluten in genetically predisposed individuals characterized by the presence of several variables (17). Yet not fully endorsed by official guidelines, it has been proposed that a patient could be diagnosed with celiac disease by using the “four out of five” rule (18):

- Typical symptoms associated with celiac disease
- Carrying any of the HLA-DQ2 and/or HLA-DQ8 haplotypes
- A positive serological test for tissue transglutaminase autoantibodies (tTGA), endomysial autoantibodies and/or antigliadin antibodies
- Duodenal biopsy showing typical features of celiac disease
- Improvement of symptoms on a gluten-free diet
Clinical presentation

The clinical presentation of celiac disease is diverse (Figure 1). Typical symptoms are abdominal discomfort, diarrhea, constipation, impairment of growth, and failure to thrive (16). These symptoms are often more pronounced in younger children whereas older children and adolescents can present with extra-intestinal manifestations such as anemia, delayed puberty and fatigue (19).

![Figure 1. "The celiac iceberg model" accoring to Lionett et al, published in International Reviews of Immunology 2011 (20)](image)

However, celiac disease can also be present with clinically asymptomatic variants despite a damaged mucosa (17). Indeed, recent screenings have shown that the majority of patients are diagnosed based on active case findings rather than based on the clinical presentation (21). Screenings of at-risk groups such as first-degree relatives (FDRs) with celiac disease and patients with type 1 diabetes (T1D), autoimmune thyroid disease, or Down and Turner syndromes have demonstrated that many cases of celiac disease are virtually clinically silent at diagnosis (16, 22). In addition, many genetically at-risk individuals can have positive serology but a normal mucosa as an early sign of an on-going autoimmunity and such individuals have been defined as having potential celiac disease and being at risk of developing celiac disease later in life (17).
The occurrence of celiac disease

Based on the criteria of celiac disease according to the ESPGHAN (17), the mean prevalence of this condition in the general population has been estimated to be 1% among Western countries (23, 24). However, there are regional variations and the highest prevalence reaching a level of 5.6% has been found in the Saharawi population in Western Sahara (25). In Europe, prevalence has been estimated to be 1-3% with the highest proportion in Sweden (3%) followed by Finland (2%) (24, 26). In the United States, the prevalence has almost doubled during the last 30 years and is now estimated to be about 1% (20, 27). Still, the true prevalence is difficult to establish, because the majority of cases are detected only in screenings; for each patient diagnosed with celiac disease, an average of 5-10 cases remain undiagnosed (25). Although the prevalence of the disease differs in many countries, the reported incidence seems to be increasing, both in Sweden and worldwide (23, 28). Plausible explanations for this rise include a true upturn in prevalence in combination with an increased awareness of celiac disease and greater availability of highly sensitive and specific serological tests, although the findings of recent longitudinal studies suggest that the prevalence can fluctuate over time (23, 29).

Pathogenesis

Mechanisms leading to disease

Although the discoveries of gluten, autoantigens, and the phenotype are well described, the pathogenesis of damage to the villi in the small intestine caused by chronic inflammatory reaction induced by gluten peptides is still mainly unresolved (Figure 2). In a simplified model, gluten peptides cross the intestinal mucosal barrier to reach the lamina propria via either increased permeability of epithelial tight junctions, or epithelial transcytosis and subsequent events leading to tissue damage (20).

Tissue transglutaminase (tTG) is a deamidating enzyme that can enhance the immunostimulatory effect of gluten and it also serves as a target autoantigen in the immune response (30). This enzyme is naturally involved in formation and repair of the extracellular matrix, and its expression is induced by tissue destruction and inflammation (30). Gliadin serves as an ideal substrate for tTG, and the enzyme can modify gluten peptides through deamidation (conversion of non-charged glutamine to negatively charged glutamic acid). In vitro studies have shown that deamidated gluten peptides enhance the ability to bind to HLA-DQ2 or HLA-DQ8 on antigen presenting cells and thereby further induce an enhanced CD4+ T-cell response, and in this context the HLA-DQ2 heterodimers have the capacity to bind a larger number of gluten peptides compared to other HLA-DQ genotypes (31). In turn, activated
CD4+ T-cells produce high levels of pro-inflammatory Th1 cytokines, predominantly interferon-γ, which activates macrophages, cytotoxic T-cells and natural killer cells and hence leads to mucosal damage with an accompanying increase in intraepithelial lymphocytes (IELs) and villous atrophy (19). CD4+ T-cells also drive the activation and expansion of celiac disease specific B cells, through maturation into plasma cells which produce autoantibodies to tTG (tTGA) (32, 33). Also, tTGA may bind to tTG and hence have an impact on the activity of the enzyme (34). The role of the celiac disease-specific autoantibodies in the pathogenesis of the disease is still unclear but it is suggested that tTGA are involved in the development of epithelial alterations and increased intestinal permeability contributing to mucosal damage (20, 35) Also, levels of serum tTGA correlates with mucosa damage and when gluten is excluded from the diet, the tTGA most often disappears (36).

Figure 2. Schematic diagram of the pathogenesis of celiac disease, from Kagnoff in J Clin Invest (37) (© 2007 American Society for Clinical Investigation)

Genetics

Celiac disease is highly heritable. The concordance is 80% between monozygotic twins and approximately 15-20% between dizygotic twins (24, 38). Dizygotic twins have the similar risk as a sibling and having a first-degree relative (mother, father
or sibling) with celiac disease has been shown to increase the risk of the disorder by approximately 10-20% (39). Moreover, females are at two-fold higher risk of celiac disease than males and it has been speculated that females may be more vulnerable to environmental exposures that influence the immunological processes (40-42).

Of all the known genes, human leukocyte antigen (HLA) represents the greatest genetic risk factor for celiac disease. The genetic contribution to the development of this disorder is well known, and the strongest association is with the genotype HLA-DQ2 (43, 44). HLA genotypes, specifically DQ2 and DQ8, constitute the dominant genetic factor linked to celiac disease, and they are located on the short arm on chromosome 6p21 that encodes for the major histocompatibility complex (MHC), a gene cluster that contains hundreds of genes involved in the immune system (24). The MHC is further divided into classes I, II, and III, and HLA belongs to MHC class II which encodes the MHC class II receptor found on all antigen presenting cells. HLA class II, constituted of an alpha (α) and a beta (β) chain, are specified by genes in the HLA-D region that comprehends DP (DPA1 and DPB1), DQ (DQA1 and DQB1) or DR (DRB1 and DRA) genes.

HLA molecules bind gluten peptides and present them to T lymphocytes. HLA class I molecules are specifically recognized by CD8+ T cells, and those cells activate a cytotoxic response. HLA class II heterodimers are recognized by the T cell receptor on CD4+ T cells that, in turn, trigger an immune response (45).

Approximately 90-95% of patients with celiac disease carry HLA alleles DQA1*05:01 and DQB1*02:01 that are in linkage equilibrium (called the DQ2.5 haplotype), DQA1*02:01 and DQB1*02:01 (called the DQ2.2 haplotype), and the remainder bear DQA1*03:01 and DQB1*03:02 alleles (called the DQ8 haplotype) (45). Patients with this disorder rarely carry any of the other mentioned haplotypes. The risk of celiac disease differs between the two genotypes (46) (Figure 3). HLA-DQ2.5 shows the strongest association with the disease, and individuals homozygous for HLA-DQ2.5 have a five-fold increased risk of developing the disorder compared to individuals heterozygous for HLA-DQ2.5 (47, 48). HLA-DQ8 is associated with a moderate risk (24).

However, it has been estimated that 25-40% of the Caucasian population are carriers of at least one of the HLA-DQ2 or HLA-DQ8 haplotypes (23, 43).
In recent years, genome-wide association studies (GWAS) have identified non-HLA genes linked to increased risk of celiac disease, which serve as a complement to HLA. The non-HLA genes, approximately 40 loci, are coding for cytokines, chemokines and their receptors, cell adhesion molecules, and T and B cell activators (49). Together, these non-HLA loci include 115 different genes (50), and a majority of the identified loci harbor immune-related genes. To date, 57 independently associated non-HLA single-nucleotide polymorphisms (SNPs) have been determined to be associated with celiac disease. Approximately 54% of the genetics of this disorder can now be explained by HLA together with non-HLA SNPs, and 40% can be explained by HLA alone (19, 46, 51). Thus these proportions suggest
that celiac disease is a polygenetic multifactorial disorder that can be triggered by environmental factors in genetically susceptible individuals.

**Environmental and lifestyle factors**

The onset of celiac disease can occur at almost any age throughout life, which suggests that various environmental and lifestyle factors are probably involved in the pathogenesis (Figure 4).

<table>
<thead>
<tr>
<th>Structural factors</th>
<th>Dietary recommendations</th>
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<tbody>
<tr>
<td>Associated factors</td>
<td>Socioeconomic factors, season of birth</td>
</tr>
<tr>
<td>Component factors</td>
<td>Fetal life Nutrition Infections</td>
</tr>
<tr>
<td>Required factors</td>
<td>Gluten (gliadin, glutenin), genetic susceptibility (HLA-DQ2 and/or HLA-DQ8)</td>
</tr>
</tbody>
</table>

**Figure 4.** A disease with a multifactorial ethiology. The figure is modified from Ivarsson et al (52).

Maternal diet during pregnancy has been associated with the risk of autoimmune disorders in the offspring although one study revealed no connection between maternal gluten consumption and the risk of celiac disease in the offspring (53). It is also possible that a cesarean section, particularly if the procedure is elective, can increase the risk of celiac disease by modifying the infant’s microflora (54, 55). The composition of the gut microbiota is influenced not only by type of delivery, but also by aspects such as breastfeeding (whether ever done or not), timing of introduction of solid foods, and use of antibiotics (56, 57). Furthermore, it has been shown that the microbiota in individuals with celiac disease and gastrointestinal symptoms differs from the microbiota in healthy controls (58). Perhaps changes in microbiota can have a driving effect on the rise of celiac disease and other autoimmune conditions. Differences in microbiota composition between celiac patients and healthy controls have been observed, but it remains unclear whether the disparities are a cause or a result of the intestinal inflammation (59). In addition,
studies have shown that infections, particularly recurrent episodes of infections in early childhood are associated with increased risk of celiac disease (60-63). Also, Myléus et al (60) found that an interaction between discontinued breastfeeding, high gluten intake, and recurrent infectious episodes in early life resulted in an increased risk of celiac disease.

