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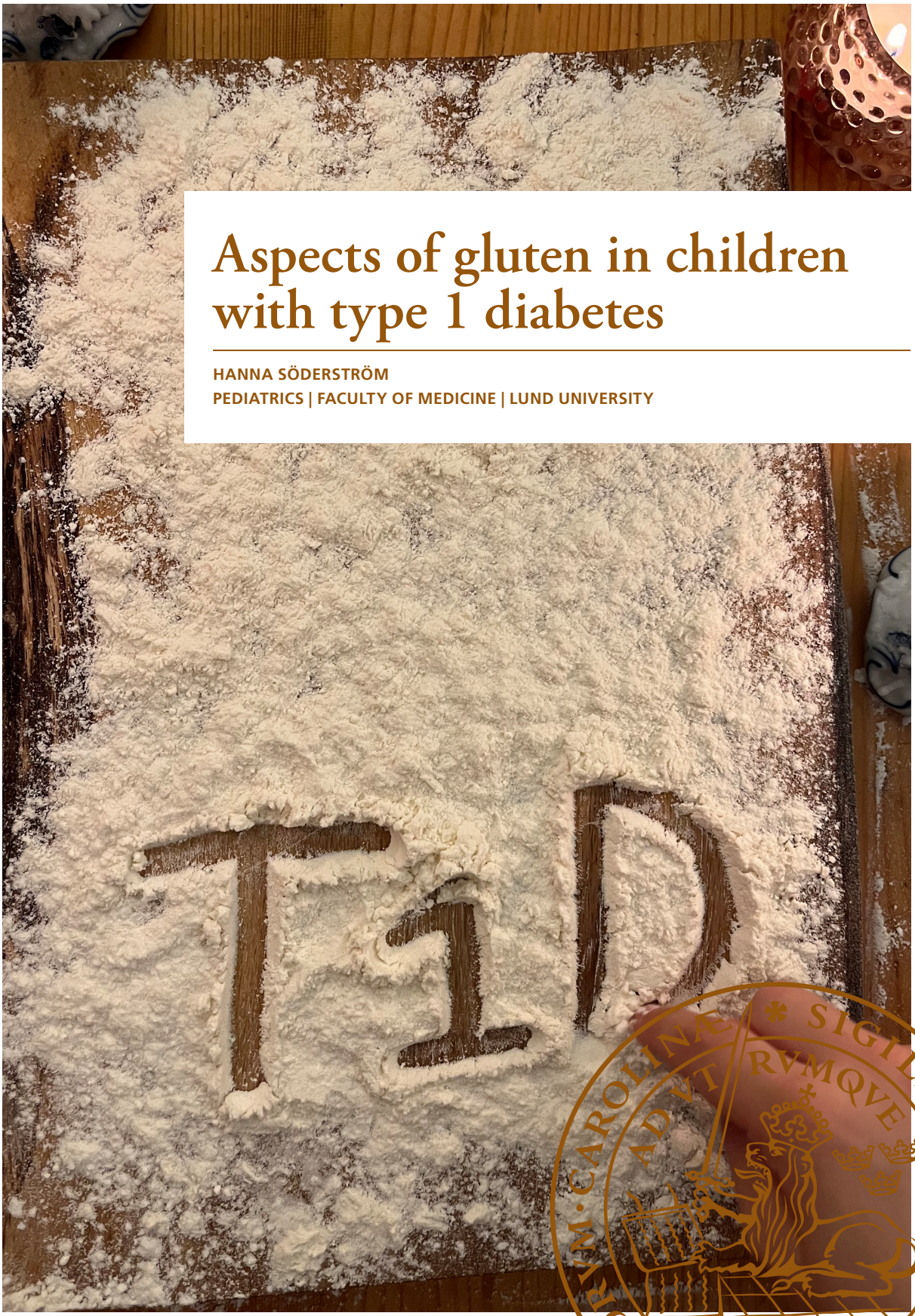

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Aspects of gluten in children with type 1 diabetes

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LUND UNIVERSITY

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Abstract

Background: Over the past two decades, there has been increasing evidence of an association between type 1 diabetes (T1D) and gluten. A gluten-free diet (GFD) has been linked to a decreased risk of T1D in mice, and a higher gluten content in the diet of pregnant women has been linked to an increased risk of T1D in offspring. Furthermore, gluten has been found to be more immunogenic in patients with T1D, and a case study showed that a boy newly diagnosed with T1D who started a GFD could stop insulin treatment.

Aim: To study whether GFD is associated with glycaemic control and growth in children with T1D with and without CD. To study whether the prevalence of CD in children with T1D was affected by the Swedish celiac epidemic. To study compliance with a GFD in children with CD and T1D and whether it is associated with glycaemic control and growth.

Methods: In Study I, children with newly diagnosed T1D were invited to a two-armed clinical trial with the choice of 12 months of GFD or to continue a normal diet (ND) while being evaluated for remaining insulin production (C-peptide), glycaemic control (HbA1c and IDAA1c), growth (BMI-SDS), adherence to GFD (Greens Questionnaire), and quality of life (QoL). Results were compared groupwise. In Studies II and III, cohorts of children with T1D registered in the BDD study (a study that registers >90% of children with T1D in Sweden) were followed for 5 (Study II) and 7 (Study III) years respectively. In Study II, children with T1D and CD were compared to children with T1D without CD for glycaemic control (HbA1c), diabetic ketoacidosis (DKA), and growth (BMI-SDS). In Study III, children with T1D and CD were evaluated for compliance with a GFD using transglutaminase immunoglobulin A antibody (Ttg-IgA) levels. Groups of different compliance levels were then compared in relation to glycaemic control and growth. In Study IV, we compared the prevalence of CD in children with T1D in two Swedish national cohorts: one cohort with children born during the Swedish Celiac Epidemic, and one cohort born after the Swedish Celiac Epidemic. The cohorts were constructed by merging information from five different national registries for the identification of cohort and diagnosis (The Swedish Longitudinal Patient Quality Register for Childhood Diabetes (Swediabkids), The National Diabetes Register (NDR), The Swedish National Patient Register (SNPR), Statistics Sweden, and the Better Diabetic Diagnostics Study (BDD-study)).

Results: In Study I, children with T1D and GFD had significantly lower HbA1c levels at 6 months compared to controls. HbA1c at 12 months and IDAA1c at 6 and 12 months were not significantly better; however, group-wise comparison using Rank-Biserial Correlation showed moderate effect sizes, indicating better glycaemic control in the GFD group. Ninety% of the children with GFD showed satisfactory adherence to the GFD. There were no significant differences in QoL between the groups. In Study II, no differences were found in HbA1C or DKA between children with T1D and CD compared to children with T1D without CD, but BMI-SDS was significantly decreased in children with CD (diagnosed before and at T1D diagnosis) during the first 2-5 years of follow-up after T1D diagnosis, compared to controls. In Study III, 68% of the children showed satisfactory compliance. Better compliance was significantly associated with lower HbA1C and no DKA after T1D diagnosis. In Study IV, no differences in CD prevalence in children with T1D were observed between the cohorts.

Conclusions: In Study I, we found that strict GFD can be maintained in children with T1D and may have positive effects on glycaemic control. In Study II, we found no difference in glycaemic control in children diagnosed with CD and T1D, but a decreased growth in the first years following CD diagnosis compared with children with T1D without CD. In Study III we found that children with T1D and CD had lower compliance with a GFD than previously reported in children with CD in general. We also found that better compliance was associated with better glycaemic control and less DKA.

In Study IV, we found that the prevalence of CD was not increased in children with T1D during the Swedish celiac epidemic in contrast to the prevalence of CD in Swedish children in general.

Key words type 1 diabetes ,celiac disease, glycaemic control, growth gluten, gluten free diet, compliance

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När jag blir nyfiken står tiden still

Table of Contents

List of publications

This thesis is based on the following papers, which will be referred to in the text using Roman numerals that have been appended at the end of this thesis.

I. **Söderström H**, Cervin M, Dereke J, Hillman M, Tiberg I, Norström F, et al. Does a gluten-free diet lead to better glycemic control in children with type 1 diabetes? Results from a feasibility study and recommendations for future trials. Contemporary Clinical Trials Communications. 2022;26:100893.

II. **Söderström H**, Lindgren M, Elding Larsson H, Forsander G, Cervin M, Carlsson A. Glycemic control and growth in children with Type 1 diabetes, with and without celiac disease. Longitudinal National Cohort Study. Manuscript

III. **Söderström H**, Rehn J, Cervin M, Ahlstermark C, Bybrant MC, Carlsson A. Compliance to a gluten-free diet in Swedish children with type 1 diabetes and celiac disease. Nutrients. 2021;13(12):4444.

IV. Bybrant MC, Palmkvist E, **Söderström H**, Lingren M, Hildebrand H, Norström F, Carlsson A. The prevalence of having coeliac disease in children with type 1 diabetes was not significantly higher during the Swedish coeliac epidemic. Acta Paediatrica 2023 Oct;112(10):2175-2181.

Acronyms

T1D	Type 1 Diabetes
CD	Celiac Disease
GFD	Gluten-Free Diet
ND	Normal Diet
HbA1c	Haemoglobin A1c
IDAA1c	Insulin Dose-Adjusted A1c
HLA	Human Leukocyte A
MMTT	Mixed Meal Tolerance Test
QoL	Quality of Life
p-glu	Plasma glucose
DKA	Diabetes Ketoacidosis
BMI	Body Mass Index
Ttg-IgA	Tissue transglutaminase IgA-antibodies
EMA	Endomysial antibodies
DGP	Deamidated Gluten Peptides
AGA	Gliadin antibodies
CI	Confidence Interval
ICD	International Classification of Diseases
NDR	The Swedish National Diabetes Register
Swediabkids	The Swedish National Pediatric Diabetes Register
BGL	Blood glucose level
ER	Endoplasmic reticulum
APC	Antigen presenting cell
ESPGHAN	The European Society for Paediatric Hepatology and Nutrition
FU	Follow-up
GIP	Gluten Immunogenic Peptides
GWAS	Genome Wide Association Study

Introduction

Diabetes

Type 1 diabetes is a common chronic disease in children and its incidence is rapidly increasing worldwide (1-3). Management is lifelong, involves burdensome daily treatment, and complications range from mild to life-threatening (4). T1D is an autoimmune disease in which a combination of genetic and environmental risk factors seems to be involved. However, the main triggering cause has yet to be discovered. Several environmental factors such as viral infection (5), dairy products (6), polyunsaturated fatty acids (7, 8), hygiene in infancy, and gluten consumption (9) have been proposed. Animal studies have shown promising results that diminish the risk of T1D in NOD mice fed a gluten-free diet (GFD) (10-12), but few studies have been conducted on humans (13, 14). In search of a deeper understanding of T1D aetiology and clinical course, this thesis aims to further explore the relationship between T1D and gluten.

History

Ancient descriptions of diabetes are found in Egyptian papyrus scrolls as well as in the Vedas and dates back at least 3500 years. Throughout the millennium, a wide range of theories of aetiology and treatments have replaced one another, but until a century ago, pathology and treatment remained elusive. Then, in 1889, Merging and Minkowski discovered the role of the pancreas and, in 1921, insulin was discovered. Only one year later, it was purified from the pancreas of a cow and, finally, an effective treatment was available (15). However, its aetiology remains largely unknown.

Classification of Diabetes

Approximately 540 million people are estimated to have diabetes mellitus worldwide (16). The disease is characterised by hyperglycaemia due to insufficient insulin secretion, insulin resistance, or a combination of both. Diabetes mellitus is mainly subdivided into type 1 diabetes (T1D) and type 2 diabetes (T2D). However, several other subtypes also exist.

Out of all patients with diabetes, 90% have T2D (17). In 2017, approximately 426 million individuals were diagnosed, corresponding to 6.28% of the global population (18). T2D is a metabolic disorder characterised by insulin resistance in combination with an inadequate compensatory insulin response, initially usually treatable through lifestyle and diet changes (17).

T1D, the second largest subtype, is an autoimmune disease in which the loss of insulin production leads to lifelong dependence on exogenous insulin. During childhood, T1D is the most common type of diabetes in the Western world (16), accounting for over 90% of childhood diabetes globally (19). In Sweden, approximately 95% of all children with diabetes are diagnosed with T1D (20).

Figure 1. Diabetes is most likely a continuum in which the autoimmunity of type 1 diabetes represents one end of the spectrum and the metabolic dysfunction of type 2 diabetes represents the other end. Reprinted from Molecular and Cellular Endocrinology, Volume 382, 2014, Leif Groop et al, Genetics of diabetes – Are we missing the genes or the disease? with kind permission from the Elsevier (21).

Although historically clearly separated, research is beginning to look at the two first groups more in terms of conditions on a continuous spectrum (21, 22), see Figure 1 for an illustration. The US-based SEARCH study of children with diabetes found 54.5% of them had autoimmunity and insulin sensitivity (T1D), 15.9% had non-autoimmunity and insulin resistance (T2D), 10.1% had non-autoimmunity and insulin sensitivity and 19.5% had autoimmunity and insulin resistance (T2D superimposed on T1D) (23). Nevertheless, classification is key to determining therapy and adapted education (22).

A third important category is monogenic diabetes (originally called *maturity onset of diabetes in the young* (MODY). Monogenic diabetes is a group of monogenic disorders in which different genes involved in the development or functions of the β cell are often affected. The largest category is MODY with at least 0.5-5% of non-autoimmune diabetes (24). Different types of MODY are often misdiagnosed as T2D or T1D but are important to identify because treatment and prognosis often differ (25).

Another important subgroup of monogenic diabetes is neonatal diabetes (often onset of diabetes under six months of age).

Other less common causes of diabetes in childhood are cystic fibrosis-related diabetes, haemochromatosis, and secondary diabetes induced by drugs and toxins (22). See Figure 2 for the proportion of diabetes diagnoses.

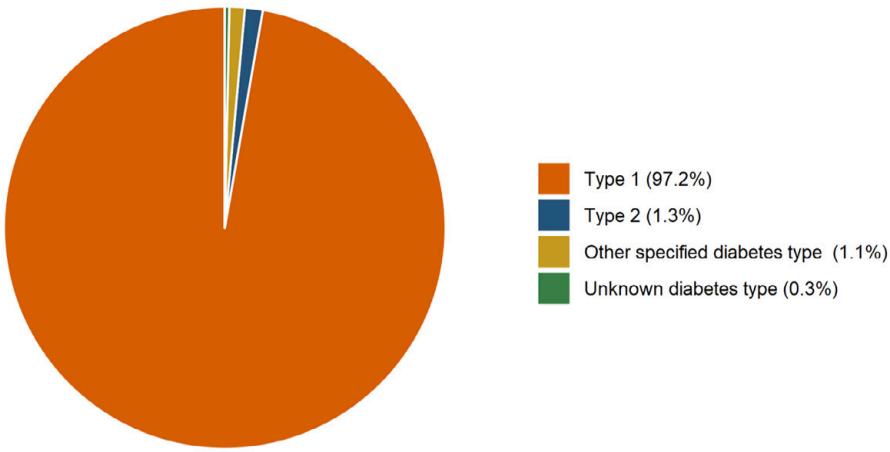

Figure 2. Proportion of diabetes diagnoses in Swedish children in 2020. Reprinted with kind permission from the Swedish National diabetes register Swediabkids (20).

Type 1 Diabetes

Epidemiology

T1D predominately develops in childhood but may develop over the entire lifetime (16). T1D is one of the most common, severe, and chronic diseases affecting children worldwide. In 2022, the prevalence was 1.2 million children (16) and the incidence is increasing (2, 3), with an annual increase of approximately 3-4% (1). However, the global prevalence shows large differences. See Figure 3 for a map of incidence rates of type 1 diabetes in children. Sweden has the world's second highest incidence rate (1). The risk is also high in other Western countries, such as in Northern Europe, Australia, New Zealand, the USA and Canada, but also in Kuwait, Saudi Arabia and Algeria, while the lowest risk is observed in East and Southeast Asia. However, the incidence pattern has changed recently. Countries with a low prevalence have the highest increase in incidence rate (1). This is a highly problematic development because these countries often have limited resources. In

low-income countries, life expectancy is 13 years after diagnosis in a 10 year old, compared to 65 years in high-income countries (26). In countries with a high prevalence, the increase in incidence seems to be levelling off. In Sweden the incidence of T1D more than doubled in the 1980s and 1990s but seems to have tapered off over the last two decades (27).

T1D is equally distributed by sex, except in the age group above 15 years, where male predominance has been noted (28). An age peak in incidence has been observed around puberty (29), and the incidence also increases during the colder season (30).

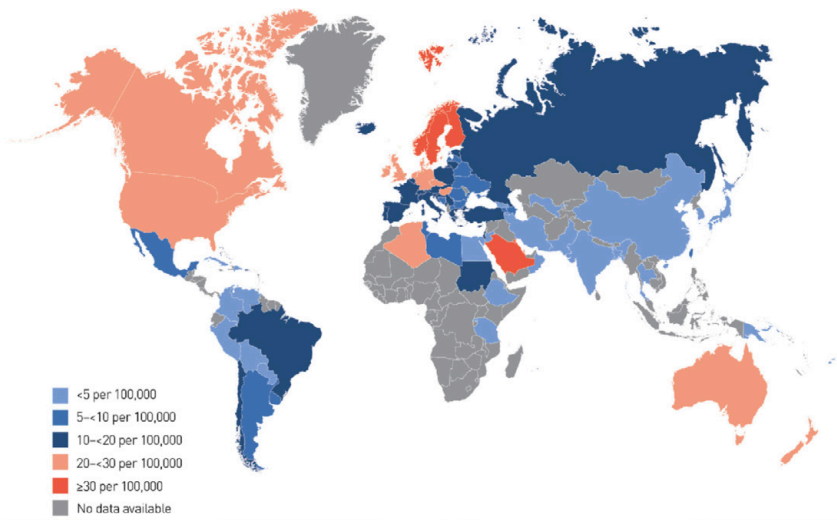

Figure 3. Map of age-sex standardised incidence rates (per 100,000) from publications of type 1 diabetes in children aged under 15 years. Reprinted from Diabetes Research and Clinical Practice, Vol 157, 2019, Christopher C. Patterson et al, Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas, 9th edition, reprinted with kind permission from Elsevier (3)

Aetiology

The personal burden of heavy treatment and the risk of serious complications, in combination with a globally rising incidence, highlight the need to search for possible prevention of T1D. To prevent T1D, we need to better understand its aetiology. It is well known that genetics plays an important role. The most strongly associated risk genes are in the HLA region of chromosome 6. Genes in the HLA region are considered to contribute to approximately 50% of the genetic risk (21). These haplotypes encode antigen-presenting cell surface receptors. The highest risk haplotypes are HLA-DQ2 and HLA-DQ8. Having one or both of these high-risk haplotypes corresponds to > 90% of children with T1D in Sweden (31). Children

with both high-risk haplotypes (HLA-DQ2/HLA-DQ8) have the highest risk of developing islet autoimmunity and T1D (32). However, genes in the HLA region are not the only genes that confer the risk of T1D. Gene-wide association studies (GWAS) have identified more than 60 other risk loci that are also associated with an elevated risk of T1D (33-35).

Although important, genetic risk is not considered to contribute to T1D alone. Several reasons for the additional environmental aetiology have been proposed.

1. Only 10% of children with HLA-conferred diabetes susceptibility proceed to manifest T1D (36)
2. Lifetime risk of T1D in monozygotic twins has been estimated to be around just 70% (37, 38)
3. The incidence of T1D increases at a pace that is far too fast for genetics to be solely responsible (39).
4. The incidence of T1D for migrants moving from a low-risk region to a high-risk region increases (40).

Various possible environmental factors have been studied. However, studies have reported contrasting results.

Dietary factors have been proposed to play a role in T1D. Some researchers have found that breast milk has a protective role (5, 6), while other researchers have found no such connection (41). A more specific protective association was found in those children who were still breastfed while being introduced to cereals (42).

The role of cow's milk has been widely studied, with contrasting results. Several studies have reported no association between early exposure and T1D risk (6, 41). Even so, some studies have found that a higher intake is associated with an increased risk of progression to T1D (43, 44) while another study has shown a decreased risk (45).

Vitamin D has been shown to downregulate the T helper 1 immune response (46). Since this cell is thought to play an important role in the destruction of the β cell in the development of T1D, it has led researchers to examine it as a potentially protective factor. Some studies have focused on the vitamin D levels in pregnant women. One study showed an association between higher levels of vitamin D during pregnancy and a lower risk of T1D in offspring (47), whereas another study found no such connection (48). Other studies have focused on later T1D development. A meta-analysis found that D supplementation in infants was associated with a lower risk of T1D (48), whereas other studies observed no such connection (45, 49).

Polyunsaturated fatty acids have been suggested to play a protective role against T1D. One study showed an association between a higher risk of islet autoimmunity

and lower concentrations of omega-6 fatty acids (7), while another study demonstrated that a higher intake of omega-3 fatty acids was associated with a lower risk of islet autoimmunity and T1D (8).

Some researchers have suggested a link between higher gluten intake and an increased risk of T1D. This subject is explored further in a separate section.

The composition of intestinal microbiota has been associated with T1D risk. (50-52). For example, children with islet autoimmunity have been shown to have lower microbial diversity than healthy children before the progression to T1D (50, 52). The microbiota is particularly interesting because its composition could be influenced by several factors proposed to play a role in the development of T1D, such as different types of diets, or other factors behind the "hygiene hypothesis."

The theory that improved hygiene causes a decrease in childhood infections which, in turn, leads to an increase in autoimmune diseases, such as T1D, is called the *hygiene hypothesis*. However, there is little evidence of its accuracy. One study reported no association between infection and T1D (53). Other studies even showed a higher number of infections associated with an increased risk of T1D; one study showed a higher risk of T1D with an increased number of early respiratory infections (54) and another study linked a higher risk of T1D to more enterovirus-induced gastrointestinal infections (55). Several viral infections have been suggested to cause T1D but, thus far, enterovirus provides the most compelling evidence, with many studies reporting an association between enterovirus, islet autoimmunity and T1D (5). For example, evidence of low-grade persistent enteroviral infections has been found in children recently diagnosed with T1D (56).

The accelerator theory proposes that T1D is linked to high birth weight and early rapid weight gain (57-59). The theoretical explanation could be that weight gain leads to insulin resistance and high BGL which, in turn, would lead to β-cell stress and then to T1D. This hypothesis would also be a suitable explanation for the increased incidence of T1D in recent decades due to an increase in childhood overweight worldwide (60). It could also explain some of the associations between T1D and T2D (23).

In summary, although not fully understood, genetics factors, and most probably also environmental factors play a role in the development of T1D. unknown.

T1D Development

In T1D, insulin-producing β cells in the islets of Langerhans in the pancreas are ultimately destroyed by the immune system, leading to insulin deficiency and hyperglycaemia. The development of T1D is thought to be a lengthy process, and the disease pathology can be described in three separate stages. (61, 62). See Figure 4 for an illustration of the development of T1D.

1. Two or more diabetes-associated autoantibodies
2. At least two diabetes-associated autoantibodies and elevated BCL without apparent symptoms
3. Two or more diabetes-associated autoantibodies, insulin deficiency and hyperglycaemia - meeting the criteria for T1D diagnosis.