Season of birth is another factor that appears to be associated with celiac disease, possibly due to the impact of seasons with recurrent episodes of infections during early childhood (64-67). There is also evidence of a connection between socioeconomic status and celiac disease, although there are no consequent patterns in this potential relationship, considering that both low and high status have been linked with the disorder in different populations (53, 68-71). The causes of the observed disparities are still a matter of speculation.

Theoretically, breastfeeding and the mode of gluten consumption (when and how) during infancy and childhood might influence the age at onset of celiac disease (72). Time to first introduction to gluten has long been considered a risk factor for this condition (52). In Sweden, the infant feeding recommendations were changed in the mid-1980s to support delaying introduction of gluten from age 4 to 6 months in an attempt to impede the development of celiac disease, but this instead led to an increase in the incidence of reported cases among children under 2 years of age (73). Unfortunately, the new recommendations overlapped with a change in the contents of commercial produced porridges and follow-on formulas. In order to reduce the protein content in products, milk powder was replaced with wheat flour which resulted in an increase in gluten amount. However, when the recommendation was subsequently changed back to 4 months, the incidence decreased, an indication that both time and amount may impact the risk of the disorder. In the United States, Norris et al. (74) prospectively followed infants that were genetically at-risk and found that being introduced to gluten very early (age < 3 months) and later (age > 6 months) increased the risk of celiac disease.

**Infant feeding recommendations**

The World Health Organization (WHO) states that optimal nutrition during the first 2 years of life is crucial to improve child survival and lower morbidity and mortality, and to reduce the risk of chronic diseases, and it also fosters better development overall (75). Since 2002, the WHO recommendations for infant feeding indicate that breastfeeding should be initiated within the first hour of birth and should be exclusive for 6 months, and continued up to 2 years of age; complementary feeding should be started around the age of 6 months when the infant’s energy and nutrient needs start to exceed what is provided in breast milk. The ESPGHAN recommends exclusive breastfeeding for 6 months and that complementary feeding should not be
started before 17 weeks (< 4 months) or after 26 weeks (> 6 months) (76). Most of the European countries have adopted the WHO recommendations on a national level although some countries also follow the ESPGHAN recommendations.

In the United States, the American Academy of Pediatrics (AAP) recommends exclusive breastfeeding for 6 months, followed by introduction of complementary foods with continued breastfeeding for up to 1 year or longer (77, 78). Single grain cereals, such as rice cereal or oatmeal, are usually introduced first to American infants, and many pediatricians recommend starting with vegetables before fruits. In Europe, the recommendations are fairly similar in many countries. The National Food Administration (NFA) in Sweden states that breast milk or infant formula is the food of choice for infants during the first 6 months, and from 4 months of age, at the earliest, it is acceptable to start with tasting portions of solid foods, but the recommendations also emphasize that is important that full breastfeeding or feeding with infant formula can be continued until 6 months of age (79). The recommendations in Sweden do not indicate that any particular food is best when starting introduction, but they do mention that it is common to begin with purées based on potatoes, root vegetables, rice, or fruits. In Germany, exclusive breastfeeding is recommended for the first 4-6 months and that partial breastfeeding along with complementary feeding and solid foods should be introduced at age 5-7 months (80). The German recommendations also indicate that it is preferable to start with vegetables, potatoes, meat or fish purées, followed cereal meals made with cow’s milk or milk-free cereal-and fruit meals. In Finland, the national recommendations are provided by the National Institute of Health and Welfare (THL), and assert the following: infants should be exclusively breastfed for the first 4-6 months; tasting portions (defined as less than 2 tea spoons) of solid foods consisting of purées of potatoes, vegetables, fruits and berries can be given from the age of 4 months and the diet can be complemented with cereals, meat, fish and eggs by the age of 5 months (81).

### Infant feeding recommendations for gluten

In 2008, the ESPGHAN published a position paper on the health effects of complementary feeding (76). A specific section concerning introduction of gluten recommended that both early (age < 4 months) and late (age ≥ 7 months) to be avoided. This paper also indicated that the risks of celiac disease, type 1 diabetes and wheat allergy might be reduced by introducing gluten gradually while the infant is still being breastfed. Many European countries have adopted these recommendations in their national infant feeding programs. In Sweden, it is recommended that gluten can be introduced at 4-6 months of age (79), whereas in Finland and Germany it is suggested that small amounts of gluten should be introduced at age 5-7 months (80, 81). The only specific recommendation in the
Unites States is that wheat-based cereals should be avoided until the age of 8 months (78).

In 2016, the ESPGHAN working group for celiac disease issued revised infant feeding recommendations with a focus on introduction of gluten and risk of celiac disease (82). This guidance was based primarily on two independent randomized controlled trials (RCTs) and several prospective observational studies that had been published up to 2015. The recommendations state that gluten can be introduced into an infant’s diet at any time between 4 and 12 months of age. The ESPGHAN also points out that gluten should not be given to an infant in large quantities during the first weeks after it is introduced, although the optimal level of gluten intake is yet to be determined.

Breast milk

Human breast milk is a dynamic fluid that varies in composition between populations, and mothers, and depending on occurrence of pre-term birth and also over the lactation period (83). The first fluid produced by the mammary glands after delivery is colostrum; which is rich in immunological components (secretory IgA, lactoferrin, leukocytes, and growth factors). After the first few days postpartum, the mother begins to produce transitional milk which is very similar to colostrum but undergoing changes to support the nutritional and developmental needs of the rapidly growing infant. By 4-6 weeks postpartum, human milk is considered to be fully mature, and the composition remains relatively consistent throughout the lactation period. Mature human breast milk contains factors such as macronutrients (protein, fat, and lactose), vitamins, and minerals required to fulfill the nutritional needs of the infant at the specific time of lactation. Breast milk also has non-nutritive functions that are, for example related to its numerous immune-related components (cytokines, growth factors, and hormones) and content of live microorganisms (e.g. probiotics and prebiotics), as well as its anti-inflammatory properties (84). In addition, breast milk contains growth factors (i.e. epidermal growth factor [EGF]), which are found at the highest concentrations the early milk and decrease over the lactation period, and are involved in the maturation and healing of the intestinal mucosa (83).

Breastfeeding

There are many positive health effects associated with breastfeeding, such as prevention of infections and reduced risk of diseases later in life (85). There is also strong evidence that lack or short duration of breastfeeding is associated with higher risk of gastrointestinal and respiratory tract infections, otitis media, and sudden
infant death (86), whereas longer duration of breastfeeding has been associated with decreased risk of allergies and obesity later in life (86-88). Furthermore, it is possible that breast milk affects tolerance induction in infants through exposure to antigens (dietary antigens, self-antigens, maternal allogenic antigens), and inflammatory molecules. Breast milk also contributes to a more diverse microbiota and stimulates infant gut maturation, and this effect is apparent long after cessation of breastfeeding (89).

Despite all the benefits of breastfeeding, there is a declining trend in the rates of this practice in the Western world. WHO estimates that between 2006 and 2012, only 25% of the infants in Europe were exclusively breastfed for 6 months. The rate of exclusive breastfeeding at 6 months is 15% in Sweden, 19% in the United States, 22% in Germany and 44% in Finland (86, 90). One explanation for the observed decline is that the national recommendations in many countries approve complementary feeding from the age of 4 months. It should also be mentioned that discrepancies in paid maternity leave may have an impact on the possibility for mothers to breastfeed longer and it is known that determinants for the initiation and duration (continuation) of breastfeeding are associated with sociodemographic factors (91-94).

Gluten

Gluten-containing cereal grains such as wheat, rye, barley, and oats, together with other grains such as rice, maize (corn), millet, and durra belong to the family Gramineae (grass plants), and the seeds of these plants have been used in food production and as part of the human diet for about 10,000 years. Cultivation of wheat began to spread beyond the Fertile Crescent (in the current Middle East) in 8000 BC, and today, wheat is the third most widely cultivated cereal grain in the world after maize and rice, with an estimated production of over 700 million tons per year (95). Wheat, rye, and barley belong to the tribe Triticeae, whereas oats belong to the closely related tribe Avenae. Most of the wheat grown today is bread wheat (Triticum aestivum), and that species has been preferred because it has larger seeds.

The term “gluten” refers to a mixture of storage proteins that are present in wheat. The main storage proteins are gliadin (a prolamin protein) and glutenin (a glutelin protein). Depending on the cereal, the prolamins have different names, being called gliadin in wheat, secalins in rye, and hordeins in barley (96). Oats contain the protein avenin, which is genetically less similar to gliadin and the other prolamins. Of all the gluten proteins, gliadin is the most immunologically potent peptide and avenin the least harmful peptide with regard to activating celiac disease (97).
One of the characteristic properties of gluten is to provide elasticity, which is an essential feature in the preparation of dough. In addition, the role of gluten in the food processing industry has led to increased use of added gluten (vital gluten) to augment the elasticity and stability of food products (95, 98).