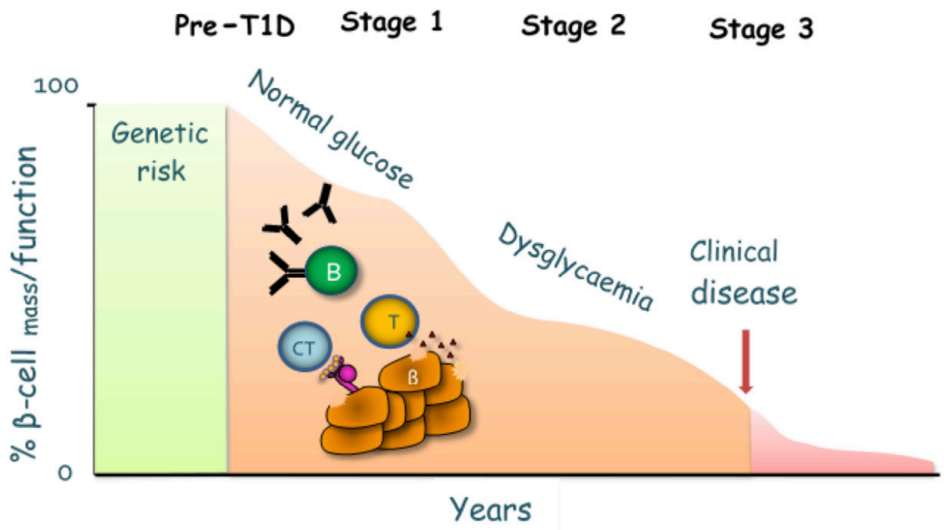

Figure 4. Progression to T1D. A possible environmental trigger can activate an autoimmune response in individuals with a genetic predisposition to T1D, resulting in the production of autoantibodies followed by insulitis which impairs β-cell function, leading to clinical disease onset. Published with kind permission from Dr Sefina Arif.

Stage 1: The first step is the prodromal, asymptomatic phase, with the appearance of autoantibodies. Four specific islet autoantibodies are known to precipitate T1D: glutamic acid decarboxylase 65 autoantibodies (GAD) autoantibodies, tyrosine phosphatase-related islet antigen 2 (IA2), insulin autoantibodies (IAA), and Zinc Transporter 8 Antibodies (ZnT8). These autoantibodies have been shown to appear months to years before any clinical signs of T1D. Not all individuals with one autoantibody develop T1D. However, there is a clear increased risk associated with an increased number of autoantibodies. If positive for two or more antibodies, the 10-year risk of T1D has been shown to be 70% (63), and the 15-year risk to be at least 85% (64), whereas the same risk if positive for only one islet autoantibody is only 15% (63). Despite evidence of the involvement of autoantibodies, their exact function in the development of T1D remains unclear.

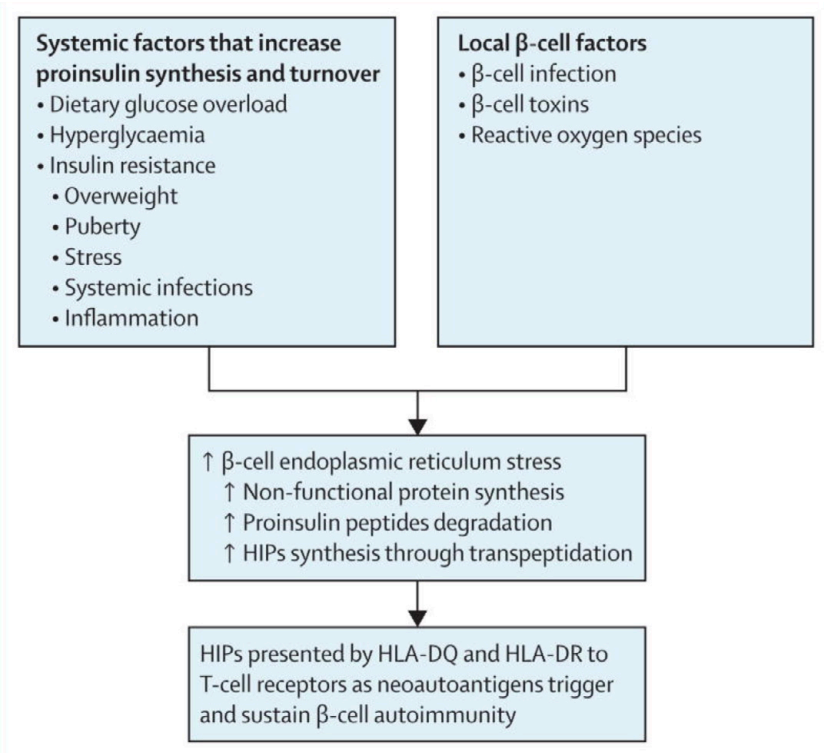

Figure 5. A unified model between environmental factors, β-cell endoplasmic reticulum stress, generation of neoautoantigens (HIPs), and loss of immune tolerance that triggers islet autoimmunity. (HIP= hybrid insulin peptide) Reprinted from The Lancet, Volume 387, 2016, Marian Rewers et Prof. Johnny Ludvigsson, Environmental risk factors for type 1 diabetes, reprinted with kind permission from Elsevier (65).

Stage 2: In the next stage, there are still no clear symptoms, but glucose intolerance with occasional hyperglycemia is present, in addition to autoantibodies. This stage is characterised by a loss of β cells. At stage 2 of T1D -development, the lifetime risk of T1D diagnosis is thought to be 100% (61).

During this stage, the β-cell stress theory proposes that different environmental factors of increased insulin demand, such as infections, psychological stress, overweight, rapid growth, low physical activity and diet with a high GI, stress the β cells (59). This type of stress can affect the endoplasmic reticulum (ER) of the β cell, leading to lower insulin synthesis and β cell apoptosis (66) A quite recent model aimed to explain the link between β-cell stress and autoimmunity is post-translation modification of islet proteins (such as insulin and pro-insulin) (65, 67, 68). In such a process insulin can be presented with new epitopes and proinsulin can be modified to hybrid insulin peptides (HIPs). Neoantigenic epitopes of insulin as well as HIPs are considered neoautoantigens, and while there is no immune tolerance to these, they lead to autoimmunity (69). See Figure 5 for a unified model

between the environmental factors, β-cell endoplasmic reticulum stress, generation of neoautoantigens (HIPs), and loss of immune tolerance that triggers islet autoimmunity. Post-translational modifications have also been observed in other autoimmune diseases, such as celiac disease, multiple sclerosis, rheumatoid arthritis, and lupus erythromatosis (70).

Stage 3: In the third stage of the development of T1D, β-cell destruction reaches a critical threshold of insulin deficiency, leading to a high, symptomatic BGL. Because of the high BGL and inability to use sugar as an energy substrate, typical symptoms include polyuria, polydipsia, nocturia, enuresis and weight loss, thereby meeting the criteria for T1D diagnosis.

Diagnosis of Diabetes

Diagnostic criteria for childhood diabetes according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) (22) are the presence of overt symptoms (polyuria, polydipsia, nocturia, enuresis and weight loss) and an elevated blood glucose level (BGL) of

1. random plasma glucose ≥11.1 mmol/L (200 mg/dl) or
2. fasting plasma glucose ≥7.0 mmol/L (≥126 mg/dl)

If the diagnosis of diabetes is unclear, for example, because of missing symptoms or acute stress, repeated BGL testing is required. The presence of islet autoantibodies confirms the T1D diagnosis, although negative autoantibodies do not exclude type 1 diabetes (71).

Treatment

Insulin is needed to lower the BGL and transport sugar into the cells, where it can be used as energy. When the criteria for T1D are fulfilled, the patient's own insulin production is usually so low that insulin replacement is immediate required. Thus, T1D treatment comprises lifelong, substitution of insulin. Good glycaemic control has been shown to be of great importance to reducing acute complications, such as diabetic ketoacidosis (DKA) and hypoglycaemia, as well as long-term complications, such as micro and macrovascular disease (72-74). Physical activity and diet also influence glycaemic control and, in combination with meticulous blood glucose monitoring, are an important part of T1D treatment.

Even if most patients with newly diagnosed T1D do not have sufficient endogenous insulin production, the variability rate of decline in β-cell function is high. Some patients can still have some function years or even decades after T1D diagnosis.

Such function has been shown to be very valuable as the remaining insulin production has been linked to better metabolic control and fewer long-term complications, such as nephropathy, neuropathy and retinopathy (75). To prevent further metabolic decompensation, insulin treatment should start right at diagnosis. The β cells of the pancreas in healthy individuals secrete continuous low-level insulin, and a temporary high-level insulin after meals to maintain a normal BGL (76). Thus, insulin replacement treatment should mimic physiological patterns as closely as possible. Basal and prandial insulin should be administered via multiple daily injections or pumps (77). Increasingly more technological devices are being used. Automatic pumps in tandem with automatic blood glucose measurements are available in wealthier countries, such as Sweden.

Despite the use of insulin and the latest devices, T1D is a burdensome disease with potentially dangerous short and long-term complications, even in children with excellent access to insulin, the latest devices, and care from their families and diabetic teams. For children in low-income countries, who often have limited access to insulin and self-management education, T1D can lead to severe disability and early death (3, 78)

Gluten

Gluten is the Latin word for glue, referring to its characteristics of holding grains together and giving bread and pasta a flexible and chewy nature. Another distinctive property of gluten is its ability to make bread rise. Gluten is heat stable and, in combination with its binding and extending capacity, it is also often used in foods other than bread, pasta and patisseries. For example, it is used to improve texture and retain moisture in processed foods such as vegetarian meat substitutes, reconstituted seafood and processed meat. Gluten is also used as a thickener, emulsifier and gelling agent in confectionery butter, ice cream and coatings (79).

History

Gluten has been part of the human diet for the last 10,000 years, ever since humans first began harvesting grains in the Fertile Crescent of Assyria, Mesopotamia and Egypt (80). Since wheat was first domesticated, over 25,000 accessions have been developed.

Importance

Cereals containing gluten are an important nutritional source with a protein content of 8–15% (79), see Figure 5 for an approximate breakdown of wheat components.

Approximately 50% of the calories consumed are derived from cereal and the global consumption of wheat is increasing (81). One slice of bread contains around 4 g of gluten and a typical western diet contains approximately 5-20g of gluten a day (79, 82).

"Gluten"

Wheat, barley, and rye contain storage proteins belonging to a family of glycoproteins called prolamins. These glycoproteins are called *prolamins* because of their high levels of proline and glutamine. In wheat, they are called gliadin and glutenin, while the prolamins in barley are called secalins and the prolamins in rye are called hordeins (83). The term "gluten" often includes the prolamins of rye and barely but gluten per se is the prolamin (gliadin and glutenin) of wheat. Glutenin is polymeric and can be subdivided into high and low molecular weights, and gliadin is monomeric and can be further classified into: α- gliadin, β-gliadin, γ-gliadin and ω-gliadin (84).

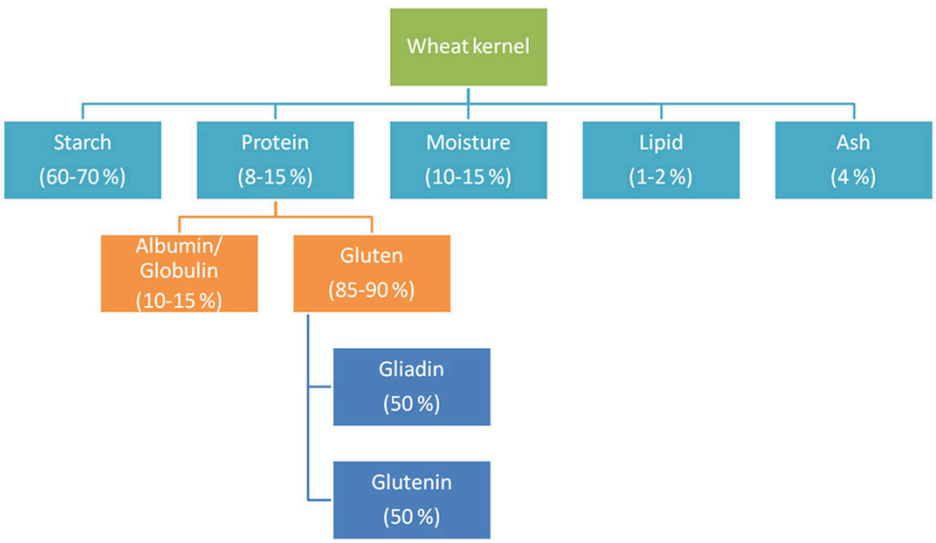

Figure 6. Approximate breakdown of wheat components. Reprinted from Journal of gastroenterology and Hepatology, Volume 32, 2017, Jessica R Biesiekierski, What is Gluten?, with the kind permission from Wiley. (79)

The prolamins of wheat, rye and barley differ from the proteins found in other types of cereal as they have a higher molecular mass and are present in larger amounts, but also by their previously mentioned high content of proline (20%) and glutamine (38%). Proline and glutamine create tight and compact structures that many

proteases cannot cleave (85). Proline and glutamine are hydrophobic repeated sequences that are insoluble in water. “Gluten” can be prepared by washing dough in water, which removes soluble and particulate matter and leaves a stretchy mass (86, 87).

Except for being a globally important protein source and a valuable ingredient in various foods, gluten is known to be involved in several diseases, including gluten ataxia, dermatitis herpetiformis, wheat allergy, non-celiac gluten sensitivity and celiac disease (79).

Celiac Disease

History

The first modern description of celiac disease (CD) was provided by Gee Samuel in 1887. Gee described CD as a chronic disease characterised by abdominal distension and muscular weakness that could only, if at all, be cured by diet. Many different diets were tried, but the specific connection to a protein component in wheat, barley and rye called *gluten* was not made until 1950, when it was published in Dr. Dickes’s PhD dissertation (88).

Epidemiology

CD is a recognised global health issue. The estimated prevalence of CD is approximately 1% (89) and the overall incidence of CD is increasing at a rate of 7.5 %/year (90). Awareness of CD has also increased. Even so the increase is believed to be true (91).Many researchers have attempted to ascertain the reason for this increase. However, no reliable data have been presented.

There are considerable geographical differences, with a higher prevalence in Northern Europe and the lowest prevalence in South America (89). These geographical differences are believed to be due to genetic and environmental differences.

Girls have a 1.5 times higher prevalence than boys, whereas children have a 50% higher prevalence compared to adults (89).

Aetiology

CD is a chronic autoimmune disorder caused by the intake of dietary gluten in genetically predisposed individuals (83).

Genetics is an important factor in CD. This is clearly demonstrated in twin studies in which monozygotic twins have been found to have a proband-wise concordance rate of 83-86%, while the corresponding number for dizygotic twins has been shown to be markedly lower, 17-20% (92, 93). The most important genes are found at the DQ locus in the HLA region of chromosome 6 (94). DQ2 and DQ8 are the most important haplotypes. Almost everyone with CD has either one or both of these haplotypes: DQ2 (95%) and/or DQ8 (5%). Patients with CD without one or both haplotypes represent less than 1% of the population (95). However, the important haplotypes DQ2 and DQ8 are also very common in the general population, ranging from 25-40% depending on country, while only 4% of this population develops CD (96) Therefore, because only 4% of genetically susceptible individuals develop CD despite gluten exposure in the general population being close to 100%, additional factors need to be considered.

In addition to exposure to gluten, no environmental causes have been established. Some studies have suggested that the mode of delivery is associated with CD (97), but more recent research has not confirmed such associations (97). Other studies have found connections between the season of birth (98) and gastrointestinal infections, such as rotaviruses (99). Finally, connections between CD and the mode of gluten introduction in infancy have been suggested. The early introduction of gluten during breastfeeding has been proposed to diminish the incidence of CD (100). However, later studies have not been able to confirm such a connection (101-103) but instead, the proposed level of gluten content during weaning seems to be important (104).

The Swedish celiac epidemic

The Swedish celiac epidemic refers to the period between 1984 and 1996. Children born during this period were found to have a fourfold higher incidence of CD than those born before and after the epidemic (105, 106). Interestingly, the national dietary recommendations for infants changed during the same period. During the celiac epidemic, gluten was recommended to be introduced at six months rather than the previously recommended four months. At the same time, the food industry decided to increase the gluten content in infant formula. At the very end of the celiac epidemic, feeding recommendations and the gluten content in infant products changed again to virtually the same recommendations and the same lower levels, as before the epidemic. Gluten was again recommended to be introduced gradually during breastfeeding, and the gluten content in infant products was reduced. Thus, changes in incidence of CD have been associated with parallel changes in national feeding recommendations, such as the timing of the introduction of gluten, the relationship between the introduction of gluten and breastfeeding, and the level of gluten content (105).

Even so, more recent studies have failed to identify any association between CD risk and the timing of the introduction of gluten (101, 102) and the introduction of gluten in relation to breastfeeding (103). However, the *amount* of gluten before two years of age still appears to be associated with an increased risk of CD (104).

Pathogenesis

The digested gluten is broken down into the prolamins: glutenin and gliadin peptides. These peptides trigger the innate immune system by interacting with the epithelial cells in the intestinal mucosa. This interaction triggers the release of the interleukins IL-8 and IL-15. In turn, these interleukins start a cascade of immunologic reaction including T-cell stimulation, enhanced Th1 production of IFN-γ, and activation of cytotoxic cells (107, 108). Gluten-derived peptides, with a high glutamine and proline content, are difficult for proteases in the intestine to break down why they remain quite large. However, gliadin also interacts with CXCR3 receptors in the epithelium, leading to zonulin release which, in turn, leads to an increased intestinal permeability of macromolecules (109).Thus, it is easier for large gliadin and glutenin peptides to cross the intestinal barrier into the lamina propria.

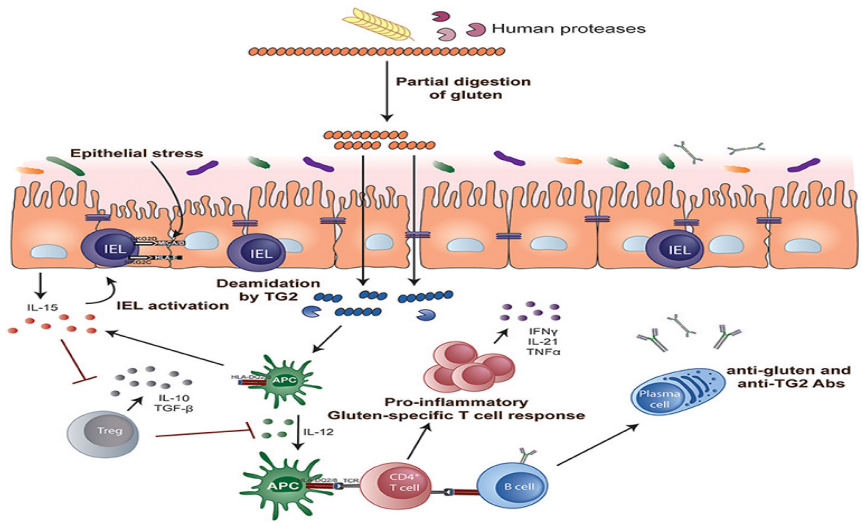

Figure 7. Key steps in the pathogenesis of celiac disease. Reprinted from Frontiers of pediatrics, Volume 6, 2018, Jason A. Tye-Din et al, Celiac Disease: A Review of Current Concepts in Pathogenesis, Prevention, and Novel Therapies, reprinted with kind permission from Frontiers.

In genetically predisposed individuals, prolamin peptides are taken up by antigen-presenting cells (APC) in the lamina propria, where they are presented on the cell surface by HLA-DQ2 and/or HLA-DQ8 molecules (110). In the lamina propria, tissue transglutaminase (tTg) deamidates gliadin peptides to glutamic acid, resulting in more effective HLA-DQ2 antigen presentation (111). These molecules activate CD4+ cells which, in turn, secrete cytokines that initiate a cascade of immunologic reactions with massive activation of T cells and B cells, leading to epithelial damage and further increased intestinal permeability, as well as anti-gliadin and anti-tTg antibodies (Ttg-IgA) (110).

Overall, inflammation causes epithelial damage in the small intestines, which deepens crypts and flattens villi. The typical clinical presentation of CD is malabsorption due to the epithelial changes described above. These symptoms include abdominal pain and distention, vomiting, diarrhoea and failure to thrive. Extraintestinal symptoms include aphthous stomatitis, dermatitis herpetiformis, iron deficiency anaemia, hepatitis and neurological manifestations such as irritability and chronic headaches (110, 112, 113).

Diagnosis

To diagnose CD in children in Sweden, the guidelines of the European Society for Pediatric Gastroenterology, Hapatology, and Nutrition (ESPHGAN) are followed. The measurement of IgA antibodies against transglutaminase 2 (Ttg-IgA) and total serum IgA is recommended as an initial step. In individuals with total IgA deficiency, IgG-based tests such as deaminated gliadin peptide antibodies (DGP-IgG) can be used instead of Ttg-IgA. If Ttg-IgA or DGP-IgG is positive, a second test is required. If the test results are >10 times higher than the lowest positive level, endomysial antibodies (EMA) should be tested. If they are also positive, CD diagnosis can be confirmed without a small bowel biopsy. If Ttg-IgA levels are <10 times higher than the lowest positive level, a biopsy must be performed to confirm the diagnosis of CD (114).

In CD the intestinal mucosa is damaged, leading to crypt hyperplasia, villous atrophy and an increased number of intraepithelial lymphocytes. These changes have been described and classified by Marsh (115). The purpose of biopsies is to histologically identify damage that often is graded using the Marsh-Oberhuber criteria (116).

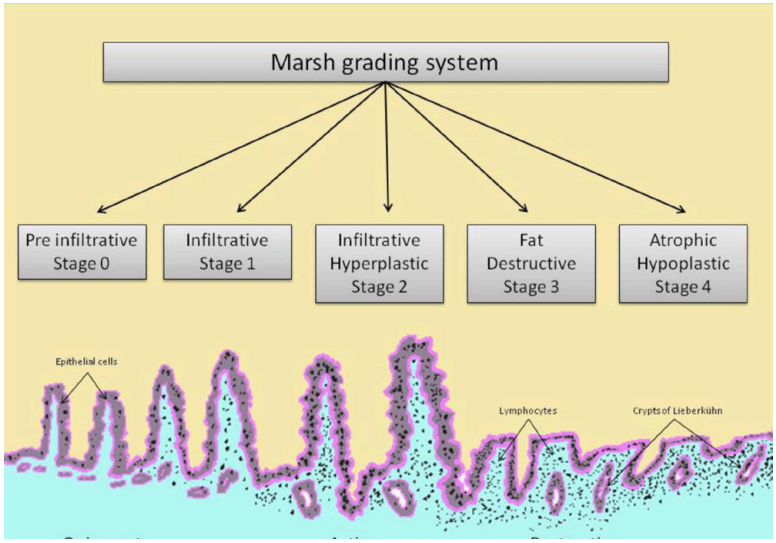

Figure 8. Reprinted with kind permission of H. Rodriguez Requne.

Unfortunately, deciding on who to test is not as straightforward as the testing procedure itself. There is great interpersonal variation in symptoms (117), as well as seemingly symptomless patients (118). Because of this, and because there are well-defined risk groups, it is important to screen high risk populations. These populations include diseases with high comorbidity of CD, such as T1D (119).

Treatment

The only currently available treatment for CD is a lifelong, gluten-free diet (GFD). A GFD offers significant improvements in symptoms and normalisation of antibodies (120). Clinical symptoms are the first to improve, often within two to four weeks. TGA-IgA is closely related to the degree of epithelial damage (121, 122) and often normalises within one year (123). Complete histological recovery is seen after two years in 95% of children (124)

GFD treatment is considered safe, whereas gluten has no great nutritional value. Nevertheless, GFD products may be of lower quality and nutritional value (125). For example, GFD products have been found to contain less thiamine, riboflavin, and niacin than gluten-containing equivalents (126, 127). Another aspect of a GFD is an elevated glycaemic index (GI) (125, 128), which is reflected in the relatively higher dietary GI of children with CD (129, 130).