**Sources of gluten in the infant diet**

In the infant diet, the main sources of gluten are porridges, gruels, breads, pastries, pasta, cereals and granolas. In Sweden, the use of commercial infant porridges and gruel is very common. Gruel is best described as a semi-liquid follow-on formula based on flour mixed with water or cow’s milk. Historically, gruel has been widely used as food for both children and adults. In ancient times, infant formula was made from cereals grains such as wheat, which represented a staple food among all social classes. Maize gruel was previously an important part of the diet in Central America and today is typical in parts of southern Africa, whereas rice gruel is common in East and South-East Asia.

Since before the industrial age, gruel has been a common weaning food among farm families in Sweden. In those early days, gruel was made of cow’s or goat’s milk boiled with barley flour or oatmeal depending on the grain that was grown in the local area. The milk used was diluted with water and a little salt was often added, and the gruel was eaten with a wooden spoon.

In 1867, Henri Nestlé (in Switzerland) launched what he called “flour milk”, which was a mixture of condensed milk, wheat flour and sugar, and this product was used for infants that could not be breastfed (99). In 1899, a German confectioner named Joseph Hipp prepared a cereal-based infant formula when his wife had problems nursing their twins (100). In short, he mixed pumped breast milk with crushed rusk flour to make the first baby cereal. Hipp subsequently also used a mixture of cow’s milk, water, rusk flour, and honey, and this formula became popular when he sold it to family members and friends. As the demand grew, the family business evolved into an international enterprise.

In Sweden, industrial production of gruel was initiated in the mid-1900 and hence the use of homemade gruel virtually ceased. Feeding with a spoon was replaced with a baby bottle, which meant that babies could easily ingest large amounts of gruel and even hold the bottle themselves.

In 1933, the Swedish nutritionist Ninni Kronberg developed a method for production of spray-dried milk powder (101). There was too much milk in Sweden in the 1930s but it was difficult to sell the surplus to other countries due to difficulties keeping the milk from souring. In 1941, a product called “välling” (gruel) was mentioned for the first time in correspondence between the company
called Semper and the Swedish National Institute for Preventive Health Care (Statens Institut för folkhälsa). After World War II, there was no longer a shortage of milk powder and Semper collaborated with the milling industry to develop a new product in which milk powder and flour were mixed in dry form. By the beginning of the 1950s, the commercial gruel products were available in most stores in Sweden.

Gluten and risk of celiac disease

Based on previous assumptions that the risk of celiac disease is increased by both early (at < 4 months of age) and late (at > 6 months of age) introduction of gluten (74, 102-105), it has been suggested that there might be a “window of opportunity” during which gluten introduction can induce tolerance. To reduce the risk of celiac disease, today it is generally recommended that both early and late gluten introduction should be avoided and that introduction should instead be done gradually while the infant is still being breastfed.

Extensive efforts have been made to determine an optimal time point to begin feeding infants gluten. Two studies indicated elevated risk in infants with early or late introduction of gluten (74, 106), although this was not confirmed in other prospective birth cohorts (103, 107, 108). Also, two independent RCTs demonstrated that at-risk infants were not protected from celiac disease by gradually introducing very small amounts of gluten or by postponing the age at gluten introduction from 6 to 12 months (109, 110).

Thus far, attempts to find a threshold for daily gluten intake that can be recommended as safe or defined as hazardous in at-risk subjects at this point is yet to be determined. The study design and dietary assessment methods used in previous investigations have differed, which makes it difficult to interpret and compare results. One of the few studies that focused on effects of the amount of gluten intake was conducted on children living in the northern parts of Sweden (106). This retrospective case-control study used a food frequency questionnaire (FFQ) to collect information on gluten consumption using semi-quantitative information using portion sizes (3 levels). The results show that, among children diagnosed with celiac disease before the age of 2 years, larger amounts of gluten were consumed by cases than by controls at the age of 7 months; in this evaluation, > 58g of flour per day (corresponding to approximately 4.6g of gluten per day) was equivalent to a large amount. Another cross-sectional screening study in Sweden assessed two birth cohorts comprising children born in 1993 and 1997, respectively (111). The subjects in the 1993 cohort were born during a period when many young children in this country were diagnosed with celiac disease. Use of the same FFQ as in the study mentioned above (106), revealed differences in gluten consumption between the two
birth cohorts. In children < 2 years of age, the daily consumption of flour (in milk- and cereal based follow-on formulas) during weaning amounted to 38g in the 1993 cohort and 24g in the 1997 cohort, indicating that an observed lower prevalence of celiac disease in the latter cohort was associated with lower gluten consumption. In a European RCT (112), a 7-day food record was used to estimate gluten consumption in at-risk children from five countries at the age of 11 months and onwards after dose escalation was ended. The mean daily gluten intake increased progressively from the age of 12 months and onwards in all countries with the highest intake in the older age groups (25-36 months) but there were large differences between the countries, ranging from 2.8g in Spain to 9.2g in Hungary reported in the 12-months food records. Thus the question remains to be determined whether the amount of gluten consumption during early childhood has an impact on the development of celiac disease in genetically at-risk children.
Aims

The overall objective of the present research was to determine whether infant feeding practices are associated with celiac disease in genetically susceptible children.

The specific aims were as follow:

- To investigate the effects of HLA genotype on the risk of developing celiac disease by the age of 5 years (Paper I).
- To describe the characteristics of infant feeding practices and to identify the sociodemographic factors associated with the duration of breastfeeding and introduction of complementary feeding (Paper II).
- To ascertain whether the timing of gluten introduction is associated with the risk of celiac disease (Paper III).
- To examine whether the amount of gluten in the diet during the first 2 years of life is linked to the risk of celiac disease (Paper IV).
Subjects and methods

Overall study design

The research underlying this thesis used data from the project entitled The Environmental Determinants of Diabetes in the Young (TEDDY), which is a multinational birth cohort study being conducted at six clinical centers in Europe (in Finland, Germany, and Sweden) and at centers in three states in the United States (Washington, Colorado, and Georgia) and also includes a data coordinating center (DCC) located in Tampa, Florida (USA).

The primary objective of TEDDY is to identify environmental factors (i.e. infectious agents, diet, lifestyle, and stress) that are associated with increased risk of islet autoimmunity and type 1 diabetes (113, 114).

Children identified with HLA risk genotypes were enrolled in the follow-up study before the age of 4.5 months. Written informed consent for genetic screening and participation in the prospective follow-up was obtained separately from the parents or primary caregivers of all the participating children.

All children enrolled in TEDDY are followed with clinical visits every 3 months for the first 4 years of life and thereafter biannually until the age of 15 years or until diagnosed with T1D (114). At each visit, venous blood is drawn for processing into serum, plasma, erythrocytes, peripheral blood mononuclear cells (PBMCs), buffy coats, and mRNA. Blood samples are analyzed for autoantibodies against islet antigens, which are markers of T1D. Autoantibodies to glutamic acid decarboxylase (GADA), insulinoma-associated protein-2 (IA-2A), insulin (IAA) and Zinc Transporter 8 (ZnT8A) are measured as primary endpoint in TEDDY because the presence of at least one of the autoantibodies (defined as islet autoimmunity, IA) precedes diagnosis of T1D (115, 116). If venous blood is not available, capillary blood is drawn. Serum and plasma samples are stored and later analyzed for enterovirus, rotavirus, additional infectious agents, vitamin D, and fatty acids. Stool samples are collected monthly up to the age of 48 months and thereafter biannually. Weight and height are measured at each clinic visit. Furthermore, toenail clippings, urine samples, and salivary cortisol and physical activity measurements, as well as drinking water samples are collected regularly.
At each clinic visit, a study nurse conducts standardized interviews with the child’s parents or primary caregiver. The questionnaires cover information regarding the child’s medical history, infant feeding practices, infectious illnesses, immunizations, medications, consumption of dietary supplements, and life stress since the last visit. Demographic data are updated every second year.

**HLA-screening and typing**

Between September 2004 and February 2010, requests were made for newborn infants from families in the general population and from families with a FDR with T1D to participate in HLA screening before the infants reached the age of 4.5 months. Screening was generally done using a cord blood sample collected at birth, although potential participants could also be screened using a heel stick or capillary sample. The HLA genotypes were analyzed by polymerase chain reaction (PCR) at five of the six international centers included in TEDDY; the typing for both the German and Finnish centers was conducted in Finland, on either a dried blood spot punch or a small volume of whole blood.

HLA genotypes of interest in TEDDY are shown in **Table 1**. Infants from the general population were eligible for the study if they had one of the following four genotypes: DR3-DQ2/DR4-DQ8, DR4-DQ8/DR4-DQ8, DR4-DQ8/DR8-DQ4 and DR3-DQ2/-DR3-DQ2 (indicated in bold type in **Table 1**). Infants with a FDR (i.e. mother, father, or sibling) with T1D were also eligible for enrollment if they had one of the above-mentioned HLA genotypes or one of five additional genotypes: DR4/DR4b, DR4/DR1, DR4/DR13, DR4/DR9, and DR3/DR9.

**Table 1.** High-risk HLA genotypes followed in TEDDY

<table>
<thead>
<tr>
<th>HLA genotypes</th>
<th>Abbreviation</th>
<th>First-degree relative with type 1 diabetes</th>
<th>General Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR3-DQAI<em>05:01-DQ8I</em>02:01</td>
<td>DR3-DQ2/DR4-DQ8</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>B. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR3-DQAI<em>03:0X-DQ8I</em>03:02</td>
<td>DR4-DQ8/DR4-DQ8</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR8-DQAI<em>04:01-DQ8I</em>04:02</td>
<td>DR4-DQ8/DR8-DQ4</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>D. DR3-DQAI<em>05:01-DQ8I</em>02:01 / DR3-DQAI<em>05:01-DQ8I</em>02:01</td>
<td>DR3-DQ2/DR3-DQ2</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>E. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR4- DQAI<em>03:0X-DQ8I</em>02:0X</td>
<td>DR4/DR4b</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>F. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR1- DQAI<em>01:01-DQ8I</em>05:01</td>
<td>DR4/DR1</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>G. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR13-DQAI<em>01:02-DQ8I</em>06:04</td>
<td>DR4/DR13</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>H. DR4-DQAI<em>03:0X-DQ8I</em>03:02 / DR9- DQAI<em>03:0X-DQ8I</em>03:03</td>
<td>DR4/DR9</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>I. DR3-DQAI<em>05:01-DQ8I</em>02:01 / DR9- DQAI<em>03:0X-DQ8I</em>03:03</td>
<td>DR3/DR9</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>
Study population

Screening of newborns for T1D associated HLA genotypes was conducted from September 2004 to February 2010. Recruitment of study participants was done at several locations in the surroundings of the six study clinics. First contact was taken with pregnant mothers at healthcare or maternity clinics or with mothers at hospitals directly after their babies were born. In Sweden, recruitment was done through five maternity clinics in the southernmost part of the country (Skåne). In Finland, newborns were recruited in three cities: Turku in the south and Tampere and Oulu in the north. In Germany, the study center is located in Munich, but screening of participants was done in all regions of the country. Most members of the German study population were recruited from all over the country through a network of hospitals (obstetric and diabetes departments) and pediatric general practitioners. In the United States, screening for recruitment of study subjects was done in three states as follows: in Colorado, at St Joseph’s hospital and 10 additional Denver hospitals in the Denver Metropolitan area; in the state of Washington, at the obstetrics wards of hospitals in the Puget Sound area; in Georgia, in hospitals located in Augusta and Atlanta, but also at a hospital in Gainesville (Florida).

A total of 21,589 (5%) of the 424,788 children that were screened met the inclusion criteria based on HLA genotyping (Table 2). The geographic distribution of infants with an HLA-eligible genotype by country or US state was as follows; 7.7% in Sweden, 6.1% in Finland, 5.8% in Colorado, 4.6% in Germany, 4.4% in Washington and 3.7% in Georgia.

<table>
<thead>
<tr>
<th>Country</th>
<th>All countries</th>
<th>Finland</th>
<th>Germany</th>
<th>Sweden</th>
<th>U.S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, (%)</td>
<td>n, (%)</td>
<td>n, (%)</td>
<td>n, (%)</td>
<td>n, (%)</td>
</tr>
<tr>
<td>HLA eligible infants</td>
<td>21 589</td>
<td>3 681</td>
<td>1 671</td>
<td>3 725</td>
<td>12 512</td>
</tr>
<tr>
<td>Excluded</td>
<td>5 213 (24.0)</td>
<td>80 (0.2)</td>
<td>297 (17.8)</td>
<td>155 (4.2)</td>
<td>4 681 (37.4)</td>
</tr>
<tr>
<td>Refused to enroll</td>
<td>7 674 (35.5)</td>
<td>1 767 (48.0)</td>
<td>777 (46.5)</td>
<td>1 032 (27.7)</td>
<td>4 098 (32.8)</td>
</tr>
<tr>
<td>Enrolled</td>
<td>8 676 (40.2)</td>
<td>1 832 (49.8)</td>
<td>594 (35.5)</td>
<td>2 526 (67.8)</td>
<td>3 724 (29.8)</td>
</tr>
</tbody>
</table>
Parents of the 21,589 HLA-eligible infants were invited to enroll the child in the TEDDY follow-up study. In all, 5,213 (24%) of the eligible infants were not enrolled due to one or more of the following protocol exclusion criteria (117):

- The child had an illness or birth defect that prohibited long-term follow-up or was involved in a treatment that might alter the natural history of T1D.
- The family refused storage of biological samples.
- The infant’s first TEDDY clinic visit did not occur before the child was 4.5 months old.

The parents of 7,647 (36%) of the HLA-eligible infants refused enrollment in the follow-up study. At the conclusion of screening, a total of 8,677 infants (40%) were enrolled in the final study (Table 2).

A study protocol requiring frequent assessment visits up to the age of 15 years can be perceived as very demanding in a pediatric study population and indeed the most common reasons for refusing enrollment were related to protocol characteristics (i.e. blood draw, demanding protocol) or family circumstances. Almost 70% agreed to participate in Sweden compared to 36% in Germany. Based on demographic variables collected during screening and later analyzed, the results show that infants born in Europe with an older mother, and having a FDR with T1D were more likely to be enrolled in TEDDY (117). In a longitudinal study, it is important that the enrolled families remain in the study. Considering TEDDY, these are the most common reasons given by parents or caregivers for staying in the study (118); continuous monitoring of the children with regard to development of T1D, being able to help scientists discover the cause of T1D, and being informed of the children’s autoantibody results.

It should also be noted that parents of enrolled children in studies investigating health and lifestyle and other environmental factors might take their own precautions to prevent their children from developing a disease, which is why this potential factor is continuously recorded in TEDDY. At the 6-month clinic visit, 30% of the mothers reported at least one preventive action related to early infant feeding practices (119). The actions most often reported by mothers include decreasing consumption of sweets, avoiding cow’s milk, and other dietary changes (21%). Moreover, 6% of the mothers reported prolonging duration of breastfeeding in an effort to prevent their children from developing T1D. The mothers who reported taking preventive actions were older, had a higher education, and had a FDR with T1D, and the TEDDY child was their first offspring.

Information on ethnicity and race is not requested in the European countries. However, this aspect must be controlled for in the Unites States, and the distribution in the US study population is as follows: 88% with a Caucasian background, 6% of
mixed races, and 3% African Americans. Controlling for ethnicity showed that 19% of the US TEDDY population belongs to the Hispanic or Latino community.

The three studies described in Papers I – III included children from all the TEDDY countries, whereas only children in Sweden were included in the case-control study reported in Paper IV.

The first study (Paper I) evaluated children carrying one of the major HLA genotypes of interest (HLA types A-D) shown in bold type in Table 1. A total of 6,403 children screened at least once for tTGA were included in the analysis.

The second study (Paper II) comprised all HLA-eligible children followed for more than 2 years and selection of the study population is depicted in Figure 5. Sixty percent of the participants were from Europe and the remaining 40% from the United States.

The third study (Paper III), included a total of 6,672 children that were screened at least once for tTGA (Figure 6).
The fourth study (Paper IV) used a nested case-control study design and included only children in Sweden (Figure 7).

Figure 6. Flowchart showing selection of the study population reported in Paper III. Abbreviations: CD, celiac disease; CDA, celiac disease autoimmunity

Figure 7. Flow chart showing study enrollment and participation in the Swedish TEDDY birth cohort described in Paper IV.
Three controls per case were randomly selected from subjects that met the matching criteria’s (HLA-genotype, gender and birth year). At the time of data analysis, 147 children were diagnosed with biopsy verified celiac disease. The analysis included 436 pairs from 146 cases (one child had no eligible controls).

**Dietary assessment**

The aim of the dietary collection in TEDDY is to identify dietary factors that predispose to or protect against IA, T1D and celiac disease. A secondary objective is to distinguish potential differences in dietary factors in relation to the primary endpoints (IA, T1D, and celiac disease) across diverse populations and ethnic groups. The dietary study in TEDDY is designed to test and confirm existing dietary hypotheses as well as to explore new, less well-documented hypotheses. It is possible that increased risk of islet autoimmunity and/or T1D is related to differences in duration of breastfeeding (120-122), specific time windows during introduction of cereals (123), early gluten introduction (121, 124), cow’s milk (125, 126), fruit and berries (127), and root vegetables (127, 128). There is also evidence that lower levels of serum 25(OH) vitamin D and intake of vitamin E and antioxidants (e.g., carotenoids, ascorbic acid, selenium, and omega-3 fatty acids) are associated with onset of T1D (129-131). **Table 3** summarizes the nutritional factors of interest in TEDDY on a food and nutrient level.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Nutrients and energy</th>
<th>Other nutritional factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow’s milk</td>
<td>Energy intake</td>
<td>Nitrates, nitrites and N-nitroso compounds</td>
</tr>
<tr>
<td>Cereals, gluten</td>
<td>Macronutrients (proteins, carbohydrates, fat)</td>
<td>Patulin</td>
</tr>
<tr>
<td>Soy</td>
<td>Vitamins C, D and E</td>
<td>Bafilomycin</td>
</tr>
<tr>
<td>Meat</td>
<td>Nicotinamide</td>
<td>Increased weight and/or height gain (fetal period, infancy, childhood)</td>
</tr>
<tr>
<td>Coffee and tea</td>
<td>n-3 fatty acids</td>
<td></td>
</tr>
<tr>
<td>Breast milk</td>
<td>Zinc</td>
<td></td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>Carotenoids and selenium</td>
<td></td>
</tr>
</tbody>
</table>

Information on the dietary habits (e.g. feeding pattern) of the participating infants was assessed by several different methods, such as mailed questionnaires to be completed prior to the first clinic visit, structured interviews at each clinic visit, and records kept by the parents between the visits.
At a child’s first clinic visit, at 3 - 4.5 months of age, the parents were instructed to use a special logbook to record information on the child with regard to the following dates: dates of introduction of new foods, use of dietary supplements and medications, vaccinations (immunizations), length and weight history of the child, illnesses and symptoms, doctor visits and hospitalizations, social and daycare interactions, and life events. The parents were asked to bring the TEDDY logbook to each clinic visit, every third month for the first 4 years.