Compliance

Strict compliance with a GFD is the only recommended treatment in celiac disease. Compliance rates in children with CD vary. Swedish studies of children with CD, symptomatic and screening-detected CD, showed a compliance rate of approximately 90% (131, 132), while compliance in children with screening-detected CD in the US was around just 75% (133). Even if quite high compliance is observed, accidental gluten intake seems common, as observed in one study in 86% of children who were trying to maintain a strict GFD (131).

There are several ways to measure compliance with a GFD. However, there is no gold standard. Because a lot of research have been conducted on new kinds of treatments for CD, recommendations for clinical outcome measures were stiputlated in the Tampere recommendations in 2018. These recommendations list several ways of measuring compliance, including histology, in the form of small bowel biopsies (grade B); serology, of which Ttg-IgA is recommended as being the most reliable (grade B); clinical outcome assessments (grade D); QoL (grade D); and gluten immunogenic peptides (GIPs) (grade D) (134).

Tissue transglutaminase IgA (Ttg-IgA) is an enzyme found in the lamina propria of the intestinal wall. The role of Ttg-IgA in CD pathology is to deaminate gliadin peptides (derived from digested gluten). The deaminated gliadin peptides are more effectively presented by HLA-DQ2, thus promoting autoimmunity to gluten (111). The level of tTg-IgA correlates well with the degree of damage to the intestinal mucosa (121, 122) and is therefore recommended as a measure to follow compliance with a GFD (134).

Growth

Since some of the primary symptoms of CD can be weight loss and short stature, growth is an important measure to follow and the European Society for Paediatric Hepatology and Nutrition (ESPGHAN) recommends that it is included in follow-up visits every six months (135). One way to examine growth is to use measured body mass index (BMI). Although important, there are few data on BMI in children with CD following a GFD. There are few studies, including small sample sizes, showing somewhat conflicting results. One study of 149 children with CD on a GFD found BMI to increase significantly, and that the proportion of overweight children doubled (136). Another study of 150 children with CD on a GFD found that the number of underweight children decreased by almost 50% and the number of overweight children increased by approximately 30% (137). In contrast to these two studies, a third study of 445 children did not find an increase of overweight children with CD on a GFD (138).

Concordant Celiac Disease and T1D

Epidemiology

The prevalence of CD in children with T1D varies across countries, between 1.6-12.2%, pooled prevalence of 6.2% (139). The wide variation is believed to depend on different CD diagnostics, as well as different levels of genetic CD risk in the studied populations (119). In Sweden, the prevalence of coexisting T1D and CD in children is approximately 10% (140, 141). T1D is often diagnosed first. A previous study of children with CD and T1D in Sweden showed that less than 1% of children had CD prior to T1D diagnosis, 3% had CD at T1D diagnosis, and 6% developed CD 1-5 years after T1D diagnosis (141).

T1D is equally distributed by sex (28) and CD is markedly more prevalent in girls compared to boys (90). However, the prevalence of T1D and CD is equally distributed by sex.

Aetiology

Apart from having similar environmental risk factors separately, the combination of T1D and CD is associated with caesarean section, birth in summer, Swedish ancestry, and the female sex (142).

Diagnosis

The presentation of CD in children with T1D can be classical, with gastrointestinal symptoms and failure to thrive, but often presents with only mild gastrointestinal symptoms (143), atypical symptoms or no symptoms at all (140, 144).

The diagnostic criteria for children with T1D are similar to those for children in general, as described above, with reference to the ESPHGAN guidelines from 2020. However, the recommendation to diagnose CD without a biopsy if Ttg-IgA levels are >10 times higher than the lowest positive level does not include children with T1D. Even so, two years after the last ESPHGAN guidelines were published, a Swedish study showed that CD in children with T1D and Ttg-IgA levels >10 times higher than the lowest positive level could also be diagnosed without a biopsy (145).

Screening

Identifying and treating CD is important even if the disease in children with T1D is often considered asymptomatic. One study of children with T1D and screening-detected CD found that 76% of the children actually had mild gastrointestinal symptoms at CD diagnosis and that these symptoms disappeared following a gluten-

free diet (143). Additionally, children with both diagnoses could have a higher vitamin D deficiency, as well as a greater risk of being diagnosed with autoimmune thyroid disease, depression and eating disorders (146-149).

Thus, the international ISPAD guidelines recommend screening for CD during the first year after T1D diagnosis and then at 2-5-year intervals (150, 151). Children in Sweden are screened at diagnosis and annually (141).

Diet and metabolic control

Both CD and T1D are diseases that require dietary treatment. In CD, the treatment is lifelong GFD. A GFD diet often has *higher* GI than ordinary food (125, 128). In T1D, the primary treatment is insulin in combination with tight metabolic control, including a recommended diet with a *low* GI. At the time of diagnosis of CD as well as of T1D, growth is often negatively affected.

It could be speculated that different dietary treatments and the double risk of initial weight loss might affect children with both diagnoses. Studies on the growth of children with T1D and concordant CD have reported divergent results. Some studies have shown impaired growth in children with T1D and CD at CD diagnosis compared to children with only T1D (149, 152-154). Other studies found no differences in growth between groups (155, 156). After the introduction of a GFD, one study found normalised growth within a year (152), in agreement with three recent reviews which concluded that a GFD did not have a negative effect on growth (157-159). In contrary, other studies reported continued decreased growth (149, 154, 160) after 1-9 years of follow up. A large cross-sectional study reported that children with both T1D and CD were lighter and shorter than children with T1D alone (119). Reasons for these opposing results include:

1. Generally small sample sizes (between 11 and 98 cases) resulting in low statistical power.
2. Questionable representativity. In two larger German register studies (149, 154) only 0.6% and. 1.9% were diagnosed with CD of the total T1D population, CD prevalence in this population is thought to be higher.
3. Mostly short follow-up time or cross-sectional design.
4. Usually no data on the time of T1D diagnosis in relation to CD diagnosis.

Because of these inconsistent results, we conducted Study II.

Compliance

CD and T1D are both chronic diseases requiring lifelong daily treatment, and the double burden of having both diseases has been reported to be difficult (161, 162). Studies on compliance with a GFD in children with CD and T1D have shown inconsistent results. See **Table 1** for a summary of previous studies describing compliance with a GFD in children with T1D and CD. Several studies have found fairly high compliance rates: 69-100% (152, 153, 163, 164). Other studies have shown substantially lower compliance rates: 30-44% (165, 166). Reasons for these inconsistent results include different ways of measuring compliance, small sample sizes, with resulting lack of power, and questionable representivity, which can be suspected in the only larger study that included only 39% of the eligible patients (165). Because of these inconsistencies and because there were no Swedish studies on compliance with a GFD in children with T1D and CD, we decided to conduct study III.

Table 1. Summary of previous studies describing compliance with a GFD in children with T1D and CD.

Author Year	Study Period	Study population	Study design	Method	Authors' conclusion	Limitations
Amin (152) 2002	1 year	11	Longitudinal	AGA/EMA	100% compliance All AB neg after 6 m	Small sample size
Hansen (153) 2006	2 years	31	Longitudinal	Ttg-IgA	77% Ttg-IgA neg after 3m-2 years	
Pham-Short (163) 2016		35	Cross-sectional	Questionnaire Ttg-IgA	69% compliance Ttg-IgA neg or diminishing questionnaire	Cross-sectional
Sadaah (164) 2004		21	Cross-sectional	Questionnaire	Around 80% compliance	Questionnaire Cross-sectional
Westman(166) 1998		20	Cross-sectional	Questionnaire	Around 30% compliance	Questionnaire Cross-sectional
Nagl(165) 2019	3 years	608	Longitudinal Register study DPV database	Ttg-IgA	36% Ttg-IgA neg after 3 years	Only 39% of T1D+CD in cohort CD prev 2.2%

Because of these inconsistencies and because there were no Swedish studies on compliance with a GFD in children with T1D and CD, we decided to conduct study III.

Common ground in T1D and CD

Epidemiology

T1D and CD are both common diseases in children globally. In 2019, the estimated global prevalence of T1D was 600,900 children (1) and the estimated prevalence of CD was approximately 1% (89). The overall incidence of both T1D and CD is increasing: T1D at a rate of approximately 3-4%/year (1) and CD at a rate of 7.5 %/year (90). Both T1D and CD (90) are more common in children than in adults.

Aetiology

T1D and CD share the same risk genes, DQ2 and DQ8 in the HLA region on chromosome 6 (31, 95). In Sweden, > 99% of children with CD and > 90% of children with T1D carry one or both of these haplotypes (31, 87). However, the important haplotypes DQ2 and DQ8 are also common in the general population, ranging from 25-40% depending on country (167). In addition to DQ2 and DQ8, other genes that confer risk for T1D or CD have been identified, including several genes that increase the risk of both diseases (168).Although important, the genetic risk of CD and T1D is unlikely to be the only aetiological explanation. For example, identical twins only have an approximate proband wise risk of T1D of around 70% (37, 38) and a 83-86% corresponding risk for CD (92, 93). In addition, the incidence of both diseases is increasing at a rate too high to be explained by genetic factors alone.

Thus, environmental and pathophysiological mechanisms must be considered (168). Infection (5, 56, 99, 169), altered microbiome (50-52, 170) and increased intestinal permeability (109, 171) are other environmental factors repeatedly described in both diseases.

Gluten is an essential autoimmune trigger in patients with CD. In T1D there is no such confirmed trigger, although gluten intake has also been associated with a higher risk of T1D in several studies (9, 10, 172, 173). During the celiac epidemic in Sweden, higher gluten content in infant products is thought to have been a part of the observed increase in CD in Swedish children (105). The way in which this period of higher gluten content in infant products affected the CD risk in T1D has not been extensively studied. Thus, we decided to compare birth cohorts during and after the celiac epidemic to investigate the prevalence of CD, specifically in children with T1D (Study IV).

Pathogenesis

A simplified theory of possible shared common pathogenesis, including shared risk genes and environmental factors, has been proposed. The commonly shared

haplotypes, DQ2 and DQ8, encode antigen-presenting cell surface receptors with specificity for both gliadin and glutenin (110), as well as hybrid insulin peptides (HIPs). When these substrates are presented to antigen-responsive T cells in a proinflammatory environment (by infection, altered microbiota or increased intestinal permeability), an autoimmune response is initiated. This autoimmune response ultimately leads to β-cell loss in T1D and destruction of the intestinal mucosa in CD.

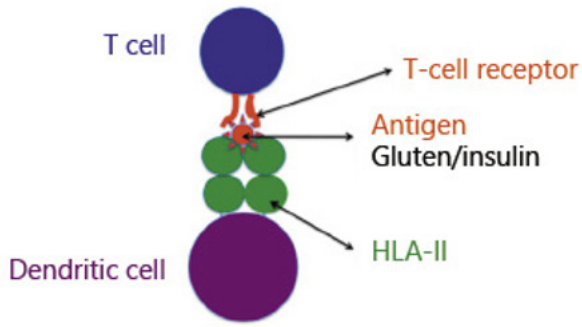

Figure 8. The immunological "synapse" of T1D and CD. Reprinted from *Horm Res Paediatr* volume 92, G Goodwin, Type 1 Diabetes Mellitus and Celiac Disease: Distinct Autoimmune Disorders That Share Common Pathogenic Mechanisms, Copyright © 2019, reprinted with kind permission from Silverchair Publisher (174).

Gluten - in T1D.

The pathogenic role of gluten in T1D has been proposed by several studies. However, although there seems to be a connection, no firm conclusion has been drawn regarding the role of gluten in the pathogenesis, onset and progression of T1D.

Inflammation

Several studies have reported an association between T1D and inflammation. Gluten seems to trigger the immune system in T1D patients compared to healthy controls. For example, upon stimulation with wheat protein or parts of wheat components, the proliferation of the T-cell response has been shown to be increased in patients with T1D compared to controls (175-177). This type of enhanced T-cell response was also observed in a study of T1D children in Finland, but only in a subset of patients (24%), and was significantly different from controls at T1D diagnosis, whereas no difference was observed in children with T1D for more than two years (178).

Microbiota

The composition of the intestinal microbiota is important in the pathogenesis of autoimmune diseases (179), including T1D (180, 181). In this context, gluten might be a disadvantage, while the microbiota appears to be changing for the better, with a higher amount of "good bacteria" and greater diversity on a GFD (12, 172).

Intestinal permeability

In T1D patients, intestinal permeability has been shown to be increased (182, 183). Several causes have been proposed for the increased permeability of T1D, including increased zonulin, particularly preceding T1D onset (184), and gluten itself (185).

The effect of gluten in different stages of T1D

Many studies of NOD mice and some studies of humans have evaluated the effect of gluten at different stages of the imagined development of type 1 diabetes.

It has been suggested that a prediabetic phase occurs during gestation. During this supposed phase, NOD mice were fed a GFD during pregnancy and lactation. The offspring of these mice were than shown to have a reduced prevalence of TID and a delayed T1D diagnosis (172). Similarly, in humans, a study of pregnant women reported the T1D risk in offspring to be increased with higher gluten ingestion (9).

Additionally, a GFD has repeatedly been shown to reduce the risk of T1D in NOD mice (10-12). In one study, a lifelong GFD in NOD mice decreased the prevalence of T1D from 64 to 15% (10). A GFD has not been shown to reduce the levels of diabetes-associated antibodies in children with a high risk of T1D (186) but individuals with a high risk of T1D put on a GFD for six months, was shown to improve insulin secretion (187).

In 2012 an association between a GFD and a prolonged remission was reported. A case-report from Denmark described a five years old boy, who soon after type 1 diabetes diagnosis started on a GFD and did not need to start insulin treatment. The boy continued with a GFD and was two years after diagnosis of type 1 diabetes still without insulin treatment (188). This report rendered our interest, and became the starting point of this dissertation and in particular for Study I.

Aims

This PhD project aimed to investigate the effects of gluten on children with T1D. The specific aims of this thesis are as follows:

I. To investigate the effect of a GFD on beta cell function and glycaemic control in Swedish children with T1D

II. To examine adherence to a GFD and effects on diabetes-related QoL in Swedish children with T1D (Study I)

III. To investigate the effect of a GFD on glycaemic control and growth in Swedish children with T1D and CD (Study II)

IV. To examine compliance to a GFD in Swedish children with T1D and CD (Study III)

V. To investigate possible associations between compliance, and glycaemic control and growth. (Study III)

VI. To investigate whether the prevalence of CD in children with T1D was affected by the Swedish celiac epidemic (Study IV)

Methods

Study Design

Study I

Study I was designed as a two-armed, non-randomised, prospective clinical intervention study to investigate whether children with newly diagnosed T1D would benefit from a one-year intervention with a GFD. Children diagnosed with T1D within the past two months were invited to participate in the study. The child and family decided whether to continue normal diet (ND) or start on a GFD.

A total of 23 children were included: 14 in the GFD group and nine in the ND group. Clinical characteristics including age at T1D diagnosis and sex were recorded. The children were followed for one year and measures of β-cell function (C-peptide), glycaemic control (HbA1c, IDAA1c), growth (length and weight), and quality of life (QoL) were recorded at inclusion and in five follow-up visits (at 3, 6, 12, 18 and 24 months) and thereafter compared groupwise.

Study II

Because of the great difficulty of including children in the first study, we decided to look at children with T1D who were presumably already on a GFD, that is, those children with an additional CD diagnosis, and compare them to children with T1D but without a CD diagnosis.

Study II was designed as a case-control study, where we used a national cohort of consecutively enrolled children diagnosed with T1D to investigate whether glycaemic control and growth differed in children with T1D and CD compared to children with T1D without CD. Clinical characteristics including age at T1D diagnosis, date at T1D diagnosis, and sex, as well as measures of glycaemic control (HbA1c and DKA) and growth (length and weight), were recorded and compared groupwise, at inclusion, and annually in a five-year follow-up.

Study III

In Study II, we presumed that children with T1D and CD were likely to follow the recommended treatment with strict adherence to a GFD. This presumption was mainly based on the high adherence found in Swedish children with CD only. To test our presumption in Study II and study compliance with a GFD in children with T1D and CD, we designed Study III as a retrospective cohort study based on medical records.

This study included children in Skåne County with T1D diagnosed between 2005 and 2012 and with an additional CD diagnosis. Clinical characteristics, including date of birth, age at T1D and CD diagnoses and sex, were recorded at inclusion. Antibodies (Ttg-IgA and EMA), HbA1c and the presence of DKA were recorded annually for a follow-up period of 1-10 years.

Study IV

In study I our original plan had included investigating the effect of a GFD on the incidence of CD in children with T1D. Since the sample size included in Study I did not allow for that we decided to look at the relation of gluten content and the incidence of CD in another way.

Study IV was designed as a register study of two national birth cohorts, one born during the Swedish celiac epidemic, when gluten content in infant products was increased, and the other born after the epidemic (when gluten content was decreased again). The two cohorts were then compared regarding the prevalence of CD in children diagnosed with T1D.

Study Population

Study I

Children 3-16 years old (n=23) diagnosed with T1D between October 2015 and April 2019 at Skane University Hospital.

Study II

All children aged 0-17 years had been diagnosed with T1D and registered in the BDD study between 2005 and 2010 (n=3612).

Study III

All children aged 0-17 years had been diagnosed with T1D and registered in the BDD study (n=743) with additional CD (n=64) in Skane County between 2005 and 2012.

Study IV

All children in Sweden born between 1992 and 1993 (n=240,844) and 1997 and 1998 (179,530) and diagnosed with both T1D (1642 and 1380) and CD (176 and 171).

Meassures

HbA1c (Study I-III)

Glycated haemoglobin A1c (HbA1c) is a measure that demonstrates the average BGL during the last 2-3 months. Erythrocytes are permeable to glucose. Thus, the beta chain of haemoglobin in erythrocytes is increasingly glycated during its lifetime. HbA1c level corresponds to the size of glycated haemoglobin in erythrocytes. In Studies I, II, and III, blood from veins or capillaries was taken and analysed in Swedish laboratories which are standardised by External Quality Assurance in Laboratory Medicine (EQUALIS).

C-peptide (Study I)

C-peptide is a byproduct of insulin production. When proinsulin is cleaved to insulin, the byproduct C-peptide is produced at equal concentrations. Thus, the golden standard recommended for measuring β-cell function is to measure levels of C-peptide (189) during a mixed meal tolerance test (MMTT) (190). An MMTT is a test in which a drink comprising mixed carbohydrates, proteins and fats is ingested, after which C-peptide and BCL are measured at 0.30, 60, 90, and 120 mins. Peak C-peptide concentration was measured at 90 mins. In Study I, 200 ml Fresubin Original Drink comprising 200Kcal, 7.6 g protein, 6.8 fat and 27.2 Cho/kg/g was used for the MMTT.

IDAA1c (Study I)

Insulin dose-adjusted A1c (IDAA1c) is used as an indirect measure of β-cell function. It is calculated using HbA1c and insulin dose using the following formula: HbA1c (%) + 4x insulin dose (units per kilogram per 24 h). During C-peptide

stimulation, IDAA1c and C-peptide have been shown to follow a linear correlation. A level of 9 has been shown to correlate with a peak stimulation C-peptide level of 300pmol/mol (191).

Ttg-IgA (Study III)

To investigate compliance with the GFD, we analysed tissue transglutaminase IgA (Ttg-IgA). Ttg-IgA is an enzyme that is found in the lamina propria of the intestinal wall. The role of tTg-IgA in CD pathology is to deaminate gliadin peptides (derived from digested gluten). A deaminated gliadin peptide is more effectively presented by HLA-DQ2 molecules, thus promoting autoimmunity to gluten (111). The level of tTg-IgA correlates well with the degree of damage to the intestinal mucosa (121, 122). There is no gold standard for analysing compliance (135), but Ttg-IgA is considered the most predictive and reproducible serological test and is recommended as a measure to monitor GFD compliance (134).

Ttg-IgA was analysed in blood samples using an EliA Celikey IgA system from Thermo Fisher Scientific. The level for a positive outcome was set at 10U/mL or higher (192).

EMA (Study I, III)

Endomysial antibodies (EMA) are another antibody strongly associated with CD and are recommended as a second confirmation test in the diagnosis of CD in children (114). At the start of the study, the test was still being used, which is why a few measurements were reported in EMA instead of Ttg-IgA. According to regional laboratory guidelines, the same cutoff level was used (positive=10 or more) (193).

BMI-SDS (Study II)

Body mass index standard deviation score (BMI-SDS) was used to evaluate growth. BMI was calculated using the formula: kg/m2. The BMI-SDS is an estimated age- and sex-adjusted BMI and was calculated based on norm data for German children and adolescents (which are almost identical to the corresponding Swedish norm data registers).

QoL (Study I)

The diabetes module of the validated questionnaires DISABKIDS 3-7 and 8-15 was used to assess QoL. Ten questions yielded a total score, in which higher scores indicated more diabetes-related QoL issues.

Compliance

Study I

To study compliance, the Green, Expert Dietician Evaluation of Gluten-Free Diet Adherence for Children was used in FUs at 3, 6 and 12 months. The questionnaire was completed with an experienced dietician during a clinical visit. Based on the answers to the questionnaire, compliance was classified into five categories: excellent, good, fair, poor and not gluten-free (194).

Study III

There is no gold standard to classify compliance to GFD using Ttg-IgA or any other entity. Thus, we created one which we judged to be a plausible way to study compliance. We classified the patients into three groups according to Ttg-IgA values 2-11 years after CD diagnosis. Two years were chosen since various studies report high normalisation after two years on a GFD (183, 184). (patients with <2 serological values more than two years after CD diagnosis were excluded):

1. Good compliance (all Ttg-IgA values <10)
2. Varying compliance (≥Ttg-IgA>10 and ≥Ttg-IgA<10)
3. Non-compliance (all Ttg-IgA values >10)

Registers

The Swedish Longitudinal Patient Quality Register for Childhood Diabetes (Swediabkids) (Study II, III and IV)

Swediabkids registers almost 100% of children from <18 years with all types of diabetes in Sweden. All clinics treating children with diabetes continuously report clinical measures to the register. Swediabkids was started in 2000 by the Pediatric Diabetes/Endocrinology group within the Swedish Pediatric Society (BLF, Barnläkarföreningen) and is a part of the NPR (195). Clinical data for Studies II and III were collected from Swediabkids. Study IV used the register for diagnostic information.

The National Diabetes Register (NDR) (Study IV)

NPR is a national register for diabetes for patients from 18 years. It was created by the Swedish Society for Diabetology to decrease the disease burden. The register comprises clinical data and diagnoses (195) and was used in Study IV for diagnostic information.

The Swedish National Patient Register (SNPR) (Studies II and IV)

The SNPR is managed by the Swedish National Board of Health and Welfare. The SNPR includes three separate registers: an inpatient register, outpatient register and day surgery register (195). These registers were used to collect data on diabetes and CD diagnoses in Studies II and IV.

Statistics Sweden (Study IV)

The Swedish government agency Statistics Sweden is responsible for official statistics. Data on the two birth cohorts used in study IV were collected from Statistics Sweden.

BDD study (Studies II and IV)

The Better Diabetes Diagnostics study (BDD) was started in May 2005 as a nationwide childhood diabetes study to collect diagnostic data at diabetes diagnosis. The study includes information on genetics and autoantibodies in >90% of children <18 years with diabetes in Sweden. The aim of the BDD is to improve diabetes diagnostics, as well as explore comorbidities and risk factors for late complications (196).

Statistics

For all studies, statistical analysis was conducted using IBM SPSS Statistics, version 25. An alpha level of ≤0.05 was applied for statistical significance.