The parents, most often the mother, answered these questions regarding breastfeeding at each visit: Was the infant breastfed? Did the infant receive any infant formula? At what age did the infant start bottle feeding? What type of formula (i.e. cow milk based, hydrolyzed, partial hydrolyzed, or soy based formula) was given? The definition of exclusive breast-feeding allowed an intake of small amounts of non-nutritious drinks such as tea, water and water-based drinks, and nutritional supplements. Based on the information collected in this manner, durations of exclusive and overall breastfeeding were calculated.

Parents were instructed to record when their child was fed anything other than breast milk or infant formula. The same question was asked at each visit: Since the last visit, has the child been given any new food items or anything besides breast milk? Parents recorded when the child was given anything new for the first time, even very small tasting portions. Several food categories were of interest: fruit and berries, potatoes, vegetables and root vegetables, rice, corn and grains, dairy products, fish, meats and meat products, and soy products. A conversion table was used to convert the child’s age from days to weeks and months to specify the time point at when a new food, or diet was started (Table 4).

Table 4. Table used to convert a child’s age as reported by the primary caregiver to be entered on the standardized questionnaires used in TEDDY, from birth to 2 years of age.

<table>
<thead>
<tr>
<th>Days</th>
<th>Weeks</th>
<th>Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 – 10</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>11 – 17</td>
<td>2</td>
<td>0.50</td>
</tr>
<tr>
<td>18 – 24</td>
<td>3</td>
<td>0.75</td>
</tr>
<tr>
<td>25 – 31</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>32 – 38</td>
<td>5</td>
<td>1.25</td>
</tr>
</tbody>
</table>
Food records

The parents were asked to keep food records to evaluate the studied children’s total and usual dietary intake. Two dietary assessment methods were used in TEDDY: a 24-hour recall and a 3-day food record. Both these methods allow cross-cultural comparisons (132, 133) and the data collected can be analyzed on both a nutrient level and a food group level. During the first clinic visit, primary caregiver (usually a mother) was trained in how to keep a 3-day food record of the child’s dietary intake. The 24-hour recall of the child’s diet was obtained at the first (3-month) visit and was collected to serve two purposes: (i) to assist in training the parents to know what types of food items they would need to record when completing the 3-day diet record; (ii) to reflect the infants diet at 3 months of age, considering that the dietary intake during the first months of an infant’s life consists mainly of breast milk and/or infant formula. As the infant diet becomes more complex, the 3-day food record is the method of choice to better capture the day-to-day variation in food intake. In general, more than 2 consecutive days are required to accurately estimate a normal dietary intake. In TEDDY, the parents were instructed to fill out the 3-day food diary immediately prior to the clinic visit. They were told to record their infant’s food intake during three consecutive days, comprising two weekdays and one weekend day, and to bring the completed record with them to the clinic. At each visit, the diet records were reviewed by trained study personnel together with the parents. Parents reported portion sizes (estimated or weighed). A booklet containing colorful pictorial illustrations of composite dishes and black-and-white shapes and scales to facilitate estimation of portion size was developed especially for TEDDY. In the event that food was provided by caregivers other than the parents, the task of keeping food records was split between parents who had received the food record training/instructions and other caregivers who had not (e.g., daycare staff, relatives, or babysitters). Thus the special portion-size booklet was intended for use by other caregivers, although no other training was given as support for this group.

The measurement of habitual food intake is one of the most challenging aspects of nutrition research. We know that the human diet is a result of an interaction between food components and cultural practices, and lifestyle and socioeconomic factors are also often predictors of the habitual diet. Unfortunately, methods used to assess the usual dietary intake often have limitations (133). Using a self-report method is prone to measurement error (i.e., the difference between the true value of a parameter and the value obtained from the reported dietary intake), although it is assumed that if records are obtained for a sufficient number of days and are of good quality, they can provide a valid estimate of the usual dietary intake. In the absence of truly objective measures of the diet, food records are often referred to as the “gold standard” of methods. Notably, food records are inexpensive to collect but expensive to code.
A potential selection bias when using food records is that the method can limit participation in some groups. For instance, the subjects must be literate and well-motivated to complete food records. Also, individuals may change their behavior due to awareness that it is being or will be measured. Examples of changes in behavior include eating different types or amounts of foods than those usually consumed. When the food record approach is applied in pediatric populations, the actual task of reporting food consumption is performed primarily by parents and other non-parental caregivers due to the limited cognitive ability of infants and children, which presents unique challenges. In TEDDY, these obstacles have been dealt with by implementing continuous training of the research staff (probing, knowledge of commercial infant foods), training of parents, and using detailed instructions given to non-parental caregivers.

In the study reported in Paper IV, gluten consumption was estimated from the 3-day food records kept by the families for the participating children at ages 6, 9, 12, 18, and 24 months. To calculate the intake of gluten, composite foods that included gluten-containing flours were broken down into ingredients. All foods and beverages consumed by the child were categorized into basic foods (single ingredients) or composite dishes (multiple ingredients). Composite dishes were broken down into their respective ingredients. For commercial baby foods, recipes were created based on the ingredient list for each brand name. The Swedish Food Composition database was used as a source of standard recipes (e.g., for bread, pizza, pancakes, and bakery sweets) (134). Special user recipes provided by the families were added and entered in the local database. The amount of food eaten by the participating children was estimated using household measurements, drawings of actual size of bread slices, and pictures in the TEDDY portion-size booklet.

Even though gluten is common in foods, it is unusual for food composition tables to include information about the analyzed gluten content of food products. Accordingly, it is still necessary to use an estimation of the gluten content, which is commonly described in the literature as the amount of vegetable protein (analyzed) in gluten-containing cereals (wheat, rye, barley) multiplied by 0.8 (96). Such approximation has been widely accepted, and this approach has been described in other publications with the aim of describing gluten intake in different study populations (135-137).
Tissue transglutaminase autoantibody (tTGA) immunoassays

Radiobinding assays (RBAs) for tTGA were performed on serum aliquots at the same two core laboratories as the analyses of diabetes autoantibodies (138). The RBA sera were stored in central laboratories at –20 °C for the duration of the study. Samples from clinical centers in the US states of Washington, Georgia/Florida, and Colorado were analyzed for tTGA at the US central laboratory (Barbara Davis Center for Childhood Diabetes [BDC], University of Colorado, Denver), and samples from centers in Finland, Germany, and Sweden were analyzed at the European central laboratory (University of Bristol Laboratory, Bristol, United Kingdom). At the BDC, anti-IgA agarose was used in RBA to capture IgA-tTGA; in Bristol, a mixture of anti-IgA agarose and protein A sepharose was used to assess both IgA-tTGA and IgG-tTGA. These two immunoassays have been shown to be highly concordant in previous tTGA workshops (139).

Screening with tissue transglutaminase autoantibodies (tTGA)

All children in TEDDY are screened annually for tTGA, starting at 2 years of age. If a child tests positive for tTGA at the 24-month visit, then all of the earlier samples (3–21 months) are retested to determine the true time of seroconversion. Children with a tTGA level of ≥ 0.05 at the BDC and ≥ 1.3 units (U) at the laboratory in Bristol are deemed tTGA positive and are re-assayed in a follow-up sample taken after 3 months in samples collected at 24 and 36 months of age, and a second follow-up sample collected after 6 months at 48 months of age and older. Children positive in the consecutive samples are defined as persistently tTGA positive. Due to the discrepancy between the detection methods in the two tTGA assays, all samples found to have IgA-tTGA levels of ≥ 0.01 units at the BDC laboratory were sent to the laboratory in Bristol for re-analysis, because the latter facility was chosen to serve as the reference laboratory (138).

Definition of celiac disease autoimmunity (CDA) and celiac disease in TEDDY

A child screened for tTGA positivity in two consecutive measurements drawn at least three months apart was defined as having CDA and was further referred to a pediatrician for evaluation of celiac disease. Determining if and when an intestinal biopsy should be performed to confirm diagnosis of celiac disease was outside the TEDDY study protocol. Nevertheless, at all the study centers, it was recommended that an upper endoscopy with multiple biopsies from the proximal duodenum including the bulb should be performed if a child had a tTGA level of > 30 units, and also regardless of tTGA level in a child with symptoms or clinical signs of celiac disease. Different stages of mucosal damage are classified according to the Marsh
score (140). Here, celiac disease was confirmed if the biopsy sample from the small intestine was classified as having a Marsh score of > 1. Marsh scores are defined as follows: Marsh 1 as normal villi with an increased number of intraepithelial lymphocytes (IELs) representing $\geq 40/100$ enterocytes; Marsh 2 as normal architecture, increased IELs corresponding to $\geq 40/100$ enterocytes, and crypt hyperplasia; Marsh 3 as various degrees of villous atrophy (gradually flattened villi) with the subsets Marsh 3a (partial villous atrophy), Marsh 3b (subtotal villous atrophy), and Marsh 3c (total villous atrophy), all of which show increased IELs ($\geq 40/\geq 25/100$ enterocytes) and crypt hyperplasia.

Although the decision to perform a biopsy was not included in the TEDDY protocol, parents and primary caregivers were asked to report biopsy results to the respective study clinic. More precisely, the information requested was to be provided by completing the “Biopsy Form”, which was the same in all the TEDDY countries and covered the following: the date of the biopsy, the type of biopsy procedure used, the facility where the biopsy was done, and the biopsy results according to histological classification. If no biopsy was performed, parents were asked to specify the reason as one of the following: parents or pediatrician refused biopsy; the child had no symptoms; the child was placed on a gluten-free diet without biopsy; a biopsy was too expensive (no health insurance).