In Study I, group comparisons for outcome measures were performed using the Mann-Whitney U test, a non-parametric test, because the data could not be assumed to be normally distributed due to the small sample sizes. In addition to P-values, rank-biserial correlation was used to estimate between-group effect sizes. A correlation of >3 was considered a moderate effect size and a correlation of >6 a strong effect size.

In Studies II and III, regression models were used to analyse associations between the CD group and outcome measures (Study II) and compliance and outcome measures (Study III).

A chi-square test was used to test the differences in prevalence rates between the two birth cohorts studied in Study IV.

Ethics

Study I

Ethical approval was granted by the Regional Ethics Board at Lund University (Dnr: 2014/349, 20140808). The study was registered at Clinical Trials (03037190; https://clinicaltrials.gov/ct2/show/NCT03037190)

Study II

Ethical approval was granted by the Regional Ethics Board at the Karolinska Institute (Dnr: 2005/476) and by the Regional Ethics Board at Lund University (Dnr:2014/476)

Study III

Ethical approval was granted by the Regional Ethics Board at Lund University (Dnr:2014/476). Additional approval was granted in 2020 (Dnr: 2020-04152)

Study IV

Ethical approval was granted by the Regional Ethics Board at Lund University (Dnr: 2014/476)

Ethical Considerations

A major ethical concern in most research on children is the age at which children should be involved in the decision to participate. The implications of such a decision could be difficult to fully comprehend and depend not only on age, but also on level of maturity. It is also a challenging task to keep communication about the study prior to inclusion neutral and not let the wish to include as many patients as possible taint information. This might be particularly important when meeting a child and a family at the vulnerable state of a recent live changing diagnosis such as T1D. In

Study I, age-adjusted information about the study was provided, both orally and in writing. Consent forms, for children when possible, and for their caregivers were also explained to the participants before they signed them.

Ethical consideration in regard to data storage is important in most types of research. In Study I, all the children had their own paper files. Care was taken to keep the files in a locked location and to anonymise the files by giving each file a code. The key to the code was kept in a separate locked location. In Study III, medical records were used. To avoid breaching the patients' integrity, only one research nurse had access to medical records and was careful to only look at and record the very few clinical measures that were needed for the study. These measures were then recorded in a completely anonymised data file. In Studies II and IV, the anonymised data from the large cohorts were collected from the registers, which made the risk of a breach negligible.

In Study I, we faced the most difficult ethical issues. The first issue was whether or not to expose children who were newly diagnosed with a chronic disease (T1D), and who were just starting burdensome lifelong treatment, to another major adjustment (GFD). Additionally, the very act of including children in a study about their disease can exacerbate their own experience of feeling unwell. Another potential risk was the four MMTTs throughout the study, in which the children would have to have an intravenous needle for blood samples. Because the research nurse was skilled and experienced, we deemed the risk of adverse events to be negligible. However, MMTT procedures may result in an unpleasant experience for the child. On the positive side, the possible benefits for the child could be a decreased risk of developing CD (if our hypothesis proved to be true), as well as possibly improved glycaemic control with potentially fewer long-term complications. Thus, because the risks associated with MMTTs were relatively negligible, the choice of exposure to a new diet was left to the child and their family – and if they decided to choose a GFD, an experienced dietician would carefully guide them through the diet change – we weighed the pros and cons in favour of our study.

Results

Study I

Between October 2015 and April 2019, we included 23 children 3-16 years of age with a T1D diagnosis <two months prior to inclusion: 14 in the GFD group and nine in the ND group, see Table 2 for background and baseline data. Three children, one after inclusion and two after the six-month visit, dropped out of the GFD group, and one child from the ND group dropped out after the three-month visit.

Table 2. Background and baseline data across study groups

	GFD n=14	ND n=9	p-value
Age at diagnosis, median (IQR)	9.14 (7.29)	8.48 (7.13)	0.896
Age at inclusion, median (IQR)	9.30 (6.83)	8.72 (7.10)	0.926
Gender, female, n (%)	8 (57%)	6 (67%)	0.648

Glycaemic control

HbA1c was significantly lower in the GFD group compared to the ND group at six months (p 0.042). Because of the small sample sizes, we also analysed effect sizes using Rank Biserial Correlation and found a moderate to strong between-group effect in HbA1c levels at 6 and 12 months (-0.568 and -0.494, respectively) with lower levels of HbA1c in the GFD group (median at 6 months = 44.0 vs. 49.5) and at 12 months=43.0 vs. 48.0). See Table 3 and Figure 9 for HbA1c comparison across groups.

IDAA1c levels did not differ significantly at any time point. However, effect sizes indicated moderate differences at 6 and 12 months (-0.386 and -0.481) with lower levels of IDAA1c in the GFD group (median at 6 months=7.09 vs 7.76 and median at 12 months= 7.64 vs. 8.75). See Table 3 and Figure 10 for IDAA1c comparison across groups.

We found no significant group differences in C-peptide levels at any time points and the effect sizes were small according to the Rank Biserial Correlations.

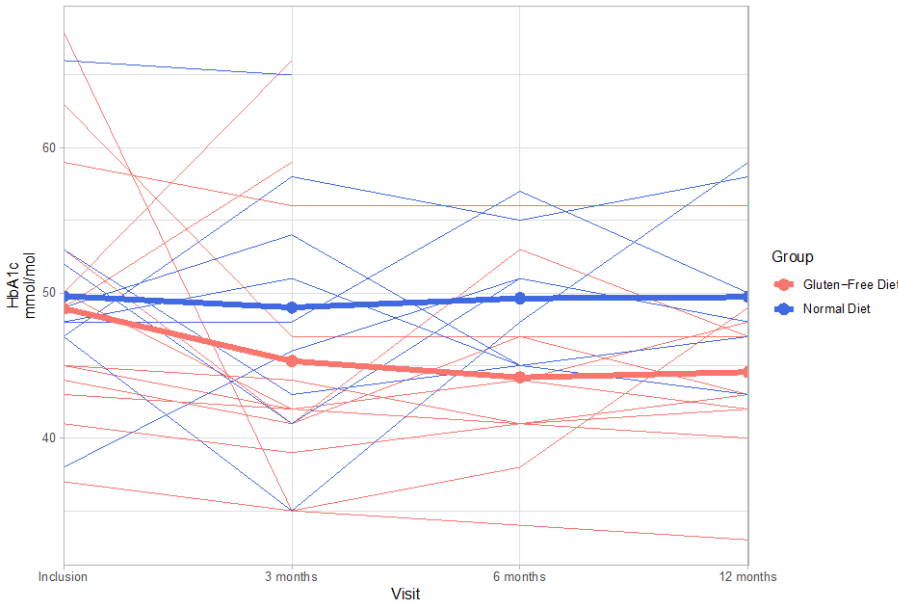

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Figure 9. HbA1c levels across the study groups at baseline and follow-up. Please note that the x-axis is truncated between a 6-12 month timepoint.

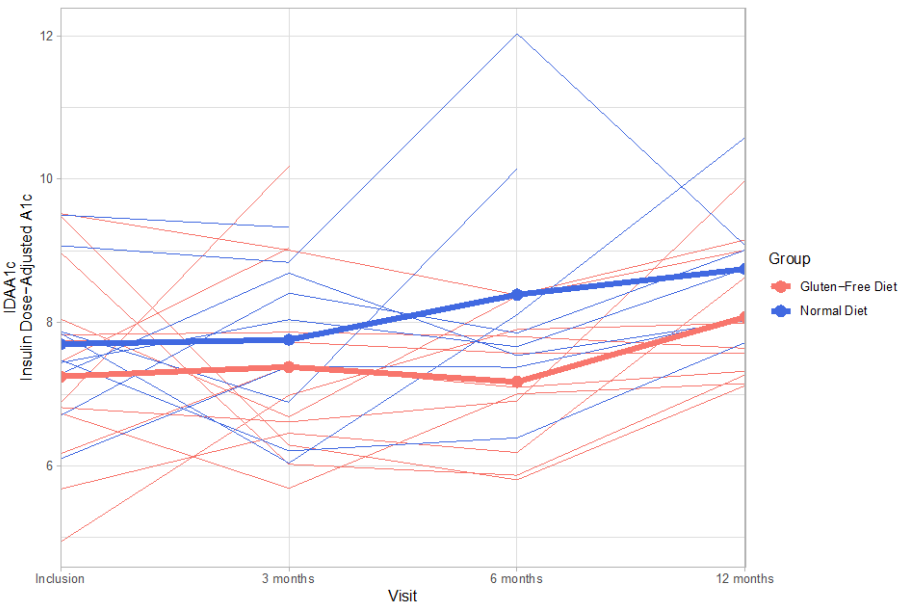

Figure 10. IDAA1c across the study groups at baseline and during follow-up. Please note that the x-axis is truncated between a 6-12 month timepoint.

Quality of Life

There were no statistically significant group differences in diabetes related QoL. Moderate effect sizes were found at inclusion and at 6- and 12-months using Rank Biserial Correlations (0.365; 0.364; 0.390), indicating a higher diabetes related QoL in the ND group at inclusion, and throughout the study.

Table 3. Results for the gluten-free and normal diet groups for HbA1c, IDAA1c, C-peptide and QoL at inclusion, 3, 6 and 12 months. Negative Rank-Biserial Correlations indicated that the GFD group had lower values. Effect sizes +- .30 are highlighted in bold.

	Gluten-free diet (GFD)		**Normal diet (ND)**			
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value	Rank-Biserial
HbA1c						
Inclusion	48.93 (9.19)	47.00 (12.00)	49.78 (7.41)	48.00 (6.00)	0.659	-0.119
3 months	45.31 (9.38)	42.00 (12.00)	49.00 (9.17)	48.00 (14.00)	0.284	-0.282
6 months	44.18 (6.37)	44.00 (6.00)	49.63 (4.69)	49.50 (9.00)	0.042	**-0.568**
12 months	44.55 (5.89)	43.00 (6.00)	49.71 (6.53)	48.00 (15.00)	0.091	**-0.494**
IDAA1c						
Inclusion	7.25 (1.47)	7.17 (2.24)	7.70 (1.06)	7.48 (1.48)	0.516	-0.175
3 months	7.38 (1.34)	6.99 (2.06)	7.76 (1.19)	8.04 (2.22)	0.471	-0.197
6 months	7.17 (0.93)	7.09 (1.72)	8.39 (1.81)	7.76 (2.22)	0.177	**-0.386**
12 months	8.08 (0.97)	7.64 (1.74)	8.75 (0.97)	8.75 (1.05)	0.104	**-0.481**
C-peptide						
Inclusion	0.46 (0.36)	0.41 (0.49)	0.52 (0.28)	0.47 (0.36)	0.571	-0.151
3 months	0.33 (0.22)	0.37 (0.45)	0.45 (0.27)	0.36 (0.54)	0.373	-0.241
6 months	0.33 (0.18)	0.33 (0.28)	0.35 (0.22)	0.33 (0.35)	1.000	-0.000
12 months	0.21 (0.12)	0.21 (0.18)	0.26 (0.16)	0.23 (0.23)	0.585	-0.183
Quality of life						
Inclusion	15.42 (6.85)	15.00 (10.00)	11.85 (2.48)	12.22 (3.89)	0.155	**0.365**
3 months	13.16 (4.79)	15.56 (4.17)	11.81 (2.84)	12.22 (4.17)	0.363	0.250
6 months	12.53 (5.71)	12.22 (6.67)	10.14 (2.33)	10.00 (2.78)	0.198	**0.364**
12 months	12.63 (4.34)	12.22 (6.67)	9.52 (3.84)	8.89 (6.66)	0.188	**0.390**
B-glu 90m MMTT						
Inclusion	13.53 (4.86)	12.50 (5.67)	11.60 (2.79)	11.20 (3.50)	0.270	0.286
3 months	15.18 (2.79)	15.10 (5.20)	14.30 (4.78)	12.70 (7.80)	0.526	0.171
6 months	16.25 (2.60)	16.65 (4.22)	16.71 (2.78)	16.25 (4.70)	0.824	-0.075
12 months	17.07 (4.47)	16.25 (9.13)	18.78 (3.20)	18.85 (3.90)	0.368	**-0.300**

Note. Mann-Whitney U tests were used to compare the groups. Effect sizes are presented in the form of Rank-Biserial Correlations. Moderate and large effect sizes, that is, Rank-Biserial Correlations > 3, are highlighted in bold. IQL = Interquartile Range. SD = Standard Deviation.

Compliance

Adherence to a GFD was excellent in 17% (n=2), good in 75% (n=9), and fair in 8% (n=1) of patients at three months. At six months, adherence was excellent in 20% (n=2), good in 70% (n=7), and fair in 10% (n=1) of patients. At 12 months, adherence was excellent in 30% (n=3), good in 60% (n=6), and fair in 10% of patients (n=1). Satisfactory adherence classified as excellent or fair was found in a

majority of the children in the GFD group: 92% at three months and 90% at 6 and 12 months. When we included dropouts and classified these as non-adherence, adherence levels were 85% at three months and 69% at 6 and 12 months.

BLC at MMTT

Comparison across groups of 90 minutes p-glu at MMTT did not show a statistically significant difference. However, the Rank Biserial Correlations demonstrated weak moderate group differences, indicating higher values in the GFD group at inclusion and lower values at 12 months (0.286 and-0.300, respectively).

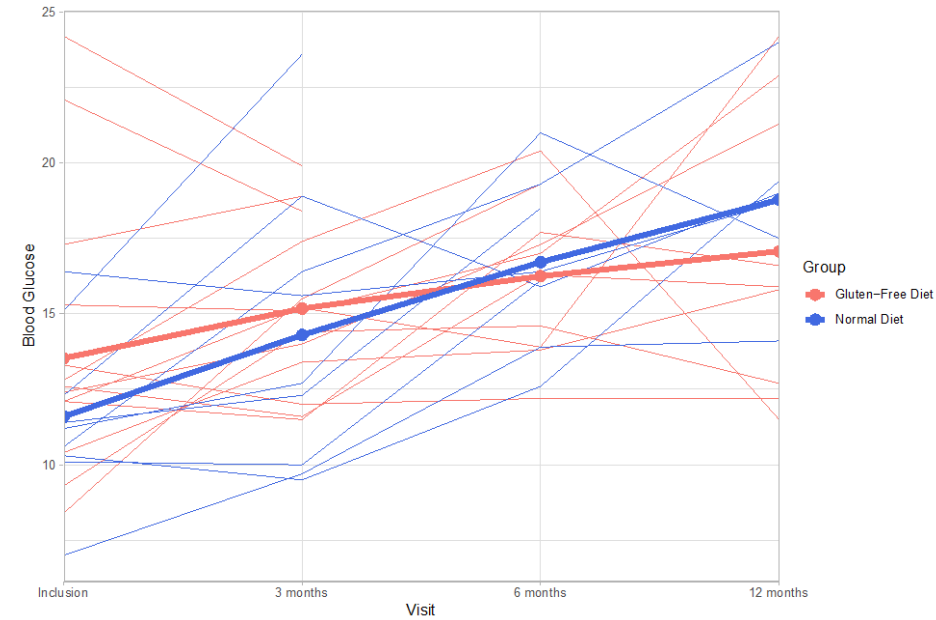

Figure 11. BLC at 90 mins during the MMTT across the study groups at baseline and follow-up. Please note the x-axis is truncated between the 6 and 12 month timepoints.

Study II

Study population

We included 3612 children diagnosed with T1D between May 2005 and December 2010. The cohort was then divided into four subgroups depending on CD status.

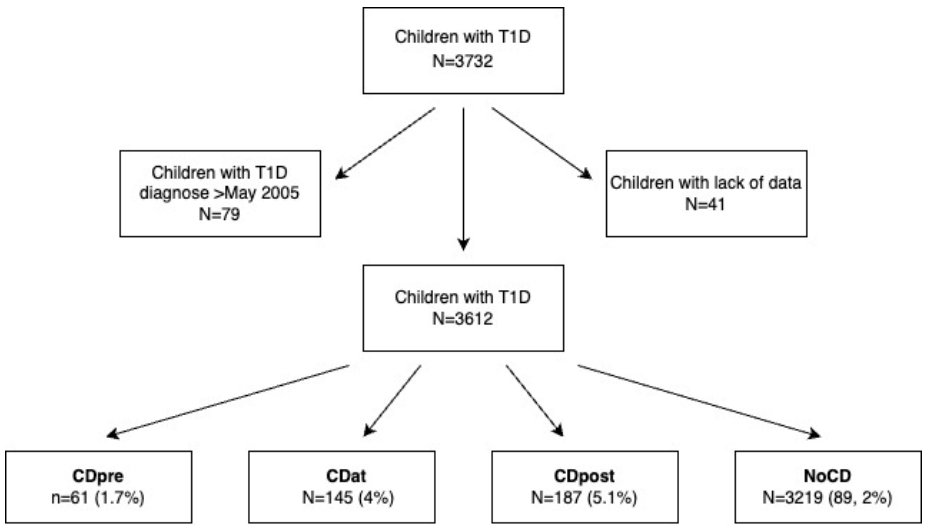

Figure 12. Flowchart of study group

CDpre= CD known at T1D diagnosis; CD at=CD within 12 months of T1D diagnosis; CDpost=CD 1-5 years after CD diagnosis; noCD=no CD diagnosis before or during the study. See Figure 12 for full study group flowchart.

Table 4. Information about the study variables in the full cohort and across CD groups.

	Full sample n = 3612	CD PRE n = 61 (1.7%)	CD AT n = 145 (4%)	CD POST n = 187 (9.2%)	No CD n = 3219 (89.1%)
Sociodemographics					
Females, n (%)	1,605 (44.4%)	29 (47.5%)	84 (57.9%)	87 (46.5%)	1405 (43.6%)
Age at T1D, M (SD)	9.85 (4.42)	10.45 (4.16)	10.21 (4.25)	7.16 (4.59)	9.97 (4.38)
HbA1c					
Baseline, M (SD)	93.92 (25.40)	96.70 (27.88)	94.29 (26.33)	88.40 (23.47)	94.17 (25.39)
1 year, M (SD)	55.10 (12.40)	56.20 (14.62)	56.89 (15.12)	55.46 (11.61)	54.97 (12.26)
2 years, M (SD)	59.52 (12.68)	62.28 (14.92)	60.06 (14.03)	59.72 (10.19)	59.43 (12.71)
3 years, M (SD)	61.57 (13.25)	64.08 (13.46)	61.49 (14.02)	60.99 (11.24)	61.56 (13.32)
4 years, M (SD)	62.87 (14.05)	64.94 (14.81)	64.09 (16.60)	61.85 (13.14)	62.83 (13.97)
5 years, M (SD)	62.94 (14.47)	64.95 (16.82)	65.60 (19.31)	62.91 (14.18)	60.98 (14.28)
BMI-SDS M=0 SD=1					
Baseline, M (SD)	-0.38 (1.24)	-0.54 (1.11)	-0.52 (1.18)	-0.38 (1.14)	-0.37 (1.25)
1 year, M (SD)	0.33 (0.94)	0.10 (0.92)	0.15 (0.89)	0.40 (0.88)	0.34 (0.94)
2 years, M (SD)	0.36 (0.92)	0.16 (0.95)	0.14 (0.85)	0.43 (0.82)	0.37 (0.93)
3 years, M (SD)	0.37 (0.93)	0.17 (0.98)	0.24 (0.93)	0.47 (0.82)	0.38 (0.93)
4 years, M (SD)	0.39 (0.96)	0.27 (0.98)	0.14 (1.08)	0.43 (0.78)	0.40 (0.96)
5 years, M (SD)	0.39 (0.98)	0.37 (1.02)	0.21 (1.09)	0.36 (0.83)	0.40 (0.99)
DKA					
Baseline, n (%)	574 (17.1%)	11 (20.8%)	21 (15.1%)	31 (17.5%)	511 (17.2%)

We found a significant difference in age at baseline (p=>0.001), with the CDpost group being significantly younger than the other groups (confirmed by Tukey-corrected post hoc test). There were significantly more girls in the CDat group (57.9%) than in the noCD (43.6% p=0.001) or CDpost (46.5% p=0.014) groups. Other significant group differences in sex were not found. At T1D diagnosis, all groups had low BMI-SDS scores compared to norm scores (BMI-SDS=-0.5-0.37) which were largely normalised at the one-year follow-up (BMI-SDS=+0.1-+0.4).

See Table 4 for information on study variables across the groups.

HbA1c and BMI-SDS

We used linear regression with multiple imputed datasets to examine whether the group affected HbA1c and BMI at baseline and each follow-up assessment. The results for HbA1c are presented in Table 5 and Figure 13 and the results for BMI-SDS are presented in Table 6 and Figure 14. No group difference was found for HbA1c at any timepoint. Differences for BMI-SDS were found for CDpre versus NoCD at 1 and 2 year follow-up, while differences for CDat versus NoCD were present at all follow-up timepoints, with lower values in the CDpre and CDat groups.

Table 5. The results of linear regression in relation to HbA1c across timepoints with imputed data. The beta coefficient for each independent variable (and its 95% confidence interval) is also presented, accounting for age and sex.

	HbA1c Baseline	**HbA1c** 1 year	**HbA1c** 2 years	**HbA1c** 3 years	**HbA1c** 4 years	**HbA1c** 5 years
CDpre vs NoCD	1.14 (-5.23, 7.51)	1.17 (-2.07, 4.42)	3.15 (-0.18, 6.47)	2.23 (-1.24, 5.71)	1.64 (-2.19, 5.48)	2.33 (-1.95, 6.61)
CDat vs NoCD	-1.28 (-5.38, 2.82)	2.02 (-0.13, 4.16)	0.58 (-1.59, 2.74)	0.23 (-2.02, 2.48)	1.30 (-1.07, 3.68)	2.08 (-0.45, 4.61)
CDpost vs NoCD	0.12 (-3.57, 3.81)	-0.17 (-2.06, 1.73)	1.24 (-0.79, 3.26)	0.73 (-1.30, 2.75)	0.61 (-1.51, 2.74)	0.45 (-1.77, 2.68)

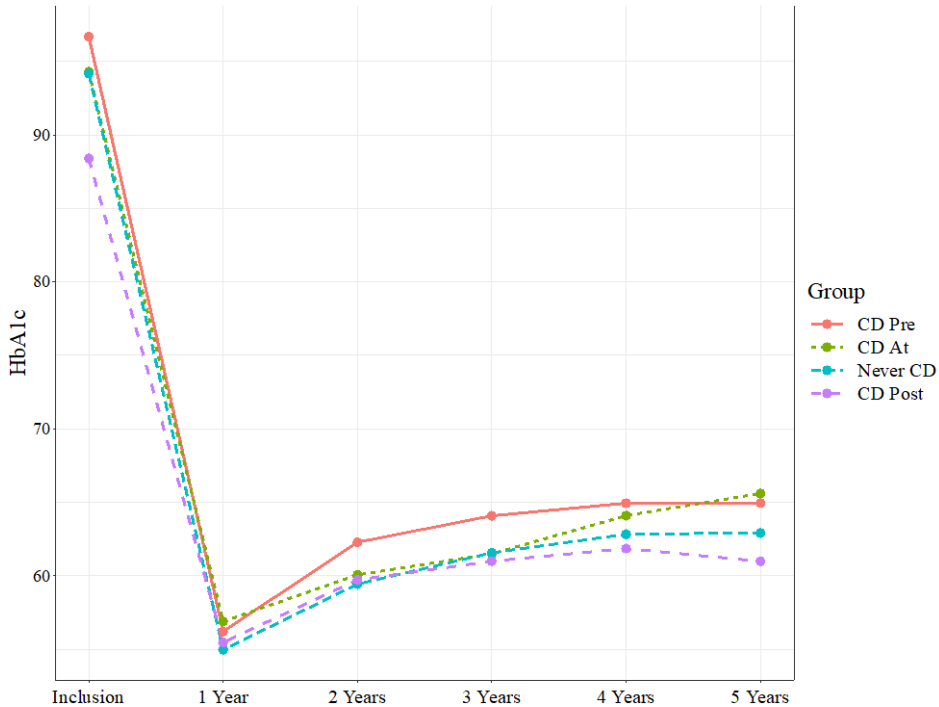

Figure 13. HbA1C over time across the four groups (non-imputed data, not adjusted for age and sex).