For the purpose of the present research, children who had a mean tTGA level of $\geq 100$ units in two consecutive blood samples at least 3 months apart, and were not investigated with intestinal biopsy, were also considered to have celiac disease. This judgment was based on reports showing that high levels of tTGA are associated with the degree of damage of the intestinal mucosa in both symptomatic and asymptomatic children (141-145).

**Statistical methods**

All statistical analyses were performed using Statistical Analysis System software versions 9.2 and 9.4 (SAS Institute Inc., Cary, NC, USA). In all four studies, a p-value of $< 0.05$ was considered statistically significant.

Paper I. The Cox proportional hazard model was used to estimate the risk of developing CDA and celiac disease. The analysis was adjusted for country of residence, gender, and having a FDR (mother, father, or sibling) with celiac disease. To estimate the cumulative risk of developing CDA according to HLA genotype, the log-rank test was used and Kaplan-Meier curves were applied to plot the results.

Paper II. The Cox proportional hazard model (stratified for country of residence) was used to estimate hazard ratios for breastfeeding duration and age at first
introduction of various types of solid foods: cow’s milk, root vegetables, fruits and berries, and cereals. Efron’s method for tied survival times was employed in the Cox analysis.

Paper III. Age at first introduction of gluten and duration of breastfeeding were presented as means, medians, and interquartile ranges (IQRs) for each country. In the main analysis, the Cox proportional hazard model was used to estimate whether risk of CDA and celiac disease was associated with age at first introduction of gluten. The analyses were adjusted for HLA genotype, country of residence, gender, and having a FDR with celiac disease. When comparing breastfeeding and age at gluten introduction, the timing of gluten introduction (as a continuous variable) was added as confounding factor.

Paper IV. A conditional logistic regression was used to compare the characteristics of cases and matched controls. Kruskal-Wallis test was applied to compare the age at tTGA seroconversion in cases by gender, birth year, and HLA genotypes. The amount of gluten was noted both as continuous variable (gram per day) and as tertiles (low, medium, and high intake). Time to seroconversion of tTGA stratified by gluten intake (categorized by tertiles) was calculated using Kaplan-Meier estimates and plotted in Kaplan-Meier curves.

**Ethical approval**

Written informed consent was obtained from a parent or a primary caregiver for all participants in the TEDDY study, and this was done separately for genetic screening and participation in the prospective follow-up. The project was approved by local institutional review boards in the respective countries and was monitored by an external advisory board formed by the National Institutes of Health (NIH). In Sweden, the entire TEDDY study was approved by the Regional Ethical Review Board, Lund, through the initial application EPN 217/2004 and the subsequent amendments 2013/291, 2013/455, 2014/193, 2014/890, 2015/718, and 2016/318.
Results

Risk of pediatric celiac disease according to HLA haplotype and country (Paper I)

Of the 6,403 children included in this study (Paper I), 1,374 (21%) were found to be homozygous for HLA-DQ2, and 2,612 (41%) were heterozygous for HLA-DQ2. Also, 786 (12%) developed CDA, and 312 of those children (4.8%) were diagnosed with celiac disease, and an additional 21 children were diagnosed with this disorder based on high levels of tTGA. By the age of 5 years, the risks of CDA and celiac disease were, respectively, 11% and 3% among children heterozygous for HLA-DQ2, but higher at 26% and 11%, in children with two copies of HLA-DQ2. Moreover, HLA-DQ2 homozygous children were at a six-fold increased risk of CDA (hazard ratio [HR] 5.70, 95% confidence interval [CI] 4.66–6.97) and celiac disease (HR 6.08, 95% CI 4.43–8.36) compared to the reference group (DQ8-DQ8 or DQ8-DQ4). HLA-DQ2 homozygosity was also associated with earlier age at seroconversion to CDA, which confirms the findings of previous studies (48, 146).

Furthermore, having a FDR with celiac disease was associated with an increased risk of CDA (HR 1.81, 95% CI 1.31–2.50) and celiac disease (HR 2.95, 95% CI 1.95–4.46). Also, girls were at higher risk of both CDA (HR 1.64, 95% CI 1.42–1.89) and celiac disease (HR 2.16, 95% CI 1.71–2.72).

The results reported in Paper I confirm observations made in similar studies of risk factors known to be associated with celiac disease (i.e., HLA type, gender, and FDR with celiac disease) (40, 147-151).

However, even after adjusting for all these known risk factors, there were still statistically significant differences in the risks of both CDA and celiac disease among the TEDDY participants in the different countries, with the highest risk noted in children in Sweden compared to those in Finland, Germany, and the United States. Although this investigation does highlight the importance of HLA, the differences found in geographically separated populations with similar HLA genotypes suggest that environmental and lifestyle factors can trigger celiac disease.

The incidence of celiac disease in Sweden is rising at an average annual rate of 4% (28).
Nonetheless, this increase is not constant, and the variation in incidence over the years strongly implies an environmental impact. Also, both intra- and international differences have been reported (23, 26). Regional disparities within Sweden have been identified with a north-south gradient (67, 152). Others have described clusters (areas) discerned as having a higher education level, older average age, higher average income, and more extensive industrial and commercial activities (153). These geographical regions have been associated with reduced risk for celiac disease. Other such factors that have been identified and may differ between populations include national vaccination programs, infectious episodes, parental smoking habits, children born by caesarian section, and socioeconomic conditions (52).

**Age at first introduction to complementary foods is associated with sociodemographic factors in children with increased risk of developing type 1 diabetes (Paper II)**

The aim of this investigation (Paper II) was to describe early infant feeding practices in the TEDDY birth cohort. The results showed differences between the participating countries with regard to timing of first introduction to complementary foods. Root vegetables were often the first solid food given to infants in the countries studied in Europe, whereas rice cereals were most often the first choice for the infants in the United States. Overall, preterm birth was associated with short duration of breastfeeding (exclusive and overall) and also, low maternal age and educational level, and mothers smoking during pregnancy were associated with both short durations of breastfeeding and early introduction to complementary foods.

Infant feeding recommendations outline how infants should be fed to enhance health later in life, but there is not always complete compliance with these guidelines. It is possible that there are specific subgroups in the general population that can be identified and targeted for use of additional preventive tools within the area of public health. The WHO infant feeding recommendations state that a child should be exclusively breastfed for the first 6 months of life, and thereafter complementary (solid) foods can be introduced together with continued breastfeeding (154).

Although it was expected that parents in all the TEDDY countries would follow these guidelines, many of the factors associated with non-compliance were the same as previously reported in the literature (155-157). For instance, variations in breastfeeding duration were observed. The median age for exclusive breastfeeding was significantly lower in the United States (1 week) than in Finland, Germany, and Sweden (3.0, 3.5, and 4.0 weeks, respectively). Considering any breastfeeding in
the TEDDY countries, median duration was 33 weeks (7.6 months), and the infants in Finland were breastfed for the longest period (8.9 months). Several studies have examined the role of duration of breastfeeding and timing of introduction of complementary foods in the development of IA, T1D and celiac disease but with inconsistent results (122, 158, 159).

In TEDDY, the initial exposure to solid foods was defined as the point at which any complementary food was given to a child for the very first time, and this even included small tasting portions. According to this definition of first exposure, approximately 20% of the TEDDY infants were introduced to complementary foods before the age of 4 months (< 17 weeks). Potatoes and root vegetables were the first solid foods to be introduced to the European participants, whereas cereals and fruits were introduced first to the US participants (Figure 8). In this study, the term “cereal” was used as a collective variable for all types of cereals (wheat, rye, barley, oat, rice, buckwheat, and millet).

Evaluation of sociodemographic factors associated with short duration of breastfeeding and early introduction of complementary foods revealed some patterns that correspond well with current knowledge on non-adherence to infant feeding recommendations (92, 160). Notably, the patterns in mothers concerning

![Figure 8](image-url)

**Figure 8.** First foods or food combinations introduced to infants enrolled in TEDDY shown by country. Proportions are expressed as percentages.
young age, low educational level, and smoking during pregnancy were all associated with early introduction of the studied food groups. To promote health later in life, it is essential to understand why complementary foods are introduced earlier than the recommended age in certain populations. In some countries, the national infant feeding recommendations have added information about developmental readiness (i.e., signs of readiness) to emphasize that it is not only the child’s age that is the most important factor when starting to give solid foods, but also the child’s readiness. Developmental readiness is described as when a child can sit up unsupported, grasp food and bring it to the mouth (with hand or spoon), and open the mouth when food is given. Mothers who have introduced complementary foods early have reported that they have done so for reasons such as the infant’s hunger or lack of weight gain, or in an attempt to change the infant’s behavior (91).

Male gender is also a factor associated with an earlier introduction of complementary foods (92). However, in the present study (Paper II), there was no relationship between gender and duration of breastfeeding, although early introduction of root vegetables, fruits and berries, and cereals was associated with male gender, indicating an impression of hunger or needing food.

Insufficient weight gain has often been given as a reason for introducing solid foods earlier, but research results in this context have been inconsistent. It is assumed that infants with a high birth weight need solid foods earlier (161). However, an association between early introduction of complementary (solid) foods and birth weight was not confirmed in the current study (Paper II). An association was found between short duration of overall breastfeeding and low birth weight (< 2500 g), which might be explained by the mothers’ ambition to increase their infants’ weight by replacing breast milk with complementary foods. On the other hand, no relationship was noted between low birth weight and early introduction to cow’s milk (i.e., infant formulas containing milk protein). Also, premature birth (gestational age < 37 weeks), which is often correlated with low birth weight, was associated with both a shorter duration of breastfeeding (exclusive and overall) and an early introduction of cow’s milk, and this can probably be explained by the fact that many preterm infants are treated at neonatal intensive care units and separated from their mothers.
Age at gluten introduction and risk of celiac disease (Paper III)

In this study (Paper III), information about infants’ age at gluten introduction and duration of breastfeeding were collected every three months by use of questionnaires and standardized interviews. A total of 6,672 children that had been screened at least once for tTGA were included in the investigation, and 773 (12%) of those subjects had CDA. Also, 307 (4.6%) of the 6,672 met the criteria for celiac disease, which was confirmed by biopsy in a majority (93%) of those children.

The results show that age at first introduction to gluten (wheat, rye, or barley) differed between the TEDDY countries. Children in Sweden were introduced earliest (mean age 5 months) compared with those in Finland (6.5 months), Germany (7.5 months), and the United States (7 months). Gluten was introduced to almost 6% of the study population before the age of 4 months (17 weeks), ranging from 11% in Sweden to 3% in Finland and the United States (Figure 9). Even though the children in Sweden were introduced to gluten earlier compared to those in the other countries, the timing of gluten introduction was not associated with increased risk of CDA and celiac disease, neither in the overall analysis nor on a country level.

Figure 9 Proportion of infants first introduced to gluten (wheat, rye or barley) shown by country. The x-axis indicates early (age < 4 months) versus late (age > 6 months) introduction to gluten-containing cereals.
Two previous studies have separately examined the timing of gluten introduction as independent risk factor for celiac disease. The first of these investigations was conducted by Ivarsson et al. (73) and followed the incidence of celiac disease in Sweden. The infant feeding recommendations in that country were changed in the mid-1980s to support delaying gluten introduction in infants from age 4 months until 6 months in an attempt to postpone the development of celiac disease. However, this strategy was not successful and instead actually led to an increase in the incidence of reported cases in children younger than 2 years of age; when then recommendation was changed back to age 4 months, the incidence of celiac disease decreased. In line with this observation, Norris et al. (74) prospectively followed genetically at-risk infants in the United States and demonstrated that being introduced to gluten very early (age < 3 months) and late (age > 6 months) increased the risk of celiac disease.

Research has also been conducted to assess introduction of gluten to infants while they are still being breastfed or using alternative approaches to determine breastfeeding practices during weaning. In a meta-analysis based on 714 patients with celiac disease and 1,255 controls, Akobeng et al. (159) demonstrated that children who were breastfed at the time of gluten introduction had lower risk of developing celiac disease. These authors proposed that a possible explanation for this finding is that breastfeeding at the time of weaning may limit the amount of gluten in the diet. It is also known that breast milk protects against a number of infections, including those occurring in the gastrointestinal tract (162). Considering the composition of human milk, especially during the first days postpartum, it would have been of interest to study infants in the TEDDY project who had never been breastfed in relation to their risk of developing celiac disease. However, only 140 (2%) children in the study population were never breastfed, and 123 (88%) of those subjects were enrolled in the United States.

The investigation reported in Paper III showed an increased risk of CDA in children breastfed for more than 1 month after introduction of gluten (HR 1.23, CI 95% 1.05–1.44), although this finding was no longer statistically significant when celiac disease was used as an outcome (HR 1.13, CI 95% 0.88–1.46). These results agree with previous studies demonstrating that neither breastfeeding nor the time of gluten introduction has any significant impact on the risk of developing celiac disease (103, 107, 163, 164).

After conclusion of the cited studies, the ESPGHAN revised its recommendations to indicate that introduction of gluten should be begun between the ages of 4–12 and completed while the infant is still being breastfed, but this was not suggested to be a means of reducing the risk of developing celiac disease (82).
Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish birth cohort (Paper IV)

In this case-control study (Paper IV), the daily intake of gluten-containing cereals were recorded in 517 children. According to the 3-day food records, the proportion of the gluten that was derived from wheat was 96% at 6 months but 90%, 81%, 79%, and 79% at 9, 12, 18 and 24 months, respectively (Figure 10). The remaining proportions of the gluten came from rye. Intake of oats was not considered in this investigation.

Median age at seroconversion to tTGA positivity was 24 months (range, 10-86 months) and median age at diagnosis was 38 months (range, 15-102 months). Median age at seroconversion differed significantly depending on the HLA genotype; 21.5 months in children homozygous for HLA-DQ2 but 36 months in those heterozygous for HLA-DQ2.

Cases and controls did not differ with regard to duration of breastfeeding or age at first introduction to gluten-containing cereals. A drastic increase in consumption of gluten-containing cereals recorded in the food records was observed at the 9-month and 12-month visits. The overall results showed that the amount of gluten consumed up to the age of 2 years was associated with at least a twofold increase in the risk of
celiac disease. The reported gluten intake was higher for the cases than for the controls at all ages, beginning at 12 months and noted again at 24 months, and the higher intake by the cases was particularly marked at 12 months (OR 1.58, 95% CI 1.17–2.13).

It has been suggested that a high gluten intake (load) can be especially critical in individuals homozygous for HLA-DQ2.5 or heterozygous for HLA-DQ2.5, because they can present large amounts of immunogenic gluten peptides (31). Therefore, the current study (Paper IV) aimed to investigate whether the amount of gluten required to exert an effect depends on the HLA genotype. The amount of gluten intake was arbitrarily estimated as low (< 3.4 g/d), medium (3.4–5.0 g/d), or high (> 5.0 g/d) based on tertiles for the study participants. Children who were homozygous for HLA-DQ2 and had a high intake of gluten (> 5g/d) had a threefold increased risk for celiac disease (odds ratio [OR] 3.19, 95% CI 1.61–6.30; p=<.0001) compared to children with lower gluten consumption. However, the findings were similar for children that were heterozygous for HLA-DQ2 (OR 2.24, 95% CI 1.08–4.62; p=.001) and those that were negative for HLA-DQ2 (OR 2.43, 95% CI 0.90–6.54; p=.079), suggesting that a high gluten intake was an independent risk factor for celiac disease in our study cohort.
Presence of any of the HLA-DQ2 and HLA-DQ8 haplotypes is regarded as necessary for the occurrence of celiac disease, although other factors must also be involved, considering that only 1–3% of the carriers of the indicated haplotypes actually develop the disorder. In the children described in this thesis, at the age of 5 years those who were HLA-DQ2 homozygous were at six-fold increased risk of celiac disease compared to the subjects in the lowest risk groups. Also, children homozygous for HLA-DQ2 developed CDA earlier than those with other HLA risk genotypes, especially non-HLA-DQ2 carriers. However, after adjusting for HLA, gender, and FDR with celiac disease, the children in Sweden were still at higher risk of both CDA and celiac disease compared to children in the other TEDDY countries, which implies that environmental factors are important determinants of this condition.

Infant feeding recommendations are structural factors that exert their influence on a societal level, and changes at that level can have a substantial impact on general public health. This was clearly demonstrated when the infant feeding recommendations in Sweden were changed in the mid-1980s to suggest that introduction of gluten to be postponed from the age of 4 months to 6 months (73). Still, there are controversies regarding whether the time to first exposure to gluten in the infant diet actually has an effect on the risk of celiac disease later in life. The ESPGHAN infant feeding recommendations for gluten that were issued in 2008 stated that early (age < 4 months) and late (age > 7 months) introduction should be avoided in order to decrease the risk of celiac disease.

The present research showed that, compared to the participants in the other TEDDY countries, the children studied in Sweden were at the highest risk of celiac disease, and they were also introduced to gluten at an earlier age. Despite these disparities, no association was found between early or late introduction to gluten and the risk of CDA and celiac disease. Since 2008, several other observational investigations have evaluated gluten introduction in relation to the risk of celiac disease, and most of the data obtained have not revealed any associations between this disorder and the time of gluten introduction or being breastfed at the time of gluten introduction (107, 108, 164, 165).
In addition, the results of two interventional studies published in 2014 demonstrated that genetically at-risk children were not protected from celiac disease by gradually introducing very small amounts of gluten or by postponing the age of the first introduction to gluten from 6 to 12 months (109, 110).

The finding that the children in Sweden were at the highest risk of celiac disease even after adjusting for other known major risk factors led to the assumption that consumption of large amounts of gluten may be an important trigger mechanism. This hypothesis was supported by the nested case-control study (Paper IV), which showed that the amount of gluten consumed did have an impact: a high gluten intake during weaning was associated with a twofold increase in the risk of celiac disease. In that study, children that developed celiac disease were reported to have higher intake of gluten at all ages (starting at 12 months) compared to matched controls. More importantly, findings were similar in children carrying any of the HLA risk genotypes, which suggests that a high gluten intake can also be hazardous in infants at moderately increased risk of developing the disease. The HLA risk genotypes for celiac disease are widely distributed in the general population, and this may prove to have an impact on future infant feeding recommendations.

In 2016, the ESPGHAN published a new position paper regarding gluten introduction and the risk of celiac disease, which provided novel evidence from several prospective studies and two RCTs that served as a basis for urging the committee to revise the existing recommendations (82). It was concluded that the risk of celiac disease is not associated with the timing of gluten introduction but may be influenced by other aspects of gluten intake.