Table 6. The results of linear regression in relation to age and sex-adjusted BMI-SDS across timepoints with imputed data. The beta coefficient for each independent variable (and its 95% CI) is also presented. Statistically significant associations are highlighted in bold.

	BMI Baseline	**BMI** 1 year	**BMI** 2 years	**BMI** 3 years	**BMI** 4 years	**BMI** 5 years
CDpre vs NoCD	-0.13 (-0.45, 0.20)	**-0.32 (-0.56, -0.08)****	**-0.29 (-0.53, -0.05)***	-0.23 (-0.47, 0.01)	-0.19 (-0.45, 0.07)	-0.21 (-0.49, 0.07)
CDat vs NoCD	-0.15 (-0.36, 0.06)	**-0.17 (-0.33, -0.01)***	**-0.17 (-0.33,- 0.01)***	**-0.18 (-0.34, -0.03)***	**-0.28 (-0.44, 0.11)*****	**-0.22 (-0.39,- 0.05)***
CDpost vs NoCD	-0.10 (-0.28, 0.09)	-0.01 (-0.16, 0.13)	-0.01 (-0.15, 0.13)	0.01 (-0.13, 0.15)	-0.01 (-0.15, 0.14)	-0.06 (-0.21, 0.10)

Notes. * $P < .05$. ** $P < .01$. *** $P < .001$.

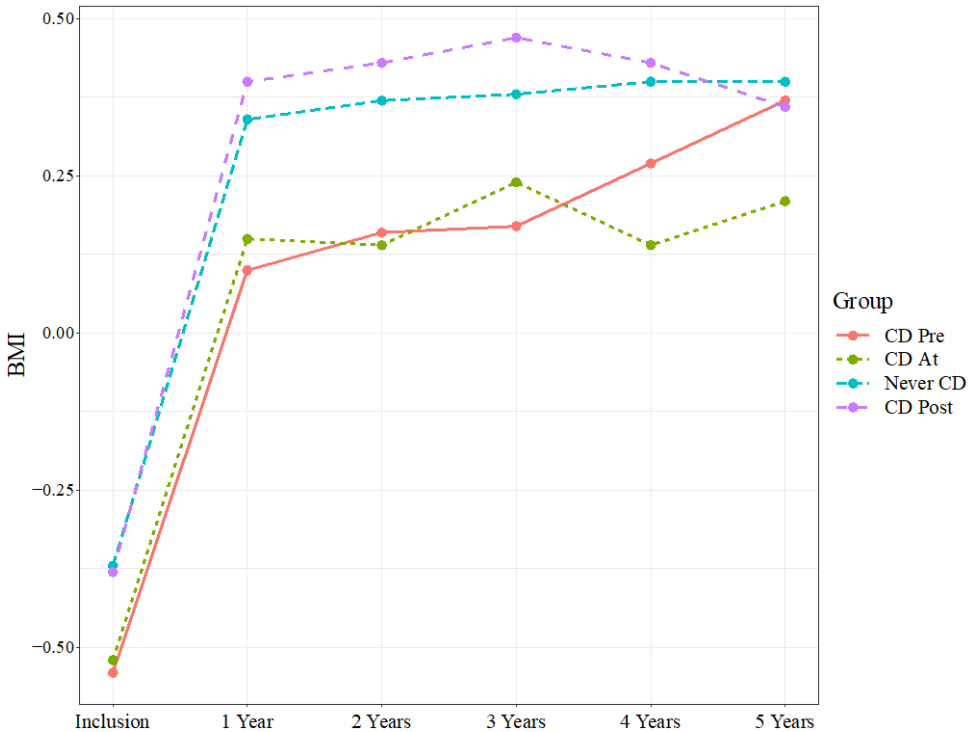

Figure 14. BMI-SDS (population mean = 0, *SD* = 1) over time across the four groups (non-imputed data).

Study III

Study population

Of the children who met the inclusion criteria (T1D diagnosis from 2005-2012 with additional CD diagnosis), 94% (n=60) were included in the study. See Figure 15 for flowchart of the study population.

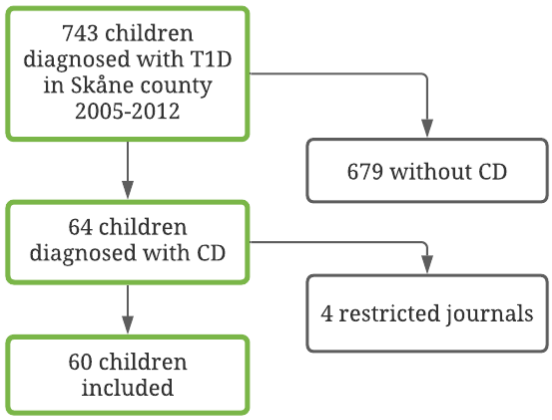

Figure 15. Flowchart of the study population.

Of the study population, 53% were female and 47% male. The mean age of the patients at T1D was 8.87 years. 19% were diagnosed with CD prior to T1D diagnosis. The children were divided into four groups according to their level of compliance. Most of them had good compliance (68%, n=34), 18% (n=9) had intermediate compliance, 14% (n=7) were non-compliant and 17% (n=10) had insufficient data to be classified. Ttg-IGA normalised within 2 years in 60% (n=30) of the patients. Among these patients, 77% (n=23) had normalised values within one year. See Table 7 for information on study variables in the full cohort and compliance groups.

Table 7. Information on the study variables in the full cohort and compliance groups.

	Full Sample	Subsamples Based on Compliance Classification			
		Good	Intermediate	Non-Compliant	Insuff. Data
	(*n* = 60)	(*n* = 34)	(*n* = 9)	(*n* = 7)	(*n* = 10)
Age at T1D, M (SD)	8.87 (4.50)	6.54 (3.68)	8.63 (2.13)	11.44 (2.58)	15.18 (2.14)
Female, *n* (%)	32 (53%)	16 (47%)	6 (67%)	6 (86%)	4 (40%)
CD pre T1D, *n* (%)	19 (32%)	12 (35%)	2 (22%)	3 (43%)	2 (20%)
DKA, *n* (%)	10 (20%) [a]	4 (13%) [b]	2 (22%)	4 (67%) [c]	0 (0%) [d]
HbA1c, M (SD)	62.36 (11.85)	59.37 (11.25)	65.95 (5.85)	69.77 (9.91)	65.31 (19.13)
SDS-BMI, M (SD)	0.72 (1.14)	0.65 (0.78)	0.48 (0.95)	1.30 (2.50)	0.76 (0.76)

A significant association was found between HbA1C and compliance, meaning higher levels of HbA1c were associated with poorer levels of compliance (OR= 1.09, CI95%=1.02-1.16, p=.008). See Figure 16 for an illustration of the association between HbA1c and compliance.

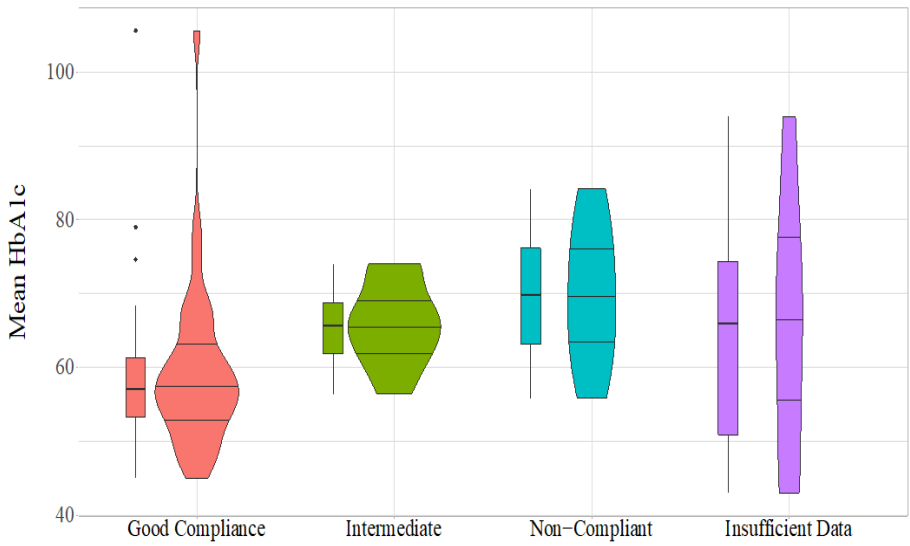

Figure 16. Association between HbA1c and Compliance.

Age was also significantly associated with compliance, meaning older age was associated with poorer level of compliance (OR= 1.41, CI95%=1.14-1.75, p=.002). See Figure 17 for an illustration of the association between Age and Compliance.

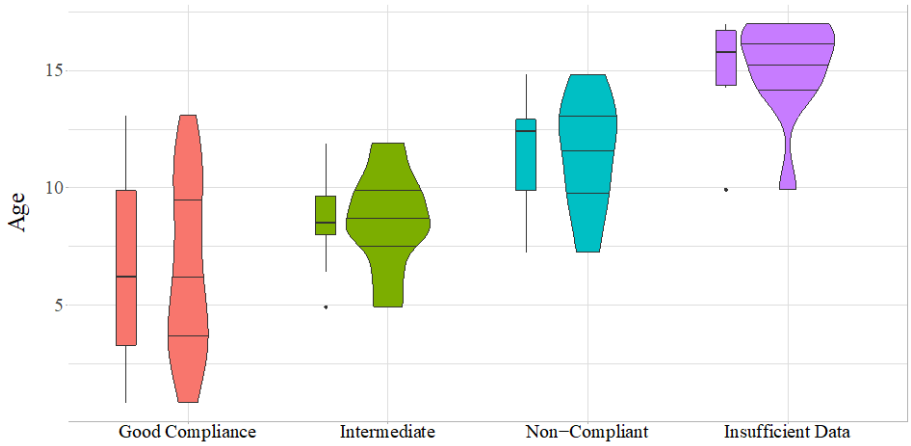

Figure 17. Association between Age and Compliance.

The mean BMI was not significantly associated with compliance. At inclusion, the mean BMI across the groups was − -0.5. At the first follow-up it was +0.4 and remained positive.

A significant association was observed between DKA and compliance. Children with at least 1 DKA after inclusion had a higher risk of poor compliance (OR= 6.22, CI95%=1.53-25.33, p=.011). See Figure 18 for an illustration of the association between DKA and Compliance.

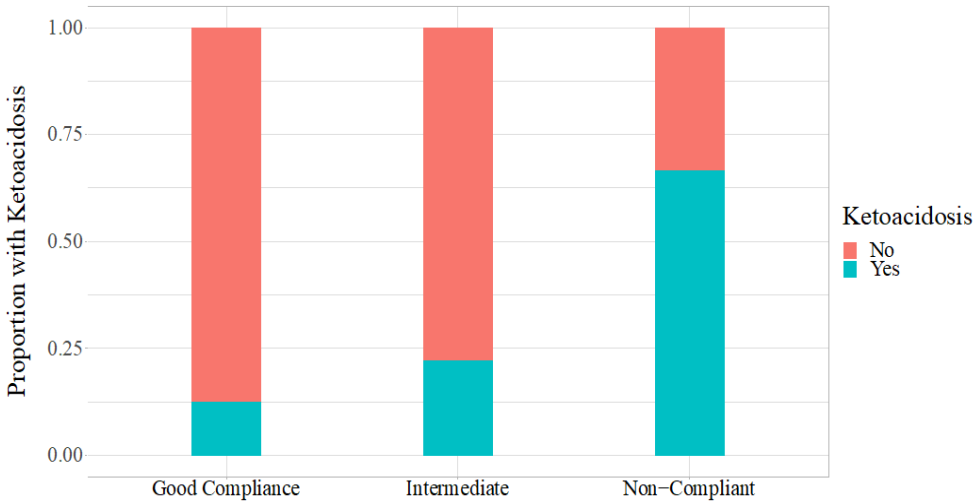

Figure 18. Association between DKA and Compliance. Figure based on ordinal regression with the compliance group as the dependent variable.

A full model, with age, sex and HbA1c as independent variables and compliance as the dependent variable also showed age and sex to be significantly associated with compliance (age, OR=1.36, CI 95%=1.07-1.734, p=0.011; HbA1c, OR=1.10, CI95%=1.02-1.17, p=0.011) while sex was not (OR=4.08, CI95%=0.085-19.73, p=0.08). The psuedo R2 (Nagelkerke) value indicated that 42% of the variance in compliance was explained by variance in age, sex and HbA1c.

We did not include DKA in the above model because of missing data. When we included DKA (as a sensitivity analysis), only age remained statistically significantly associated with compliance. Psuedo R2 indicated that 46% of the variance in compliance was explained by variance in age. To examine dependence on collinearity problems, independent T-tests were conducted. The T-tests showed a clear and significant association between experiencing DKA post T1D diagnosis and a poorer mean HbA1c (DKA, M=72.71 [12.72] versus no DKA, M=58.94 [7.90], t(45)=4.26, p<0.001). Since HbA1c and DKA were strongly associated, they may supress each other when included in the same model. Thus, high HbA1c and DKA after T1D diagnosis can be regarded as being uniquely associated with lower compliance with a GFD.

Study IV

Using two national birth cohorts of children <18 years old from 1992 to 1993 and 1997 to 1998, we found 3022 children with T1D, 1642 from the first cohort and 1380 from the second cohort. Additional CD was found in 11.5% (n=337) of children. See Figure 19 for flowchart of cohort selection.

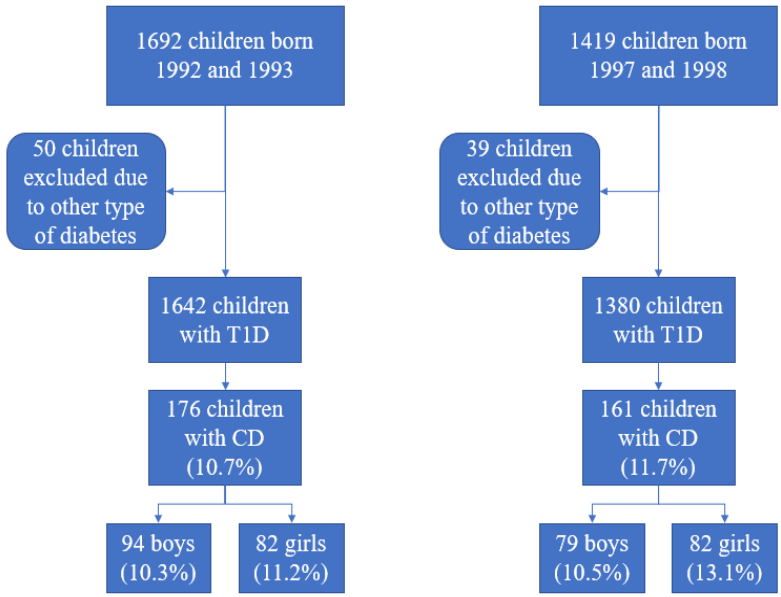

Figure 19. Selection of children <17 years from data from the National Board of Health and Welfare Sweden, diagnosed with type 1 diabetes (T1D) and diagnosed with coeliac disease (CD). Two birth cohorts: born during the Swedish epidemic of coeliac disease (1992/1993) and post epidemic (1997/1998). Patients excluded according to more precise diabetes diagnosis in the Swediabkids and NDR quality registe

The prevalence difference between cohorts was not significant: 10.7% (CI95%= 9.2-12.2%) in the 1992-1993 cohort and 11.7% in the 1997-1998 cohort. See Table 9 for prevalence across cohorts.

Mean age at T1D diagnosis was significantly lower in the group of children with CD compared to the group without CD (8.4 vs. 9.8 years, p=<.001), but age at T1D diagnosis for children with T1D and CD did not significantly differ between cohorts (8.3 vs. 8.5 years, p=.707). Mean age at CD diagnosis was significantly lower in the later cohort (9.4 vs. 11.0 years p=.002). Stratified by sex, boys in the post-epidemic group were significantly younger than boys in the epidemic group (9.8 vs. 11.9 years, p= 003), while the girls did not differ significantly between cohorts (9.1 vs. 10.2 years, p=0.127). See Table 8 for mean age across cohorts.

Table 8. Prevalence and mean age at celiac disease (CD) diagnosis in the two birth cohorts during the Swedish epidemic of CD (1992/1993) and after (1997/1998) the epidemic, as well as mean age divided by sex.

Birth Cohorts	**All with CD** n=337	**1992-1993** n=176	**1997-1997** n=161	**P-value**
Sex Male %	51	53	49	
Total prevalence of CD	11.1	10.7	11.7	0.461
Mean age CD at diagnosis	10.3	11.0	9.4	0.002
Female mean age at CD diagnosis	9.6	10.2	9.1	0.127
Male mean age at CD diagnosis	10.8	11.9	9.8	0.003

Discussion

Mainfindings

Study I: A GFD could be maintained in children with T1D and was associated with positive effects on glycaemic control.

Study II: Children diagnosed with both CD and T1D had a decreased growth in the first years following diagnosis of T1D compared with children with T1D without CD.

Study III: Children with both T1D and CD had a lower level of compliance with a GFD than previously reported in children with CD in general. A higher level of compliance to a GFD was associated with a better glycaemic control.

Study IV: In contrast to Swedish children in general, the prevalence of CD was not increased in children with T1D during the Swedish celiac epidemic.

Gluten and glycaemic control

We found that children with T1D had significantly better glycaemic control, measured with HbA1c, after six months on a GDF compared to children on an ND. Although this positive effect was not significant at 12 months, median group differences in HbA1c levels were very similar between 6 and 12 months. To avoid the possibility of not finding true differences (type 2 error), considering the small sample sizes, we analysed the group effect size and found further support for the positive effect of a GFD on glycaemic control. Using this method, we found substantial group differences with lower levels of IDAA1c and HbA1c after 6 and 12 months in the GFD group.

Our results are strengthened by the findings of two similar studies: a Danish study of 15 children with T1D on a GFD for one year (14), and a Czech study of 26 children with T1D on a GFD for one year (13). In both studies, significant positive effects on glycaemic control (measured by IDAA1c and HbA1c levels) were observed in children with T1D on a GFD. Also worth mentioning, but less directly comparable, are studies on children with T1D and CD in which good compliance with a GFD has been associated with better glycaemic control (163).

A possible explanation for better glycaemic control because of a GFD may be reduced inflammation. Gluten-derived gliadin peptides induce an inflammatory response in patients with CD (107, 108). Similarly, an increased inflammatory response has been found in T1D patients (175, 176). Additionally, as in CD, intestinal permeability has been shown to increase in T1D (182) and large gluten-derived, gliadin peptides have been found in the bloodstream and even in the pancreas (197). General inflammation as well as inflammation of the ß cell, can cause ER stress. ER stress can induce post-translational modification of islet proteins (such as insulin and pro-insulin) (65, 67, 68), which can lead to ß-cell autoimmunity (69) with subsequent ß-cell destruction. In summary, a GFD may lead to reduced inflammation and therefore fewer destroyed ß cells, a slower decrease in insulin, and a better controlled BGL.

However, we found no difference in the decrease in insulin (measured by 90 minutes level of C-peptide at MMTT), between the GFD and the ND group. This result is strengthened by the findings of the two similar studies mentioned above (13, 14). However, the somewhat larger of the two studies (13) did find a significant difference in favor of the GFD group, when they, instead of comparing groups for C-peptide levels at specific timepoints during the study, compared groups for the annual trends of fasting C-peptide decrease.

Another way in which GFD can affect glycaemic control is by improving insulin sensitivity. Improved insulin sensitivity has previously been observed in individuals with pre-diabetes on a GFD (187). If the children on a GFD had improved insulin sensitivity, one explanation for not finding an attenuated C-peptide decrease could be that those children did not need as much insulin to take care of their high BGL at MMTT. Thus, lower c-peptide levels were observed.

We did not succeed in including as many children as we intended to in Study I. Because of the significant recruiting difficulties and the lack of power in the study, we decided to try to achieve our aim from another angle. We decided to examine possible associations between gluten and T1D by studying children who were presumably already on a GFD, that is, those children with an additional CD diagnosis.

In Study II we used a large national cohort of children with T1D to compare glycaemic control in those children with T1D and CD with those children with T1D without CD. We found no significant differences in glycaemic control between the groups. The results of previous studies on glycaemic control in children with T1D and CD compared to children with T1D without CD have been contradictory. Some studies have found better glycaemic control (lower HbA1c levels) in patients with T1D and CD (149, 152, 155, 198) while many other studies found no significant differences between the groups (119, 154, 156, 199). There may be many reasons for the different results. Several of these studies had a small sample size, giving low power to the study. Many of them had short follow-up, or a cross-sectional design.

Furthermore, CD screening protocols vary across countries and because CD is a disease with malabsorption at diagnosis, which could theoretically affect glycaemic control, it is important to know how and when CD was diagnosed and the interval at which the child was screened. Additionally, different strategies to follow adherence to a GFD, if any, were applied, and the length of T1D (also affecting glycaemic control) was often unknown. However, three major recent reviews on the topic concluded that glycaemic control is not negatively affected by a GFD in children with T1D and CD (157-159) and it was also found that good compliance to a GFD might have positive effects on glycaemic control (HbA1c) (157).

Unfortunately, we did not have the opportunity to look at compliance with a GFD in our second study (since it was a retrospective cohort study and the registers we used did not include information on measures to follow compliance). Thus, we were unable to determine whether the results with no significant effect on glycaemic control in the GFD group depended on:

1. No effect of a GFD on glycaemic control, or
2. Poor compliance and thus a negative effect of an unhealed intestinal mucosa outlasting a potential positive effect of a GFD.

We knew from previous studies that compliance with a GFD for children with CD in Sweden in general was high, around 90% (131, 200) but compliance in children with additional T1D had scarcely been studied, and not at all in Sweden. Thus, we decided to look at a subgroup (children from Skåne County) of the cohort from Study II (somewhat extended in time from 2005 to 2012) in which we were able to include compliance by withdrawing information on tTg-IgA status from the medical records.

In Study III, we found poorer compliance to be associated with worse HbA1c and a higher risk of experiencing a DKA. The following association between a higher degree of compliance and better glycaemic control is supported by other studies (152, 165, 201), including a RCT in which children with T1D and CD were randomised to have either ND or GFD for 12 months (202). However, the sample size of the mentioned RCT was small (15 children in each arm) and a second small similar RCT found no difference in glycaemic control between groups (13). Regarding other studies on the association between compliance and DKA we only found one other study and that study did not see any association between the two (165).

It could be argued that the association between higher compliance and better glycaemic control could depend on more effective treatment of CD. Theoretically, an effective treatment would lead to better healing of a damaged intestinal mucosa which, in turn, would decrease inflammation with positive effects on the beta cells and less insulin resistance. However, data from our second study showed that

children with an undiagnosed (untreated) CD at T1D diagnosis did not differ in glycaemic control from children without a CD diagnosis at T1D diagnosis. Nor did these children differ in glycaemic control at FUs 1, 2 or 3, even though mucosal healing can take more than three years in children with CD and concordant T1D (122, 123, 153). These findings contradict the theory that a damaged mucosa in CD would negatively affect glycaemic control. This strengthens the theory of a positive effect of a GFD. Nevertheless, future studies of a GFD in children with T1D but without CD would be easier to analyse.