There are some indications that large quantities of gluten can increase the risk of celiac disease. However, few studies have examined the effects of amounts of gluten consumed during the first years of life, and the current evidence grade is low. Only one retrospective study on this topic has been conducted thus far, and it included subjects born in Sweden during the “epidemic of celiac disease” and showed that consumption of “a large amount” of gluten during weaning, indicated as > 58g of flour (equivalent to approx. 5g of gluten), was associated with an increased risk of celiac disease (106). Interestingly, this level corresponds to the amount that was arbitrarily defined as high gluten intake based on tertiles in Paper IV, which supports the notion that further investigations are warranted to identify a maximum level of gluten consumption that can be considered safe during infancy. As regarding quantity, little research has concerned the type of gluten (i.e., in wheat, rye, or barley) in relation to risk of celiac disease. Accordingly, the ESPGHAN and several systemic reviews have concluded that no recommendations can currently be made regarding the type of gluten that should be used at introduction and during weaning (72, 163, 166, 167).
Thus additional prospective studies on gluten intake (amount and type) and risk of celiac disease during childhood are needed that employ the same method to compare habitual dietary intake in the general population in different countries. To investigate diet as an independent variable in terms of how it affects a future dependent variable such as celiac disease, it will be necessary to use open methods such as food records (3, 4, or 7 days) or repeated 24-hour recalls. Food frequency questionnaires (FFQs) may represent a suitable option but will have to be tailored to fit the food habits and the types of foods available on the market in the countries where a study is conducted.

Clearly, future studies should be focused on whether genetically at-risk children that develop celiac disease in countries other than Sweden have been exposed to a higher gluten intake compared to healthy children in the same areas. More importantly, longer follow-up studies are needed to ascertain whether a high gluten intake is also a risk factor in older children. Such investigations are in progress and will be included in TEDDY in the near future. The results will make it possible to establish whether the habits of gluten intake among children in Sweden differ from those in the corresponding age groups in other countries, and also to determine whether gluten intake (amount and type) is an independent risk factor for celiac disease.
Conclusions

- HLA was found to be the strongest risk factor for CDA and celiac disease, and HLA-DQ2 homozygous children were at six-fold increased risk of celiac disease compared to children carrying standard risk HLA genotypes.

- Other risk factors in the present study were female gender, having a FDR with celiac disease, and country of residence, with children in Sweden at higher risk of CDA and celiac disease compared to children in the United States by the age of five years.

- There were country differences in duration of breastfeeding and first introduction to complementary foods. Non-adherence to infant feeding recommendations was associated with young maternal age, low educational level, and smoking during pregnancy.

- Neither duration of breastfeeding nor time to first introduction of gluten was associated with increased risk of celiac disease in genetically susceptible children.

- A high intake of gluten before 2 years of age increased the risk of celiac disease, indicating that amount of gluten might be a trigger for celiac disease in genetically susceptible children.
Populärvetenskaplig sammanfattning

Vad är celiaki?

Celiaki (även kallad glutenintolerans) är en av de vanligaste kroniska sjukdomarna hos barn. Sjukdomen drabbar ca 1% av befolkningen i västvärlden och i Sverige beräknas upp emot 2% av befolkningen ha celiaki. Personer med celiaki tål inte gluten som finns i sädeslagen vete, råg och korn. Den enda kända behandlingen idag består av livslång glutenfri kost.


Celiaki är starkt kopplat till HLA-komplexet som är beläget på kromosom 6 och specifikt då till haplotyperna (en del av en kromosom) DQ2 eller DQ8 HLA (humant leukocytantigen) som kodar för molekyler som uttrycks på antigenpresenterande celler, vilka i sin tur aktiverar T-hjälpceller i immunförsvaret. Närmare 90–95 procent av alla celiakipatienter är bärare av DQ2 och resterande av DQ8. De specifika HLA-generna är således nödvändiga för att celiaki ska utvecklas, men endast en mindre andel av bärarna utvecklar sjukdomen. Man brukar säga att HLA bidrar med cirka 40% av den ärfliga risken för celiaki. Den som har en nära släktning med sjukdomen löper ytterligare ökad risk för sjukdomen. Om ens förälder, syskon eller barn har fått diagnosen ökar den egna risken med 10-15%.

Hur gick forskningen till?

Alla studier som ingår i denna avhandling är baserad på barn, deltagare i forskningsstudien The Environmental Determinants of Diabetes in the Young
Studien är en multicenterstudie som genomförs i fyra länder, Finland, Tyskland, Sverige och USA (i delstaterna Colorado, Washington och Georgia). Studien följer ett gemensamt protokoll där barn som screenats positivt för HLA-typer associerade med ökad risk för typ 1 diabetes och celiaki bjuds in att delta i en 15-årig uppföljningsstudie. Under perioden 2004-2010, screenades 424,788 barn och bland dessa var 21,589 (5%) bärare av någon av de specifika HLA-typerna. Totalt accepterade 8,676 (40%) familjer att deras barn kunde delta i uppföljningsstudien. Barnen följs med regelbundna besök på sin TEDDY-mottagning, var 3:e månad upp till 4-års ålder och därefter 2 gånger per år. Vid varje besök görs standardiserade intervjuer, upprepade blodprover som analyseras för förekomst av autoantikroppar mot betacellsautoantigen samt från två års ålder årligen för transglutaminas autoantikroppar (tTGA). En förhöjdd nivå av dessa tTGA kan tyda på att individen har celiaki. Information om bland annat amning, introduktion av nya livsmedel till barnets kost samlas in vid varje besök.

Vilka är resultaten och slutsatserna?

Syftet med denna avhandling är att studera spädbarnskostens påverkan på risken att utveckla celiaki. I arbete I ville vi studera förekomsten av celiaki autoimmunitet (CDA) och celiaki hos barn, bärare av HLA DQ2 och/eller DQ8 genotyper upp till 5-års ålder. Vi studerade effekterna av HLA-typ, kön, första grads släkting med celiaki och bosättningsland för risken att utveckla celiaki. Barn homozygota för haplotypen DQ2 hade sex gånger så hög risk att utveckla CDA och celiaki i tidig ålder jämfört med barn som bar på standardrisk genotypen. Andra icle-genetiska variabler som kön (flicka) och att ha en släkting med celiaki var också betydande riskfaktorer. Risken att utveckla CDA och celiaki var störst i Sverige jämfört med andra länder, även efter att ha justerat för HLA, kön och FDR med celiaki.

I arbete II, ville vi beskriva den tidiga spädbarnskosten i studiepopulationen samt hitta sociodemografiska faktorer som var associerade med kort amningslängd och tidig introduktion av fast föda. Vi fann att amningslängd och tidpunkt av introduktion av fast föda skiljer sig mellan länder. Europeiska barn introduceras först till potatis- och rotfrukter medan amerikanska barn introduceras först till ris och rotfrukter. Cirka 20% av studiepopulationen introduceras till fast föda före 4 månaders ålder, 27% i Finland och 10% i USA. Faktorer associerade till tidig introduktion var mammans ålder (< 25 år vid barnets födelse), låg utbildningsnivå (kortare än 12 års skolgång), och rökning under graviditeten. Samma faktorer var också associerade vid kort amningslängd (både exklusiv amning samt total amningslängd).

I arbete III ville vi undersöka om tidpunkten för glutenintroduktion var associerat med risk för CDA och celiaki hos barn med genetisk ökad risk att utveckla celiaki.
Vi fann stora skillnader mellan länderna vad gäller tidpunkt för glutenintroduktion. Baserat på fynden i arbete I, där svenska barn har den största risken att utveckla CDA och celiaki, så var den ökade risken inte associerad med tidpunkt för glutenintroduktion, trots att de svenska barnen introducerades tidigast. Svenska barn introducerades till gluten vid 5 månader ålder jämfört med 7.5 mån i Tyskland, 7 mån i USA och 6.5 mån i Finland. Resultaten från arbete III bekräftar tidigare studier som visar att varken amningslängd eller tidpunkten för glutenintroduktion påverkar risken för celiaki.

I sista arbetet, delarbete IV, ville vi undersöka om mängden gluten som de svenska barnen ätit under de två första levnadsåren var en riskfaktor för utveckling av celiaki. I en fall-kontroll studie där totalt 517 barn ingick kunde vi se att en hög mängd gluten ökade risken för celiaki. I jämförelse med friska barn rapporterade barn med celiaki ett högre intag av gluten, detta speciellt tydligt vid 12-månaders ålder men även vid 18 och 24 månaders ålder. Det har föreslagits att individer bärare på den HLA-typ som innebär högst risk att utveckla celiaki (homozygota för HLA-DQ2) skulle vara extra känsliga för ett högt glutenintag. Vi studerade om mängd gluten hade olika påverkan på risk att utveckla celiaki beroende på HLA-typ. Det rapporterade intaget delades upp i tertiler (låg, mellan och hög). Risken att utveckla celiaki var lika hög bland de som åt ett högt intag (> 5 gram per dag) oavsett HLA-typ. Vi kunde även se att bland de barn som hade rapporterat det största glutenintaget även utvecklade tTGA tidigare än de som åt mindre mängd gluten. Barn som åt mer än 5 gram gluten per dag hade en dubbelt så hög risk att utveckla celiaki jämfört med de barn som åt en mindre mängd.

Slutsatsen är att HLA-typ är en mycket stark riskfaktor för utveckling av celiaki. Svenska barn har en högre risk att utveckla sjukdomen jämfört med amerikanska barn. Spädbarnskosten kan spela roll då vi ser skillnader mellan länder. Varken amning eller tidpunkt för glutenintroduktion är i denna studiepopulation riskfaktorer för celiaki, däremot verkar det som om mängd gluten i kosten hos svenska barn spelar en stor roll för utveckling av sjukdomen.

Nästa steg är att inkludera barn från övriga länder i TEDDY-studien och med en längre uppföljningstid studera om barn med celiaki har åtit mer gluten än friska barn. Det finns då en möjlighet att undersöka om svenska barns glutenintag skiljer sig från barn i övriga länder och därmed slå fast om glutenintag (mängd och typ av gluten) är en oberoende riskfaktor för celiaki.
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