As described above, the association between better glycaemic control and a higher degree of compliance could depend on the effect of a GFD with decreased inflammation, preserved β-cell mass, more insulin and better regulated BGL. Another reason for better glycaemic control with a higher compliance to a GFD could be higher overall compliance to healthcare treatment and recommendations for these patients. In this case, higher compliance to a GFD would be accompanied by more meticulously followed T1D treatment, which would result in better glycaemic control regardless of level of compliance to a GFD. This possible confounding factor calls for RCTs in children with T1D and CD, where not only compliance to a GFD, but also T1D regimen are carefully followed and controlled for.

Development of CD in children with T1D

One of our original ideas with Study I, beyond investigating the effect of a GFD on glycaemic control, was to follow-up the children after five years to see whether a GFD for one year after T1D diagnosis would affect the incidence of CD. The prevalence of CD in children with T1D is high, with a prevalence between 1.7 and 12.2% compared to 1% in children in general (139). Most children diagnosed with TID and CD are diagnosed with CD at T1D diagnosis or in the years immediately following. Gluten has been shown to be immunogenic in patients with T1D (175-177), particularly at diagnosis (178). Thus, our hypothesis of a possible effect of a GFD on the incidence of CD in children with T1D was as follows: Removal of gluten from the diet at a time when gluten has been proven to be the most immunogenic in T1D patients, and the risk of developing additional CD for T1D patients is the highest, would decrease the risk of the development of CD in children diagnosed with T1D. Unfortunately, because our study population was too small to study a potential decrease in incidence, we had to abandon that aim.

In order to find another way to study the associations between gluten and the prevalence of CD, we compared the prevalence of CD in children with T1D born during and after the Swedish celiac epidemic. The Swedish celiac epidemic refers to a period between 1984 and 1996 when the prevalence of CD in Swedish children below two years of age was fourfold compared to before and after the epidemic (105, 106). The incidence increase has been associated with a higher gluten content

in infancy (105), a theory that has also been confirmed in a later study (104). Thus, according to our hypothesis that gluten withdrawal at T1D diagnosis could decrease the risk of developing CD, we expected the prevalence of CD to be equally or more increased in the cohort of children with T1D compared to the overall cohort. Surprisingly, and contrary to Swedish children in general (100), there was no increase of CD in children with T1D born during the celiac epidemic compared to children with T1D born after the CD epidemic.

One reason for this unexpected result could be timing. During the celiac epidemic, gluten content was generally higher in infant products. Since most children with T1D are diagnosed at a later age, with an incidence peak around puberty (29), the extra gluten in their early years might not have been that relevant. Even so, bad timing would only explain why the increase in incidence was not *more* elevated in children with T1D than in children in general at the time. The question of why gluten amounts in early childhood would be *less* relevant to children with T1D than to Swedish children in general remains unanswered.

In Study IV we discussed the possibility of a genetic explanation. The genetic aetiology is strong and largely shared between T1D and CD, with >90% of children with T1D and >99% of children with CD, being monozygotic or homozygotic for DQ2 and/or DQ8 in the HLA region of chromosome 6 (31, 87). Thus, it is possible that the different effect of the celiac epidemic on the incidence of CD in children with T1D compared to children in general depended on different genetics.

Since the amount of gluten in infancy did not seem to increase the CD risk in children with T1D compared to children in general, perhaps a strong genetic predisposition could have outlasted potential environmental factors such as gluten.

However, genetic risk is unlikely to be the only aetiological explanation for CD in T1D since almost all children with T1D have at least one of the genes necessary for CD (>90% of children with T1D have one or both DQ2 and/or DQ2) while the prevalence of CD in children with T1D is only between 1.7 and 12.2%. Further, a previous study compared children with T1D and CD in Sweden and Denmark and found an increased CD risk in the Swedish children with T1D, independent of HLA, pointing towards a difference in environmental exposure (203).

One explanation for our results could be that some environmental factors affect children with T1D differently than children without T1D (possibly because of different genetics). For example, the specific combination of T1D and CD has been associated with caesarean section, birth in summer, Swedish ancestry and the female sex (142).

Overall, we do not know why the incidence of CD in children with T1D does not seem to increase with a higher gluten content in infancy, as would seem to be the case in children without T1D. It could depend on the timing of gluten intake, or that

different genetics lead to different effect of environmental factors on children with T1D compared to children without T1D.

CD, GFD and association with growth

In children with T1D, we found growth to decrease during the first years following a CD diagnosis. In Study II we examined the possible effects of CD on growth by comparing children with T1D and CD with children with T1D but without CD. Children with CD prior to T1D diagnosis had diminished growth during the first two years after T1D diagnosis, while the effect for children diagnosed with CD at T1D diagnosis remained during the five years of follow-up.

Our data are supported by previous studies on children with T1D, which also found decreased growth after CD diagnosis (149, 154, 160). Two studies (149, 154) found decreased growth several years post-CD diagnosis (4.5 vs. 9 years), while the third study only followed children for one year post-CD diagnosis (160). However, the first two studies were large register studies with questionable representativity because of the unexpectedly low number of double-diagnosed children (0.6% vs. 1.9%), while the third study was a prospective case-control study, but quite small, with only 29 children having both T1D and CD. We also found studies that did not agree with our results. One small study (49 children with T1D and CD) followed children with T1D 2.5 years after CD diagnosis and found no difference in growth compared to children with T1D who did not receive a CD diagnosis (155). Another smaller study (11 children with T1D and CD) found lower growth at CD diagnosis, but no remaining differences one year post-CD diagnosis, compared to children with T1D without CD.

At CD diagnosis, the intestinal mucosa is damaged with crypt hyperplasia and villous atrophy (115), causing malabsorption and nutritional deficit. Treatment with a strict GFD usually resolves enteropathy. Children with CD who start treatment with a GFD usually do most of the growth catch-up within six months (204). Within two years the intestinal mucosa has often healed (123, 124), even if healing sometimes continues for three years (135). In our study, after five years, there was still significantly lower growth in the group diagnosed with CD at T1D diagnosis than in the group with no CD diagnosis. However, the group with CD prior to T1D had caught up with the group without CD at the third-year follow-up. The reason for the seemingly extended duration of decreased growth in children with additional T1D compared to children with only CD could be due to a slower rate of healing of the intestinal mucosa. Levels of Ttg-IgA are known to correspond well with mucosal damage (121, 122) and study of Ttg-IgA normalisation in children with CD showed a significantly longer normalisation time in the subgroup with additional T1D, over three years compared to a little over one year (1204 vs. 403 days) (123).

The reasons for a possibly slower rate of healing in children with T1D and CD are unknown. One reason could be lower compliance to GFD in children with T1D and additional CD than in children with CD in general. Unfortunately, we had no data on compliance in Study II. However, when we looked at compliance in Study III we found the level of compliance to be substantially lower (68%) than the level of compliance previously reported in Swedish children with CD in general (around 90% (131, 200). Even so, we found no significant associations between compliance and growth in Study III, although there was a trend towards a higher BMI-SDS with a higher compliance. Since the differences we saw in growth in Study II were rather small, the sample size in Study III (60 children) might have been too small for any potential difference to be significant.

An alternative interpretation of the lower BMI-SDS in the group with CD and T1D compared to the group with T1D without CD would be that a diet regimen of a GFD is actually healthier. In Study II no group was underweight during the follow-ups (BMI-SDS values between +0.10 and +0.47). Thus, since the group on a GFD was closer to "ideal", they may have had a healthier diet. However, a GFD is known to have a higher GI than an ND (125, 129, 130). Additionally, children with CD in general have not shown decreased weight in a GFD (136-138). In contrast, some studies report that they are more often overweight (136, 137). Overall, the explanation of a healthier diet seems far-fetched.

In Study II we found significant differences in growth at follow-up but not at CD diagnosis. Our results are supported by other studies (155, 160) although most studies found decreased growth in children with T1D at CD diagnosis compared to children with T1D without CD (149, 152, 153, 199). As mentioned above, most previous studies were small or had questionable representativeness. One reason why we did not find a decreased growth at CD diagnosis could be that the children in the group, in which we looked at growth specifically at CD diagnosis (CDat), had been simultaneously diagnosed with T1D. As expected, all children in our study had a low BMI-SDS at the time of T1D diagnosis. It is possible that the catabolic status at T1D diagnosis outlasted the effects of malabsorption in untreated CD patients. Studies that found a lower growth at CD diagnosis had no information on time of T1D diagnosis in relation to CD diagnosis.

Important advantages of our study are:

1. a large study sample (3612 children with T1D, of which 393 children have T1D and CD)
2. a good representativeness (>90% of all children diagnosed with T1D in Sweden between 2005 and 2010).
3. and a well defined cohort. (The annual CD screening programme for children with T1D in Sweden and the BDD register, made it possible to

subdivide the children according to when they were diagnosed with CD in relation to their T1D diagnosis.)

One limitation of the study is that we did not have a reference group of patients with CD but without T1D. The growth of such a group would have been interesting to compare with the growth of children with CD and T1D, to better understand the influence on the growth of CD and T1D.

Future studies should use two control groups to compare the growth of children with T1D and concordant CD: one with T1D and no CD, and one with CD and no T1D.

Quality of life

In Study I we aimed to investigate not only how a GFD affected glycaemic control and growth in children with T1D, but also how it affected their life in general. In order to achieve this, we used the QoL questionnaire DISABKIDS at inclusion, 3, 6 and 12 months, and compared the results groupwise. There were no significant differences between groups but the group of children on a GFD had lower QoL scores at all timepoints. Unfortunately, since the sample size was small, and the groups already differed at inclusion, it was difficult to draw any firm conclusions from our data. However, our results indicated that a GFD has no significant effect on QoL. This interpretation is strengthened by the fact that there were few dropouts in the group of children on a GFD and that most children complied well with the GFD. To the best of our knowledge, there is only one other study of children with T1D on a GFD including QoL, and that study did not either find any difference in QoL between children with T1D on a GFD compared to children with T1D on a ND. (13). However, that study only measured QoL at 12 months, making it unclear whether the groups were comparable at baseline.

Compliance with a GFD and methodological challenges

As discussed above, we investigated compliance with a GFD in children with T1D and CD to better understand whether glycaemic control in these children was associated with a GFD. In Study III, involving a representative cohort of all children in Skåne County diagnosed with T1D between 2005 and 2012 with an additional CD diagnosis, we found good compliance in 68% of the children. Some similar studies found good compliance to be somewhat higher, between 69 and 100% (152, 153, 163, 164), while other studies on compliance with a GFD in children with CD and T1D showed good compliance to be substantially lower at 30-44% (165, 166). There are several possible reasons for the wide range of results.

Reasons for different results include variations in sample size and different degrees of sample representativity. Many of the mentioned studies had smaller sample sizes and fewer follow-ups than our study. A smaller sample size provides less power.

The only large study we found had quite questionable representativity since 61% of the children were excluded and CD prevalence was only 2.2% (165). In contrast, our sample was quite representative since we included almost all the children who met the inclusion criteria in the region at the time (prevalence rate 8.6%).

However, the most significant reason for the different results on compliance to a GFD is probably the lack of a standardised way of measuring compliance. There are no completely reliable measures (205) and different studies use different measures, making direct comparison difficult. The measures used include: intestinal biopsy, serology, clinical outcome assessment, QoL and GIPs. Because of the high amount of research on new kinds of CD treatment, *The Tampere Recommendations* on compliance measures were written in 2018 to advise researchers about ways of measuring outcome. In *The Tampere Recommendations* biopsy and serology using Ttg-IgA are rated the most reliable measures (grade B), while the other measures listed above were given grade D (134).

Thus, we used one of the best measures available in Study III. Ttg-IgA, an enzyme in the lamina propria of the intestinal wall, promotes autoimmunity to gluten (111). Intestinal damage in CD correlates well with Ttg-IgA levels (121, 122) and Ttg-IgA is recommended as a tool for measuring the success of CD treatment. However, Ttg-IgA is far from being a perfect measure. First, many different types of tests are available that show varying Ttg-IgA levels, although analysing the same sample (206). Second, Ttg-IgA has been shown to underestimate intestinal mucosal damage (207). Furthermore, Ttg-IgA response also depends on the duration of a GFD and the amount of ingested gluten (208).

There is no standardised way of interpreting fluctuations in Ttg-IgA in relation to compliance. In Study III, we decided to disregard one deviating Ttg-IgA value if there were at least a total of five values. If, for example, a child was Ttg-IgA negative for all follow-ups except one, the positive value was disregarded, and compliance interpreted as good. Some other researchers have chosen a stringent way in which good compliance does not allow for any positive Ttg-IgA values. Since studies on compliance in children with CD show that accidental gluten intake is > 80%, such an interpretation of compliance is harsh (131).

In Study I we could not use Ttg-IgA or intestinal biopsy since the children we studied did not have CD. Thus, they were not expected to produce Ttg-IgA or CD histopathological intestinal mucosal changes in response to gluten. We therefore used Green - a questionnaire regarding gluten exposure, completed by a dietician at clinical visits at 3, 6 and 12 months, and scored them into five different compliance categories. In Study I we found good compliance with a GFD at around 90% throughout the year. Our adherence rate was strengthened by a similar rate in the

Danish study of a GFD in children with T1D without CD, which also measured compliance to a GFD using questionnaires (14). Unfortunately, dietary questionnaires are not a very sensitive way of measuring gluten exposure (209). However, a similar Czech study of children with T1D on a GFD also found a high adherence rate, using a newer and direct measure: gluten immunogenic peptides (GIP) (13). Even if the three individual studies are small, they all show a high compliance to a GFD in children with T1D without CD, which adds substantially to their credibility. Overall, it shows that a GFD is feasible in children with newly diagnosed T1D.

For future studies, gluten immunogenic peptides (GIPs) are a very interesting alternative to study compliance to a GFD (134). GIPs include a large 33-mer peptide derived from α-gliadin, which is resistant to digestion (210). Thus, GIPs measured in urine or stools provide a direct measure of gluten intake (209) and not the body's response to gluten, (such as Ttg-IGA and histopathological changes in intestinal mucosa). Thus, they are useful in patients both with and without CD.

Other methodological challenges

Recruiting patients for Study I

A setback during this thesis project was the challenge in recruiting patients for Study I. To obtain sufficient power, we planned to include 160 children, 80 on a GFD and 80 continuing with their ordinary diet. However, after three years of ongoing inclusion we only managed to include 23 children, corresponding to approximately 10% of potentially eligible patients.

To understand why so few children were included, we analysed what we could have done better. When we started preparing for the study, we sent an individual letter with information about the study to all physicians in Skåne County working with paediatrics. All physicians working in diabetic paediatrics were also orally informed. Even so, almost all children in the study were included by the physician responsible for the study. A reason for this could be a lack of reminders. Most physicians have a high workload and in such an environment a single information mail might not be enough. Another reason could be reluctance on the part of the physicians to propose a potentially burdensome global change in the diet to a child who recently has been diagnosed with a life changing and serious chronic disease such as T1D. A third reason might be a similar reluctance on the part of the family and child.

We propose that future studies use a trial coordinator to ensure that all eligible children are invited to participate. It is also important that the trial coordinator is well informed about the study to be able to explain the rationale behind it to

physicians and eligible families. Further, the trial coordinator needs to have enough time at their disposal to be able to remain in contact with the sites and the families.

Bias

Another shortcoming of Study I is that the included children were not randomised. Instead, the choice of diet was left to the child and their family. Our rationale for the decision to use a non-randomised design was that we were concerned that compliance would be negatively influenced if the choice of diet was not left to the child and their family. The two previously mentioned similar studies used the same method. This may potentially explain the rather high compliance in our study, as well as in the two other studies. However, this could also be a reason for confounding. Choosing a GFD is perhaps associated with a generally high ability to adjust to healthcare recommendations, thus also being more prone to meticulously following T1D treatment advice, which could then be a confounding factor for better glycaemic control in the group on the GFD.

To be able to better understand why glycaemic control was better in the group on a GFD, future studies need to include more children, a higher percentage of eligible children, and use a randomised design.

Conclusions and Future remarks

We found possible associations between gluten and T1D. In Study I improved glycaemic control was associated with a GFD in children with T1D. In Study II growth was decreased in children with T1D and CD compared to children with T1D without CD. In Study III glycaemic control in children with T1D and CD improved with better compliance to a GFD. In Study IV, we surprisingly found that the prevalence of CD was not increased in children with T1D during the Swedish celiac epidemic, as it was in the general Swedish child population at the time.

Additionally, we found compliance to a GFD to be lower in children with TID and CD (Study III).

Our results need to be viewed in the light of several limitations. The connections we found between gluten and T1D were associations and were therefore sensitive to confounding factors. Further, our sample size in Study I was underpowered and the measures of compliance in Studies I and III were imperfect.

The results of an improved glycaemic control with a GFD in children with T1D in Study I are strengthened by two other small studies that found the same association (14). We therefore suggest that any similar future study should have a randomised design to avoid confounding, and a sample size of 30-40 children in each arm, which would give 80% power to detect the smallest clinically significant effect we found. To further study associations between gluten and the prevalence of CD, it would also be interesting to include our original idea of a five-year follow-up in such a study, to continue to look at the possible effect of a GFD on the incidence of CD.

In Study III we found improved glycaemic control with better compliance. Although these children also had CD, the presumption that a damaged intestinal mucosa would affect glycaemic control is unlikely, since the children with an undiagnosed (untreated) CD in Study II did not differ in glycaemic control from T1D children without CD. However, the association between glycaemic control and higher compliance could depend on a higher overall compliance to healthcare treatment and thus a more meticulously followed T1D treatment, resulting in better glycaemic control regardless of compliance. These possible confounding factors demonstrates the need for RCTs in children with T1D and CD, where not only compliance with a GFD, but also T1D regimen are carefully followed and controlled for. A future RCT should also consider including a control group of children with T1D without CD, as well as a control group of children with CD without T1D. In such a study the

possible effects of a GFD would be easier to separate from the effects of CD and T1D per see.

To better understand the broader effects and interactions between diet and disease, it would also be a good idea to include QoL. For example, such an understanding might help to improve compliance with a GFD in children with CD and T1D.

Populärvetenskaplig sammanfattning

I min avhandling har jag undersökt huruvida gluten påverkar diabetes typ 1 hos barn. Särskilt har jag studerat om, och hur, en glutenfri kost påverkar barn som precis insjuknat i typ 1 diabetes. För att göra det har jag tittat på barn med typ 1 diabetes, och barn med typ 1 diabetes och celiaki, eftersom de äter en glutenfri kost.

Typ 1 diabetes

Typ 1 diabetes är en av de vanligaste, och allvarligaste, kroniska sjukdomar hos barn i världen, idag. År 2022 beräknades runt 1,2 millioner barn vara drabbade och allt fler barn insjuknar. Hur vanlig sjukdomen är skiljer sig åt mellan länder. I Sverige är typ 1 diabetes näst vanligast i världen efter Finland. Typ 1 diabetes är ungefär lika vanliga hos pojkar som hos flickor. Man kan drabbas under hela livet men oftast debuterar typ 1 diabetes i barndomen. Typ 1 diabetes är en autoimmun sjukdom där kroppen bildar autoantikroppar som förstör de celler som bildar insulin. Med mindre insulin kan kroppen inte tillgodogöra sig socker lika bra längre. I stället kissar man ut mer och mer av sockret, blir törstig och går ned i vikt. Ju mindre insulin man tillverkar desto allvarligare sjuk blir man. Helt utan insulin dör man och därför var upptäckten att man kunde tillföra insulin utifrån livräddande. Alla med typ 1 diabetes behöver livslång behandling med dagliga injektioner av insulin även om de flesta har kvar en del av sin insulinproduktion något år efter diabetesdebuten. Dessutom behöver man vara noga med att hålla sin blodsockernivå jämn så att man inte drabbas av komplikationer. Komplikationer kan vara akuta och livshotande (insulinkoma och syraförgiftning) eller kroniska med allvarliga funktionsnedsättningar som tex syn och känselnedsättningar till följd.

Celiaki

Celiaki är liksom typ 1 diabetes en vanlig sjukdom. Ungefär 1 % av världens barn beräknas vara drabbade och liksom typ 1 diabetes blir celiaki vanligare och vanligare. Fler flickor än pojkar får celiaki men hos barnen med typ 1 diabetes är celiaki ungefär lika vanligt hos pojkar som hos flickor. Celiaki är också en autoimmun sjukdom som triggas av gluten, ett protein som finns i vete, råg och

korn. Vid celiaki bryter kroppen ned tarmslemhinnan i tunntarmen med bland annat magont, viktnedgång, och dålig tillväxt som följd. Behandlingen av celiaki är livslång glutenfri kost.

Gluten

Gluten har funnits i människans diet sen 10 000 år tillbaka. Gluten är ett protein som finns i vete, råg och korn. Gluten är globalt en mycket viktig proteinkälla. Konsumtionen av vete i världen ökar och 50% av kaloriintaget beräknas idag härröra från spannmål.

Vid celiaki är det gluten som triggar och driver den autoimmuna processen. När gluten plockas bort från kosten försvinner ofta symtomen på celiaki inom några veckor, och efter ett år brukar barns tarmar vara läkta. Studier tyder på att gluten även kan trigga autoimmunitet vid typ 1 diabetes.

Innan jag påbörjade mitt doktorandarbete hade en case-studie rapporterat att en pojke i Danmark, som precis fått typ 1 diabetes, kunnat sluta med insulin efter att ha påbörjat en glutenfri kost. Innan dess hade en del djurstudier redan kunnat visa ett samband mellan glutenfri mat och minskad risk för typ 1 diabetes. Studier på barn med diabetes hade dessutom visat ökade immunologiska reaktioner på gluten vid diagnos av typ 1 diabetes.

Studier, resultat och framtida studier

Studie 1

För att vidare undersöka ett möjligt samband mellan typ 1 diabetes och gluten designade vi en studie av barn med nydiagnostiserad typ 1 diabetes, där hälften av barnen fick äta glutenfri kost medan andra hälften fortsatte med sin ursprungliga kost. Vi ville undersöka om de som åt en glutenfri kost kunde behålla sin egenproduktion av insulin länge än de som åt normalkost, samt om den glutenfria kosten kunde påverka blodsockerkontroll positivt. Kostinterventionen pågick under ett år och under denna tid mättes insulinproduktion, sockerkontroll, följsamhet till kosten, och livskvalité, för att sedan jämföras gruppvis. Resultaten av studien visade att barnen med glutenfri mat verkade ha; bättre sockerkontroll, god följsamhet till den glutenfria kosten, och oförändrad livskvalité. Tyvärr lyckades inte vi få med så många barn som vi ville i studien och resultaten är därför osäkra. Våra resultat stärktes dock av 2 liknande, ungefär samtida studier, som båda visade snarlika resultat.

Studie 2

Eftersom det varit svårt att få barn att delta i vår studie, och vi ville fortsätta studera sambandet mellan typ 1 diabetes och glutenintag bestämde vi oss för att studera barn med typ 1 diabetes som redan åt glutenfri mat, nämligen barn med celiaki. Vi tittade retrospektivt på en stor kohort av barn med typ 1 diabetes som registrerats i den nationella BDD studien som inkluderar över 90% av barn med diabetes i Sverige. Vi delade in barnen i grupper utifrån om de även hade en celiakidiagnos, och i så fall när de hade fått den, i förhållande till sin diabetesdiagnos. Därefter jämförde vi grupperna avseende blodsockerkontroll och tillväxt. Vår kohort innehöll totalt 3612 barn med typ 1 diabetes. Av dessa hade över 11 % celiaki; 1.7% hade celiaki före diabetesdiagnos; 4% vid diagnos; och 5.2% 1-5 år efter diagnos. Vi hittade ingen skillnad mellan grupperna avseende blodsockerkontroll. Avseende tillväxt såg vi däremot att barnen med celiaki före diabetesdiagnos hade lägre tillväxt de 2 första åren efter diabetesdiagnos, och att barnen som diagnostiserats med celiaki vid diabetesdiagnos hade lägre tillväxt alla 5 årliga uppföljningar. Det finns forskning som visar att tarmen hos barn med celiaki och diabetes läker långsammare än hos barn med endast celiaki. Kanske var det på grund av en långsam tarmläkning barnen med celiaki hade en lägre tillväxt. Varför barn med båda diagnoserna läker sämre vet man inte men en del studier har visat sämre följsamhet till glutenfri mat hos dessa barn vilket skulle kunna vara en förklaring. Eftersom vi inte hade tillgång till mått av följsamhet till glutenfri mat i den här studien kunde vi inte avgöra om den lägre tillväxten vi såg berodde på låg följsamhet eller på den glutenfria kosten i sig.

Studie 3

För att undersöka följsamheten till glutenfrikost hos barn med diabetes i Sverige gjorde vi den tredje studien där vi tittade på 60 barn med diabetes och celiaki under 1-10 år, registrerade i den svenska BDD studien. Barnen delades in i 3 grupper efter transglutaminas nivåer (antikroppar i blodet som korrelerar med följsamhet till glutenfrimat vid celiaki); god följsamhet; varierande följsamhet och dålig följsamhet. Följsamheten visade sig vara god hos 68% av barnen, vilket är något mindre än generellt hos barn med celiaki i Sverige, där den ligger runt 90%. Resultaten visade också att de barn som hade god följsamhet hade bättre sockerkontroll än barnen med sämre följsamhet. Huruvida det var den glutenfria kosten eller en generellt god förmåga att följa behandlingsrekommendationer (alltså även rekommendationer avseende metabolkontroll och diabetes i det här fallet) som påverkade blodsockerkontrollen positivt vet vi dock inte.

Studie 4

Eftersom risken att få celiaki är mycket större hos barn med diabetes än hos barn i allmänhet hade vi i vår första studie egentligen också tänkt titta på om risken att få celiaki skulle kunna minska hos barn som fick äta glutenfrikost i anslutning till diabetesdiagnos. Tyvärr blev vår studie allt för liten för att kunna se en sådan eventuell skillnad. Därför bestämde vi oss för att undersöka eventuellt samband på ett annat sätt. Den svenska celiakiepidemin var en period mellan 1984 och 1996 då celiaki plötsligt blev fyra gånger vanligare i Sverige än det var innan. Anledningen till att barn fick celiaki i större utsträckning tror man var att glutenhalten i välling var större under denna period. När man ändrade tillbaka glutenhalten till tidigare nivåer såg man nämligen att det blev färre barn som fick celiaki igen, ungefär lika många som innan glutenhalten ökade. Dessutom har man i senare studier kunnat visa att ökad glutenhalt i maten till barn under 2 år ger större risk för celiaki.

För att titta på hur det ökade gluteninnehållet i maten kan ha påverkat förekomsten av celiaki hos barn med typ 1 diabetes jämförde vi en nationell kohort av barn med typ 1 diabetes födda under celiakiepidemin med en kohort av barn med typ 1 diabetes födda efter celiakiepidemin. Till vår förvåning var det ungefär lika många som hade celiaki i grupperna. Barn med typ 1 diabetes verkade således påverkas mindre av skillnaden i matens gluteninnehåll under småbarnsåren, än vad barn generellt gjorde. Hur det kan komma sig vet vi inte. En anledning till att prevalensen av celiaki hos barn med typ 1 diabetes inte påverkades *mer* av gluten innehåll än vad den gjorde hos barn utan typ 1 diabetes skulle kunna vara tidpunkten för det ökade gluteninnehållet. Studier har visat att gluten verkar vara mest immunogent kring diagnos av typ 1 diabetes. Eftersom typ 1 diabetes oftast diagnostiseras senare under barndomen, kanske ett högt gluten innehåll i småbarnsåren, som under celiakiepidemin, inte spelar så stor roll. Denna teori förklarar dock inte varför barnen med typ 1 diabetes skulle påverkas *mindre* av gluteninnehåll i småbarnsåren än vad barn generellt verkat göra. Eventuellt skulle skillnaden mellan grupperna kunna förklaras av genetiska skillnader då vissa gener är betydligt vanligare hos barn med typ 1 diabetes och celiaki än hos barn generellt. Hur en eventuell genetisk påverkan fungerar vet vi dock inte. Det behöver nya studier undersöka.

Sammanfattning och framtida studier

För att få säkrare resultat avseende den glutenfria kostens inverkan på blodsockerkontroll hos barn med typ 1 diabetes vore det bra att göra om studie I med fler barn, som lottas till respektive kostgrupp. I en sådan studie skulle man också kunna titta på huruvida förekomsten av celiaki 5 år efter kostinterventionen påverkas av ett års glutenfri kost efter diabetesdiagnos. För att bättre förstå den minskade tillväxten hos barn med typ 1 diabetes och celiaki jämfört barn med typ 1 diabetes utan celiaki skulle det underlättas om man i framtida studier inkluderade

en kontrollgrupp med bara celiaki. Avseende resultatet med sämre följsamhet till glutenfrikost hos barn med celiaki och typ 1 diabetes vore det bra att göra en ny studie där man inkluderar livskvalité för att förstå hur man bättre kan hjälpa dessa barn att följa sin kost.

Under doktorandarbetets gång hittade vi bättre blodsockerkontroll hos barn med typ 1 diabetes och glutenfri mat jämfört med hos barn med typ 1 diabetes och vanlig mat. Vi såg också att barn med typ 1 diabetes och celiaki, som hade en bättre följsamhet till en glutenfrikost, hade bättre blodsockerkontroll. Dessutom visade våra resultat överraskande att gluteninnehåll i spädbarnskosten verkade påverka förekomsten av celiaki hos barn med typ 1 diabetes mindre än barn utan typ 1 diabetes. Slutligen kom vi fram till att barn med celiaki och typ1 diabetes verkar ha en sämre följsamhet till glutenfri kost än barn med celiaki utan typ 1 diabetes.

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References

1. Tuomilehto J, Ogle GD, Lund-Blix NA, Stene LC. Update on Worldwide Trends in Occurrence of Childhood Type 1 Diabetes in 2020. Pediatric endocrinology reviews: PER. 2020;17(Suppl 1):198-209.
2. Tuomilehto J. The emerging global epidemic of type 1 diabetes. Current diabetes reports. 2013;13(6):795-804.
3. Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, et al. Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice. 2019;157:107842.
4. Snell-Bergeon JK, Nadeau K. Cardiovascular disease risk in young people with type 1 diabetes. Journal of cardiovascular translational research. 2012;5:446-62.
5. Rodriguez-Calvo T. Enterovirus infection and type 1 diabetes: unraveling the crime scene. Clinical & Experimental Immunology. 2019;195(1):15-24.
6. Holmberg H, Wahlberg J, Vaarala O, Ludvigsson J, Group AS. Short duration of breast-feeding as a risk-factor for β-cell autoantibodies in 5-year-old children from the general population. British journal of nutrition. 2007;97(1):111-6.
7. Virtanen SM, Niinistö S, Nevalainen J, Salminen I, Takkinen H-M, Kääriä S, et al. Serum fatty acids and risk of advanced β-cell autoimmunity: a nested case–control study among children with HLA-conferred susceptibility to type I diabetes. European journal of clinical nutrition. 2010;64(8):792-9.
8. Norris JM, Yin X, Lamb MM, Barriga K, Seifert J, Hoffman M, et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. Jama. 2007;298(12):1420-8.
9. Antvorskov JC, Halldorsson TI, Josefsen K, Svensson J, Granström C, Roep BO, et al. Association between maternal gluten intake and type 1 diabetes in offspring: national prospective cohort study in Denmark. bmj. 2018;362:k3547.
10. Funda DP, Kaas A, Bock T, Tlaskalová-Hogenová H, Buschard K. Gluten-free diet prevents diabetes in NOD mice. Diabetes/metabolism research and reviews. 1999;15(5):323-7.
11. Hansen AK, Ling F, Kaas A, Funda DP, Farlov H, Buschard K. Diabetes preventive gluten-free diet decreases the number of caecal bacteria in non-obese diabetic mice. Diabetes/metabolism research and reviews. 2006;22(3):220-5.
12. Marietta EV, Gomez AM, Yeoman C, Tilahun AY, Clark CR, Luckey DH, et al. Low incidence of spontaneous type 1 diabetes in non-obese diabetic mice raised on gluten-free diets is associated with changes in the intestinal microbiome. PloS one. 2013;8(11):e78687.

13. Neuman V, Pruhova S, Kulich M, Kolouskova S, Vosahlo J, Romanova M, et al. Gluten-free diet in children with recent-onset type 1 diabetes: A 12-month intervention trial. Diabetes, Obesity and Metabolism. 2020;22(5):866-72.
14. Svensson J, Sildorf SM, Pipper CB, Kyvsgaard JN, Bøjstrup J, Pociot FM, et al. Potential beneficial effects of a gluten-free diet in newly diagnosed children with type 1 diabetes: a pilot study. Springerplus. 2016;5(1):1-8.
15. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E. Milestones in the history of diabetes mellitus: The main contributors. World journal of diabetes. 2016;7(1):1.
16. Magliano DJ, Boyko EJ. IDF diabetes atlas. 2022.
17. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. The lancet. 2017;389(10085):2239-51.
18. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes–global burden of disease and forecasted trends. Journal of epidemiology and global health. 2020;10(1):107.
19. Shah AS, Nadeau KJ. The changing face of paediatric diabetes. Diabetologia. 2020;63:683-91.
20. Swediabkids. Fördelning av diabetesdiagnos 2020. År 2020. 2020.
21. Groop L, Pociot F. Genetics of diabetes–are we missing the genes or the disease? Molecular and cellular endocrinology. 2014;382(1):726-39.
22. Libman I, Haynes A, Lyons S, Pradeep P, Rwagasor E, Tung JYl, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatric diabetes. 2022;23(8):1160-74.
23. Dabelea D, Pihoker C, Talton JW, D'Agostino Jr RB, Fujimoto W, Klingensmith GJ, et al. Etiological approach to characterization of diabetes type: the SEARCH for Diabetes in Youth Study. Diabetes care. 2011;34(7):1628-33.
24. Bonnefond A, Unnikrishnan R, Doria A, Vaxillaire M, Kulkarni RN, Mohan V, et al. Monogenic diabetes. Nature Reviews Disease Primers. 2023;9(1):12.
25. Carlsson A, Shepherd M, Ellard S, Weedon M, Lernmark Å, Forsander G, et al. Absence of islet autoantibodies and modestly raised glucose values at diabetes diagnosis should lead to testing for MODY: lessons from a 5-year pediatric Swedish national cohort study. Diabetes Care. 2020;43(1):82-9.
26. Gregory GA, Robinson TI, Linklater SE, Wang F, Colagiuri S, de Beaufort C, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. The lancet Diabetes & endocrinology. 2022;10(10):741-60.
27. Waernbaum I, Lind T, Möllsten A, Dahlquist G. The incidence of childhood-onset type 1 diabetes, time trends and association with the population composition in Sweden: a 40 year follow-up. Diabetologia. 2023;66(2):346-53.
28. Gale EA, Gillespie KM. Diabetes and gender. Diabetologia. 2001;44:3-15.

29. Forga L, Chueca MJ, Tamayo I, Oyarzabal M, Toni M, Goñi MJ. Cyclical variation in the incidence of childhood-onset type 1 diabetes during 40 years in Navarra (Spain). Pediatric Diabetes. 2018;19(8):1416-21.
30. Gerasimidi Vazeou A, Kordonouri O, Witsch M, Hermann JM, Forsander G, de Beaufort C, et al. Seasonality at the clinical onset of type 1 diabetes—Lessons from the SWEET database. Pediatric diabetes. 2016;17:32-7.
31. Sanjeevi CB, Lybrand TP, DeWeese C, Landin-Olsson M, Kockum I, Dahlquist G, et al. Polymorphic amino acid variations in HLA-DQ are associated with systematic physical property changes and occurrence of IDDM. Diabetes. 1995;44(1):125-31.
32. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes. 2008;57(4):1084-92.
33. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nature genetics. 2009;41(6):703-7.
34. Bradfield JP, Qu H-Q, Wang K, Zhang H, Sleiman PM, Kim CE, et al. A genome-wide meta-analysis of six type 1 diabetes cohorts identifies multiple associated loci. PLoS genetics. 2011;7(9):e1002293.
35. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. Pediatric diabetes. 2018;19(3):346-53.
36. Knip M. Pathogenesis of type 1 diabetes: implications for incidence trends. Hormone research in paediatrics. 2011;76(Suppl. 1):57-64.
37. Kyvik KO, Green A, Beck-Nielsen H. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. Bmj. 1995;311(7010):913-7.
38. Redondo MJ, Jeffrey J, Fain PR, Eisenbarth GS, Orban T. Concordance for islet autoimmunity among monozygotic twins. New England Journal of Medicine. 2008;359(26):2849-50.
39. Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. The Lancet. 2008;371(9626):1777-82.
40. Söderström U, Åman J, Hjern A. Being born in Sweden increases the risk for type 1 diabetes–a study of migration of children to Sweden as a natural experiment. Acta paediatrica. 2012;101(1):73-7.
41. Couper JJ, Steele C, Beresford S, Powell T, McCaul K, Pollard A, et al. Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. Diabetes. 1999;48(11):2145-9.
42. Frederiksen B, Kroehl M, Lamb MM, Seifert J, Barriga K, Eisenbarth GS, et al. Infant exposures and development of type 1 diabetes mellitus: The Diabetes Autoimmunity Study in the Young (DAISY). JAMA pediatrics. 2013;167(9):808-15.
43. Lamb MM, Miller M, Seifert JA, Frederiksen B, Kroehl M, Rewers M, et al. The effect of childhood cow's milk intake and HLA-DR genotype on risk of islet autoimmunity and type 1 diabetes: The Diabetes Autoimmunity Study in the Young. Pediatric diabetes. 2015;16(1):31-8.

44. Virtanen SM, Läärä E, Hyppönen E, Reijonen H, Räsänen L, Aro A, et al. Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes. Childhood diabetes in Finland study group. Diabetes. 2000;49(6):912-7.
45. Rosenbauer J, Herzig P, Giani G. Early infant feeding and risk of type 1 diabetes mellitus—a nationwide population-based case–control study in pre-school children. Diabetes/metabolism research and reviews. 2008;24(3):211-22.
46. L Bishop E, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory. JBMR plus. 2021;5(1):e10405.
47. Sørensen IM, Joner G, Jenum PA, Eskild A, Torjesen PA, Stene LC. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. Diabetes. 2012;61(1):175-8.
48. Miettinen ME, Reinert L, Kinnunen L, Harjutsalo V, Koskela P, Surcel H-M, et al. Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. Diabetologia. 2012;55:1291-4.
49. Simpson M, Brady H, Yin X, Seifert J, Barriga K, Hoffman M, et al. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia. 2011;54:2779-88.
50. De Goffau MC, Luopajärvi K, Knip M, Ilonen J, Ruohtula T, Härkönen T, et al. Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes. 2013;62(4):1238-44.
51. Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC medicine. 2013;11:1-12.
52. Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen A-M, et al. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell host & microbe. 2015;17(2):260-73.
53. Cardwell C, Carson D, Patterson C. No association between routinely recorded infections in early life and subsequent risk of childhood-onset Type 1 diabetes: a matched case–control study using the UK General Practice Research Database. Diabetic medicine. 2008;25(3):261-7.
54. Rasmussen T, Witsø E, Tapia G, Stene LC, Rønningen KS. Self-reported lower respiratory tract infections and development of islet autoimmunity in children with the type 1 diabetes high-risk HLA genotype: the MIDIA study. Diabetes/metabolism research and reviews. 2011;27(8):834-7.
55. Stene LC, Oikarinen S, Hyöty H, Barriga KJ, Norris JM, Klingensmith G, et al. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). Diabetes. 2010;59(12):3174-80.
56. Dahlquist GG, Ivarsson S, Lindberg B, Forsgren M. Maternal enteroviral infection during pregnancy as a risk factor for childhood IDDM: a population-based case-control study. Diabetes. 1995;44(4):408-13.

57. Johansson C, Samuelsson U, Ludvigsson J. A high weight gain early in life is associated with an increased risk of type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1994;37:91-4.
58. Harder T, Roepke K, Diller N, Stechling Y, Dudenhausen JW, Plagemann A. Birth weight, early weight gain, and subsequent risk of type 1 diabetes: systematic review and meta-analysis. American journal of epidemiology. 2009;169(12):1428-36.
59. Cardwell CR, Stene LC, Joner G, Davis EA, Cinek O, Rosenbauer J, et al. Birthweight and the risk of childhood-onset type 1 diabetes: a meta-analysis of observational studies using individual patient data. Diabetologia. 2010;53:641-51.
60. Ferrara CT, Geyer SM, Liu Y-F, Evans-Molina C, Libman IM, Besser R, et al. Excess BMI in childhood: a modifiable risk factor for type 1 diabetes development? Diabetes care. 2017;40(5):698-701.
61. Besser RE, Bell KJ, Couper JJ, Ziegler AG, Wherrett DK, Knip M, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Stages of type 1 diabetes in children and adolescents. Pediatric diabetes. 2022;23(8):1175-87.
62. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes care. 2015;38(10):1964-74.
63. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. Jama. 2013;309(23):2473-9.
64. Anand V, Li Y, Liu B, Ghalwash M, Koski E, Ng K, et al. Islet autoimmunity and HLA markers of presymptomatic and clinical type 1 diabetes: joint analyses of prospective cohort studies in Finland, Germany, Sweden, and the US. Diabetes Care. 2021;44(10):2269-76.
65. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. The Lancet. 2016;387(10035):2340-8.
66. Cnop M, Foufelle F, Velloso LA. Endoplasmic reticulum stress, obesity and diabetes. Trends in molecular medicine. 2012;18(1):59-68.
67. McGinty JW, Chow I-T, Greenbaum C, Odegard J, Kwok WW, James EA. Recognition of posttranslationally modified GAD65 epitopes in subjects with type 1 diabetes. Diabetes. 2014;63(9):3033-40.
68. Marré ML, James EA, Piganelli JD. β cell ER stress and the implications for immunogenicity in type 1 diabetes. Frontiers in cell and developmental biology. 2015;3:67.
69. Delong T, Wiles TA, Baker RL, Bradley B, Barbour G, Reisdorph R, et al. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. Science. 2016;351(6274):711-4.
70. Doyle HA, Yang M-L, Raycroft MT, Gee RJ, Mamula MJ. Autoantigens: novel forms and presentation to the immune system. Autoimmunity. 2014;47(4):220-33.
71. Tojjar J, Cervin M, Hedlund E, Brahimi Q, Forsander G, Elding Larsson H, et al. Sex Differences in Age of Diagnosis, HLA Genotype, and Autoantibody Profile in Children With Type 1 Diabetes. Diabetes Care. 2023.

72. Control D, Group CTR. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England journal of medicine. 1993;329(14):977-86.
73. Genuth S. Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes. Endocrine Practice. 2006;12:34-41.
74. Nathan DM, Group DER. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes care. 2014;37(1):9-16.
75. Kuhtreiber W, Washer S, Hsu E, Zhao M, Reinhold III P, Burger D, et al. Low levels of C-peptide have clinical significance for established Type 1 diabetes. Diabetic Medicine. 2015;32(10):1346-53.
76. Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic β-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes. 2001;50(1):1-11.
77. Cengiz E, Danne T, Ahmad T, Ayyavoo A, Beran D, Ehtisham S, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Insulin treatment in children and adolescents with diabetes. Pediatric Diabetes. 2022;23(8):1277-96.
78. Pacaud D, Lemay JF, Richmond E, Besançon S, Hasnani D, Jali SM, et al. Contribution of SWEET to improve paediatric diabetes care in developing countries. Pediatric diabetes. 2016;17:46-52.
79. Biesiekierski JR. What is gluten? Journal of gastroenterology and hepatology. 2017;32:78-81.
80. Charmet G. Wheat domestication: lessons for the future. Comptes rendus biologies. 2011;334(3):212-20.
81. Leonard MM, Vasagar B. US perspective on gluten-related diseases. Clinical and experimental gastroenterology. 2014:25-37.
82. Hoppe C, Gøbel R, Kristensen M, Lind MV, Matthiessen J, Christensen T, et al. Intake and sources of gluten in 20-to 75-year-old Danish adults: a national dietary survey. European journal of nutrition. 2017;56:107-17.
83. Catassi C, Verdu EF, Bai JC, Lionetti E. Coeliac disease. The Lancet. 2022;399(10344):2413-26.
84. Shewry PR, Lookhart GL. Wheat gluten protein analysis: American Association of Cereal Chemists; 2003.
85. Hausch F, Shan L, Santiago NA, Gray GM, Khosla C. Intestinal digestive resistance of immunodominant gliadin peptides. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2002;283(4):G996-G1003.
86. Shewry PR, Halford NG. Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of experimental botany. 2002;53(370):947-58.
87. Shewry PR, Halford NG, Belton PS, Tatham AS. The structure and properties of gluten: an elastic protein from wheat grain. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences. 2002;357(1418):133-42.

88. Losowsky MS. A history of coeliac disease. Digestive diseases. 2008;26(2):112-20.
89. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. Clinical gastroenterology and hepatology. 2018;16(6):823-36. e2.
90. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. Official journal of the American College of Gastroenterology| ACG. 2020;115(4):507-25.
91. Lebwohl B, Murray JA. Gluten Introduction, Breastfeeding, and Celiac Disease: Back to the Drawing Board:: Statement Prepared by the Executive Council of the North American Society for the Study of Celiac Disease (NASSCD). The American journal of gastroenterology. 2016;111(1):12.
92. Greco L, Romino R, Coto I, Di Cosmo N, Percopo S, Maglio M, et al. The first large population based twin study of coeliac disease. Gut. 2002;50(5):624-8.
93. Nisticò L, Fagnani C, Coto I, Percopo S, Cotichini R, Limongelli MG, et al. Concordance, disease progression, and heritability of coeliac disease in Italian twins. Gut. 2006;55(6):803-8.
94. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. The Journal of experimental medicine. 1989;169(1):345-50.
95. Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, et al. HLA types in celiac disease patients not carrying the DQA1* 05-DQB1* 02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Human immunology. 2003;64(4):469-77.
96. Garnier-Lengliné H, Cerf-Bensussan N, Ruemmele FM. Celiac disease in children. Clinics and research in hepatology and gastroenterology. 2015;39(5):544-51.
97. Dydensborg Sander S, Hansen AV, Størdal K, Andersen A-MN, Murray JA, Husby S. Mode of delivery is not associated with celiac disease. Clinical Epidemiology. 2018:323-32.
98. Tanpowpong P, Obuch JC, Jiang H, McCarty CE, Katz AJ, Leffler DA, et al. Multicenter study on season of birth and celiac disease: evidence for a new theoretical model of pathogenesis. The Journal of pediatrics. 2013;162(3):501-4.
99. Silvester JA, Leffler DA. Is autoimmunity infectious? The effect of gastrointestinal viral infections and vaccination on risk of celiac disease autoimmunity. Clinical Gastroenterology and Hepatology. 2017;15(5):703-5.
100. Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, et al. Prevalence of childhood celiac disease and changes in infant feeding. Pediatrics. 2013;131(3):e687-e94.
101. Aronsson CA, Lee H-S, Liu E, Uusitalo U, Hummel S, Yang J, et al. Age at gluten introduction and risk of celiac disease. Pediatrics. 2015;135(2):239-45.
102. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P, et al. Randomized feeding intervention in infants at high risk for celiac disease. New England Journal of Medicine. 2014;371(14):1304-15.

103. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. New England Journal of Medicine. 2014;371(14):1295-303.
104. Aronsson CA, Lee H-S, Koletzko S, Uusitalo U, Yang J, Virtanen SM, et al. Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish birth cohort. Clinical Gastroenterology and Hepatology. 2016;14(3):403-9. e3.
105. Ivarsson A, Persson L, Nyström L, Ascher H, Cavell B, Danielsson L, et al. Epidemic of coeliac disease in Swedish children. Acta paediatrica. 2000;89(2):165-71.
106. Ivarsson A, Myleus A, Norström F, van der Pals M, Rosen A, Högberg L, et al. Prevalence of childhood celiac disease and changes in infant feeding. Pediatrics. 2013;131(3):e687-e94.
107. Ebert EC. Interleukin 15 is a potent stimulant of intraepithelial lymphocytes. Gastroenterology. 1998;115(6):1439-45.
108. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 2004;21(3):357-66.
109. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Sapone ATA, Thakar M, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scandinavian journal of gastroenterology. 2006;41(4):408-19.
110. Newton KP, Singer SA, editors. Celiac disease in children and adolescents: special considerations. Seminars in Immunopathology; 2012: Springer.
111. Molberg Ø, Mcadam SN, Körner R, Quarsten H, Kristiansen C, Madsen L, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nature medicine. 1998;4(6):713-7.
112. Van Kalleveen MW, de Meij T, Plötz FB. Clinical spectrum of paediatric coeliac disease: a 10-year single-centre experience. European Journal of Pediatrics. 2018;177:593-602.
113. Husby S, Koletzko S, Korponay-Szabó I, Mearin M, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. Journal of pediatric gastroenterology and nutrition. 2012;54(1):136-60.
114. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease 2020. Journal of pediatric gastroenterology and nutrition. 2020;70(1):141-56.
115. Green PH, Rostami K, Marsh MN. Diagnosis of coeliac disease. Best Practice & Research Clinical Gastroenterology. 2005;19(3):389-400.
116. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. European journal of gastroenterology & hepatology. 1999;11(10):1185-94.

117. Fasano A. Celiac disease: how to handle a clinical chameleon. N Engl J Med. 2003;348(25):2568-70.
118. Hujoel IA, Reilly NR, Rubio-Tapia A. Celiac disease: clinical features and diagnosis. Gastroenterology Clinics. 2019;48(1):19-37.
119. Craig ME, Prinz N, Boyle CT, Campbell FM, Jones TW, Hofer SE, et al. Prevalence of Celiac Disease in 52,721 Youth With Type 1 Diabetes: International Comparison Across Three Continents. Diabetes Care 2017; 40: 1034-1040. Diabetes Care. 2017;40(11):E168-E9.
120. Husby S, Bai JC. Follow-up of celiac disease. Gastroenterology Clinics. 2019;48(1):127-36.
121. Taavela J, Kurppa K, Collin P, Lähdeaho ML, Salmi T, Saavalainen P, et al. Degree of damage to the small bowel and serum antibody titers correlate with clinical presentation of patients with celiac disease. Clinical Gastroenterology and Hepatology. 2013;11(2):166-71. e1.
122. Singh P, Kurray L, Agnihotri A, Das P, Verma AK, Sreenivas V, et al. Titers of anti-tissue transglutaminase antibody correlate well with severity of villous abnormalities in celiac disease. Journal of clinical gastroenterology. 2015;49(3):212-7.
123. Isaac DM, Rajani S, Yaskina M, Huynh HQ, Turner JM. Antitissue transglutaminase normalization postdiagnosis in children with celiac disease. Journal of pediatric gastroenterology and nutrition. 2017;65(2):195-9.
124. Wahab PJ, Meijer JW, Mulder CJ. Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. American journal of clinical pathology. 2002;118(3):459-63.
125. Penagini F, Dilillo D, Meneghin F, Mameli C, Fabiano V, Zuccotti GV. Gluten-free diet in children: an approach to a nutritionally adequate and balanced diet. Nutrients. 2013;5(11):4553-65.
126. Thompson T. Folate, iron, and dietary fiber contents of the gluten-free diet. Journal of the Academy of Nutrition and Dietetics. 2000;100(11):1389.
127. Thompson T. Thiamin, riboflavin, and niacin contents of the gluten-free diet: is there cause for concern? Journal of the Academy of Nutrition and Dietetics. 1999;99(7):858.
128. Segura MEM, Rosell CM. Chemical composition and starch digestibility of different gluten-free breads. Plant Foods for Human Nutrition. 2011;66(3):224-30.
129. Zuccotti G, Fabiano V, Dilillo D, Picca M, Cravidi C, Brambilla P. Intakes of nutrients in I talian children with celiac disease and the role of commercially available gluten-free products. Journal of Human Nutrition and Dietetics. 2013;26(5):436-44.
130. Alzaben AS, Turner J, Shirton L, Samuel TM, Persad R, Mager D. Assessing nutritional quality and adherence to the gluten-free diet in children and adolescents with celiac disease. Canadian Journal of Dietetic Practice and Research. 2015;76(2):56-63.

131. Tapsas D, Fälth-Magnusson K, Högberg L, Hammersjö J-Å, Hollén E. Swedish children with celiac disease comply well with a gluten-free diet, and most include oats without reporting any adverse effects: a long-term follow-up study. Nutrition research. 2014;34(5):436-41.
132. Webb C, Norström F, Myléus A, Ivarsson A, Halvarsson B, Högberg L, et al. Celiac disease can be predicted by high levels of anti-tissue transglutaminase antibodies in population-based screening. Journal of pediatric gastroenterology and nutrition. 2015;60(6):787-91.
133. Mehta P, Li Q, Stahl M, Uusitalo U, Lindfors K, Butterworth MD, et al. Gluten-free diet adherence in children with screening-detected celiac disease using a prospective birth cohort study. Plos one. 2023;18(2):e0275123.
134. Ludvigsson JF, Ciacci C, Green PH, Kaukinen K, Korponay-Szabo IR, Kurppa K, et al. Outcome measures in coeliac disease trials: the Tampere recommendations. Gut. 2018;67(8):1410-24.
135. Mearin ML, Agardh D, Antunes H, Al-Toma A, Auricchio R, Castillejo G, et al. ESPGHAN position paper on management and follow-up of children and adolescents with celiac disease. Journal of Pediatric Gastroenterology and Nutrition. 2022;75(3):369-86.
136. Valletta E, Fornaro M, Cipolli M, Conte S, Bissolo F, Danchielli C. Celiac disease and obesity: need for nutritional follow-up after diagnosis. European journal of clinical nutrition. 2010;64(11):1371-2.
137. Brambilla P, Picca M, Dilillo D, Meneghin F, Cravidi C, Tischer M, et al. Changes of body mass index in celiac children on a gluten-free diet. Nutrition, Metabolism and Cardiovascular Diseases. 2013;23(3):177-82.
138. Capriati T, Francavilla R, Ferretti F, Castellaneta S, Ancinelli M, Diamanti A. The overweight: a rare presentation of celiac disease. European journal of clinical nutrition. 2016;70(2):282-4.
139. Elfström P, Sundström J, Ludvigsson JF. Systematic review with meta-analysis: associations between coeliac disease and type 1 diabetes. Alimentary pharmacology & therapeutics. 2014;40(10):1123-32.
140. Bybrant MC, Örtqvist E, Lantz S, Grahnquist L. High prevalence of celiac disease in Swedish children and adolescents with type 1 diabetes and the relation to the Swedish epidemic of celiac disease: a cohort study. Scandinavian journal of gastroenterology. 2013;49(1):52-8.
141. Larsson K, Carlsson A, Cederwall E, Jönsson B, Neiderud J, Jonsson B, et al. Annual screening detects celiac disease in children with type 1 diabetes. Pediatric diabetes. 2008;9(4pt2):354-9.
142. Adlercreutz EH, Wingren CJ, Vincente RP, Merlo J, Agardh D. Perinatal risk factors increase the risk of being affected by both type 1 diabetes and coeliac disease. Acta paediatrica. 2015;104(2):178-84.
143. Narula P, Porter L, Langton J, Rao V, Davies P, Cummins C, et al. Gastrointestinal symptoms in children with type 1 diabetes screened for celiac disease. Pediatrics. 2009;124(3):e489-e95.

144. Holmes G. Coeliac disease and type 1 diabetes mellitus–the case for screening. Diabetic Medicine. 2001;18(3):169-77.
145. Cerqueiro Bybrant M, Udén E, Frederiksen F, Gustafsson AL, Arvidsson CG, Fureman AL, et al. Celiac disease can be predicted by high levels of tissue transglutaminase antibodies in children and adolescents with type 1 diabetes. Pediatric Diabetes. 2021;22(3):417-24.
146. Setty-Shah N, Maranda L, Nwosu BU. Increased risk for vitamin d deficiency in obese children with both celiac disease and type 1 diabetes. Gastroenterology Research and Practice. 2014;2014.
147. Tittel SR, Dunstheimer D, Hilgard D, Knauth B, Fröhlich-Reiterer E, Galler A, et al. Coeliac disease is associated with depression in children and young adults with type 1 diabetes: results from a multicentre diabetes registry. Acta Diabetologica. 2021;58:623-31.
148. Tokatly Latzer I, Rachmiel M, Zuckerman Levin N, Mazor-Aronovitch K, Landau Z, Ben-David RF, et al. Increased prevalence of disordered eating in the dual diagnosis of type 1 diabetes mellitus and celiac disease. Pediatric Diabetes. 2018;19(4):749-55.
149. Kaspers S, Kordonouri O, Schober E, Grabert M, Hauffa BP, Holl RW. Anthropometry, metabolic control, and thyroid autoimmunity in type 1 diabetes with celiac disease: a multicenter survey. The Journal of pediatrics. 2004;145(6):790-5.
150. Mahmud FH, Elbarbary NS, Fröhlich-Reiterer E, Holl RW, Kordonouri O, Knip M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Other complications and associated conditions in children and adolescents with type 1 diabetes. Pediatric diabetes. 2018;19(Suppl 27):275.
151. Fröhlich-Reiterer E, Elbarbary NS, Simmons K, Buckingham B, Humayun KN, Johannsen J, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Other complications and associated conditions in children and adolescents with type 1 diabetes. Pediatric diabetes. 2022;23(8):1451-67.
152. Amin R, Murphy N, Edge J, Ahmed ML, Acerini CL, Dunger DB. A longitudinal study of the effects of a gluten-free diet on glycemic control and weight gain in subjects with type 1 diabetes and celiac disease. Diabetes care. 2002;25(7):1117-22.
153. Hansen D, Brock-Jacobsen B, Lund E, Bjørn C, Hansen LP, Nielsen C, et al. Clinical benefit of a gluten-free diet in type 1 diabetic children with screening-detected celiac disease: a population-based screening study with 2 years' follow-up. Diabetes care. 2006;29(11):2452-6.
154. Fröhlich-Reiterer EE, Kaspers S, Hofer S, Schober E, Kordonouri O, Bechtold-Dalla Pozza S, et al. Anthropometry, metabolic control, and follow-up in children and adolescents with type 1 diabetes mellitus and biopsy-proven celiac disease. The Journal of pediatrics. 2011;158(4):589-93. e2.
155. Sun S, Puttha R, Ghezaiel S, Skae M, Cooper C, Amin R, et al. The effect of biopsy-positive silent coeliac disease and treatment with a gluten-free diet on growth and glycaemic control in children with Type 1 diabetes. Diabetic Medicine. 2009;26(12):1250-4.

156. Rami B, Sumnik Z, Schober E, Waldhör T, Battelino T, Bratanic N, et al. Screening detected celiac disease in children with type 1 diabetes mellitus: effect on the clinical course (a case control study). Journal of pediatric gastroenterology and nutrition. 2005;41(3):317-21.
157. Eland I, Klieverik L, Mansour AA, Al-Toma A. Gluten-Free Diet in Co-Existent Celiac Disease and Type 1 Diabetes Mellitus: Is It Detrimental or Beneficial to Glycemic Control, Vascular Complications, and Quality of Life? Nutrients. 2023;15(1):199.
158. Mozzillo E, Franceschi R, Di Candia F, Francesco R, Leonardi L, Fedi L, et al. The impact of gluten-free diet on growth, metabolic control and quality of life in youth with type 1 diabetes and celiac disease: A systematic review. Diabetes Research and Clinical Practice. 2022:110032.
159. Burayzat S, Elsahoryi N, Freitekh A, Alzoubi O, Al-Najjar R, Tayyem R. Does a Gluten-Free Diet Affect BMI and Glycosylated Hemoglobin in Children and Adolescents with Type 1 Diabetes and Asymptomatic Celiac Disease? A Meta-Analysis and Systematic Review. Children. 2022;9(8):1247.
160. Goh VL, Estrada DE, Lerer T, Balarezo F, Sylvester FA. Effect of gluten-free diet on growth and glycemic control in children with type 1 diabetes and asymptomatic celiac disease. Journal of Pediatric Endocrinology and Metabolism. 2010;23(11):1169-73.
161. Kaur N, Bhadada SK, Minz RW, Dayal D, Kochhar R. Interplay between type 1 diabetes mellitus and celiac disease: implications in treatment. Digestive Diseases. 2018;36(6):399-408.
162. Leonard M, Cureton P, Fasano A. Managing coeliac disease in patients with diabetes. Diabetes, Obesity and Metabolism. 2015;17(1):3-8.
163. Pham-Short A, Donaghue KC, Ambler G, Garnett S, Craig ME. Quality of life in type 1 diabetes and celiac disease: role of the gluten-free diet. The Journal of pediatrics. 2016;179:131-8. e1.
164. Saadah O, Zacharin M, O'Callaghan A, Oliver M, Catto-Smith A. Effect of gluten-free diet and adherence on growth and diabetic control in diabetics with coeliac disease. Archives of disease in childhood. 2004;89(9):871-6.
165. Nagl K, Bollow E, Liptay S, Rosenbauer J, Koletzko S, Pappa A, et al. Lower HbA1c in patients with type 1 diabetes and celiac disease who reached celiac-specific antibody-negativity—A multicenter DPV analysis. Pediatric diabetes. 2019;20(8):1100-9.
166. Westman E, Ambler GR, Royle M, Peat J, Chan A. Children with coeliac disease and insulin dependent diabetes mellitus-growth, diabetes control and dietary intake. Journal of Pediatric Endocrinology and Metabolism. 1999;12(3):433-42.
167. Hadithi M, von Blomberg BME, Crusius JBA, Bloemena E, Kostense PJ, Meijer JW, et al. Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. Annals of internal medicine. 2007;147(5):294-302.
168. Hagopian W, Lee H-S, Liu E, Rewers M, She J-X, Ziegler A-G, et al. Co-occurrence of type 1 diabetes and celiac disease autoimmunity. Pediatrics. 2017;140(5).

169. Viskari H, Ludvigsson J, Uibo R, Salur L, Marciulionyte D, Hermann R, et al. Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: time trends and geographical variation. Diabetologia. 2005;48:1280-7.
170. Valitutti F, Cucchiara S, Fasano A. Celiac disease and the microbiome. Nutrients. 2019;11(10):2403.
171. Bosi E, Molteni L, Radaelli M, Folini L, Fermo I, Bazzigaluppi E, et al. Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia. 2006;49(12):2824-7.
172. Hansen CHF, Krych Ł, Buschard K, Metzdorff SB, Nellemann C, Hansen LH, et al. A maternal gluten-free diet reduces inflammation and diabetes incidence in the offspring of NOD mice. Diabetes. 2014;63(8):2821-32.
173. Antvorskov JC, Josefsen K, Haupt-Jorgensen M, Fundova P, Funda DP, Buschard K. Gluten-free diet only during pregnancy efficiently prevents diabetes in NOD mouse offspring. Journal of diabetes research. 2016;2016.
174. Goodwin G. Type 1 diabetes mellitus and celiac disease: distinct autoimmune disorders that share common pathogenic mechanisms. Hormone research in paediatrics. 2019;92(5):285-92.
175. Mojibian M, Chakir H, Lefebvre DE, Crookshank JA, Sonier B, Keely E, et al. Diabetes-specific HLA-DR–restricted proinflammatory T-cell response to wheat polypeptides in tissue transglutaminase antibody–negative patients with type 1 diabetes. Diabetes. 2009;58(8):1789-96.
176. Auricchio R, Paparo F, Maglio M, Franzese A, Lombardi F, Valerio G, et al. In vitro–deranged intestinal immune response to gliadin in type 1 diabetes. Diabetes. 2004;53(7):1680-3.
177. Troncone R, Franzese A, Mazzarella G, Paparo F, Auricchio R, Coto I, et al. Gluten sensitivity in a subset of children with insulin dependent diabetes mellitus. The American journal of gastroenterology. 2003;98(3):590-5.
178. Klemetti, Savilahti, Ilonen, Åkerblom, Vaarala. T-cell reactivity to wheat gluten in patients with insulin-dependent diabetes mellitus. Scandinavian journal of immunology. 1998;47(1):48-53.
179. Kataoka K. The intestinal microbiota and its role in human health and disease. The Journal of Medical Investigation. 2016;63(1.2):27-37.
180. Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PloS one. 2011;6(10):e25792.
181. Mejía-León ME, Petrosino JF, Ajami NJ, Domínguez-Bello MG, de La Barca AMC. Fecal microbiota imbalance in Mexican children with type 1 diabetes. Scientific reports. 2014;4(1):3814.
182. Bosi E, Molteni L, Radaelli M, Folini L, Fermo I, Bazzigaluppi E, et al. Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia. 2006;49:2824-7.
183. Vaarala O. Gut microbiota and type 1 diabetes. The review of diabetic studies: RDS. 2012;9(4):251.

184. Sapone A, De Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes. 2006;55(5):1443-9.
185. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology. 2008;135(1):194-204. e3.
186. Hummel M, Bonifacio E, Naserke HE, Ziegler AG. Elimination of dietary gluten does not reduce titers of type 1 diabetes-associated autoantibodies in high-risk subjects. Diabetes Care. 2002;25(7):1111-6.
187. Pastore M-R, Bazzigaluppi E, Belloni C, Arcovio C, Bonifacio E, Bosi E. Six months of gluten-free diet do not influence autoantibody titers, but improve insulin secretion in subjects at high risk for type 1 diabetes. The Journal of Clinical Endocrinology & Metabolism. 2003;88(1):162-5.
188. Sildorf SM, Fredheim S, Svensson J, Buschard K. Remission without insulin therapy on gluten-free diet in a 6-year old boy with type 1 diabetes mellitus. Case Reports. 2012;2012:bcr0220125878.
189. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β-cell function: report of an ADA workshop, 21–22 October 2001. Diabetes. 2004;53(1):250-64.
190. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of β-cell function in therapeutic trials in type 1 diabetes. Diabetes care. 2008;31(10):1966-71.
191. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes care. 2009;32(8):1384-90.
192. Bulow E. Vävnadstransglutaminas-antikroppar (IgA/IgG), a-tTG (IgA/IgG) http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Vävnadstransglutaminas-antikroppar%20(IgA_IgG),%20a-tTG%20(IgA_IgG)(200207).pdf2020 [
193. Bülow E. Endomysium-antikroppar (IgA), EmA (IgA). 2020.
194. Dowhaniuk JK, Mileski H, Saab J, Tutelman P, Thabane L, Brill H. The gluten free diet: Assessing adherence in a pediatric celiac disease population. Journal of the Canadian Association of Gastroenterology. 2020;3(2):67-73.
195. Emilsson L, Lindahl B, Köster M, Lambe M, Ludvigsson JF. Review of 103 S wedish H ealthcare Q uality R egistries. Journal of internal medicine. 2015;277(1):94-136.
196. Persson M, Becker C, Larsson HE, Lernmark Å, Forsander G, Ivarsson S, et al. The better diabetes diagnosis (BDD) study–a review of a nationwide prospective cohort study in Sweden. Diabetes research and clinical practice. 2018;140:236-44.
197. Bruun SW, Josefsen K, Tanassi JT, Marek A, Pedersen MH, Sidenius U, et al. Large gliadin peptides detected in the pancreas of NOD and healthy mice following oral administration. Journal of Diabetes Research. 2016;2016.

198. Acerini C, Ahmed M, Ross K, Sullivan P, Bird G, Dunger D. Coeliac disease in children and adolescents with IDDM: clinical characteristics and response to gluten-free diet. Diabetic medicine. 1998;15(1):38-44.
199. Simmons JH, Klingensmith GJ, McFann K, Rewers M, Taylor J, Emery LM, et al. Impact of celiac autoimmunity on children with type 1 diabetes. The Journal of pediatrics. 2007;150(5):461-6.
200. Webb C, Myléus A, Norström F, Hammarroth S, Högberg L, Lagerqvist C, et al. High adherence to a gluten-free diet in adolescents with screening-detected celiac disease. Journal of pediatric gastroenterology and nutrition. 2015;60(1):54-9.
201. Sanchez-Albisua I, Wolf J, Neu A, Geiger H, Wäscher I, Stern M. Coeliac disease in children with type 1 diabetes mellitus: the effect of the gluten-free diet. Diabetic Medicine. 2005;22(8):1079-82.
202. Kaur P, Agarwala A, Makharia G, Bhatnagar S, Tandon N. Effect of gluten-free diet on metabolic control and anthropometric parameters in type 1 diabetes with subclinical celiac disease: A randomized controlled trial. Endocrine Practice. 2020;26(6):660-7.
203. Adlercreutz EH, Svensson J, Hansen D, Buschard K, Lernmark Å, Mortensen HB, et al. Prevalence of celiac disease autoimmunity in children with type 1 diabetes: regional variations across the Øresund strait between Denmark and southernmost Sweden. Pediatric diabetes. 2015;16(7):504-9.
204. Zung A, Kori M. Lack of association between seroconversion and catch-up growth in children with celiac disease. Journal of Pediatric Endocrinology and Metabolism. 2012;25(1-2):131-7.
205. Moreno MdL, Rodríguez-Herrera A, Sousa C, Comino I. Biomarkers to monitor gluten-free diet compliance in celiac patients. Nutrients. 2017;9(1):46.
206. Reeves GE, Squance ML, Duggan AE, Murugasu RR, Wilson RJ, Wong RC, et al. Diagnostic accuracy of coeliac serological tests: a prospective study. European journal of gastroenterology & hepatology. 2006;18(5):493-501.
207. Silvester JA, Kurada S, Szwajcer A, Kelly CP, Leffler DA, Duerksen DR. Tests for serum transglutaminase and endomysial antibodies do not detect most patients with celiac disease and persistent villous atrophy on gluten-free diets: a meta-analysis. Gastroenterology. 2017;153(3):689-701. e1.
208. Lähdeaho M-L, Mäki M, Laurila K, Huhtala H, Kaukinen K. Small-bowel mucosal changes and antibody responses after low-and moderate-dose gluten challenge in celiac disease. BMC gastroenterology. 2011;11(1):1-9.
209. Comino I, Fernández-Bañares F, Esteve M, Ortigosa L, Castillejo G, Fambuena B, et al. Fecal gluten peptides reveal limitations of serological tests and food questionnaires for monitoring gluten-free diet in celiac disease patients. The American journal of gastroenterology. 2016;111(10):1456.
210. Shan L, Qiao S-W, Arentz-Hansen H, Molberg Ø, Gray GM, Sollid LM, et al. Identification and analysis of multivalent proteolytically resistant peptides from gluten: implications for celiac sprue. Journal of proteome research. 2005;4(5):1732-41.