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^{1}H NMR metabolic signatures of early paediatric Type 1 Diabetes

Master's thesis

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Declaration of Honour

I hereby declare that I have independently developed this thesis, entitled "^{1}H NMR metabolic signatures of early paediatric Type 1 Diabetes" under the guidance of my thesis supervisor and have used academic literature and other information sources that are duly cited within the thesis and listed in the bibliography at the end of the thesis. As the author, I affirm that in the development of this thesis, I have not infringed upon the copyrights of any third parties.

In Prague, on the submission date of April 19, 2024, ____________________________

Acknowledgements

I extend my sincere gratitude to my thesis supervisor doc. Ing. Jaroslav Havlík, Ph.D., for his guidance, scientific advice, patience, and valuable feedback throughout the course of my thesis writing. Additionally, I am grateful to my consultant, Eliška Jeníčková, MSc, for her essential support in the laboratory, her collegial demeanour and for imparting knowledge which contributed to the successful completion of my thesis.

^{1}H NMR metabolic signatures of early paediatric Type 1 Diabetes

Abstract

Background: T1D is an autoimmune disorder marked by the destruction of insulin-producing β-cells in the pancreas. Faecal metabolites, reflecting factors such as gut microbiota composition and dietary influences, may serve as indicative biomarkers for T1D status and susceptibility. Analysing faecal metabolomic profiles holds potential in diagnosing and identifying increased risk for paediatric T1D, aiming to enhance early detection and management of the disease.

Aim/ Hypothesis: This case-control study hypothesises that children at genetic predisposition for developing T1D exhibit distinct faecal ^{1}H NMR metabolic profiles compared to healthy children. We propose that these faecal metabolic differences, potentially influenced by variations in gut microbiota composition or dietary patterns, may precede the clinical onset of T1D. Understanding these variations could offer valuable insights for the clinical diagnosis and early detection of T1D.

Methods: This study analysed 87 faecal samples from 24 children (aged 4.8 years ± 1.2 years), genetically predisposed to T1D and their matched controls. The faecal metabolic profiles were evaluated using ^{1}H NMR spectral analysis. The ^{1}H NMR data were processed using NMRProcFlow 1.4 and Chenomx 9.02 software and statistically evaluated using MetaboAnalyst 6.0 to identify potential metabolic differences that could signify increased risk of T1D.

Results: The study identified a statistically significant difference ($p < 0.05$) in the levels of the metabolite 3-(3-hydroxyphenyl)propionic acid, which were higher in the T1D-predisposed group than in the control group. While the specific biochemical function of this metabolite is not yet understood, it is speculated that variations in gut microbiota composition or dietary patterns among the T1D-predisposed children may account for the observed differences in metabolite concentrations.

Conclusion: Our study findings suggest that differences in faecal metabolic profiles could aid in the early detection of T1D in paediatric populations. The identification of specific metabolites through ^{1}H NMR spectroscopy underscores its utility as a powerful tool for pinpointing biomarkers associated with T1D risk and progression.

Keywords: metabolomics, faecal metabolic profiling, ^{1}H NMR, biomarkers, T1D

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1 Introduction .

Type 1 Diabetes (T1D) is a chronic autoimmune condition characterised by the progressive destruction of insulin-producing β-cells in the pancreas (Piya & Michels 2012). Often diagnosed in children and adolescents (Atkinson & Eisenbarth, 2001), T1D requires lifelong insulin therapy (Janež et al., 2020) and significantly increases the risk of various health complications (White, 2015). The disease appears to be more prevalent in developed countries, with a rising global incidence (Gong et al. 2024). Contributing factors to T1D include viral infections, dietary habits, environmental toxins, and the composition of gut microbiota (Rewers & Ludvigsson 2016).

One of the earliest detectable biomarkers for T1D is the presence of islet autoantibodies (AAbs) which are now recognized as standard indicators that provide crucial insights into the immune response against pancreatic β-cells (Lebastchi & Herold 2012). However, the detection of AAbs, signalling active autoimmunity, actually occurs at a relatively advanced stage in the progression of the disease (Jin & She 2012). This underscores the need to advance research to identify and validate additional, earlier predictive biomarkers for T1D (Chai et al. 2022).

Recent advancements in biomedical sciences, particularly in metabolomics – the study of small molecules in biological samples – have greatly enhanced our understanding of the biochemical and molecular mechanisms underlying T1D (Qiu et al. 2023). Metabolomics is essential for detecting early disruptions in metabolic processes that precede clinical symptoms of the disease (Guasch-Ferré et al. 2016). Nuclear magnetic resonance (NMR) spectroscopy is widely recognized for its robustness and precision in identifying metabolic signatures and biomarkers (Friedrich 2012), and is crucial for analysing biomolecular structure, dynamics, and interactions (Mureddu & Vuister 2019). Despite challenges in detecting low metabolite concentrations in natural settings (Peng et al. 2024), NMR remains essential for advancing metabolic research and diagnostics (Emwas et al. 2019).

The role of gut microbiota in T1D is increasingly recognized as critical due to its significant impact on metabolic and immune responses that influence disease progression (Zheng et al. 2018). Studies show that individuals at risk for T1D have distinct microbial profiles marked by reduced diversity and an imbalance between beneficial and pathogenic bacteria (Dedrick et al., 2020) which can affect immune tolerance and inflammation, potentially triggering pancreatic β-cell destruction (Wu & Wu 2012). Specific microbial metabolites are also implicated in modulating these responses (Mokhtari et al. 2021).

Understanding these dynamics offers potential for early interventions and highlights gut microbiota as both a crucial factor in T1D pathogenesis and a target for therapy (Zhou et al. 2020).

The primary objective of this thesis is to determine whether children genetically predisposed to T1D exhibit distinct metabolic profiles that could serve as early indicators of susceptibility to the disease. Through detailed metabolomic analyses using ^{1}H NMR spectroscopy of faecal samples, this study compares the metabolic signatures of at-risk children to those of healthy controls. This study aims to identify specific metabolites and their possible pathways that may indicate an elevated risk of developing T1D. Successfully identifying these biomarkers could revolutionise the approach to early diagnosis and intervention, potentially altering the trajectory of the disease and improving outcomes for those at increased risk of T1D (Liu et al. 2013).

2 Scientific hypothesis and objectives of the thesis

The objectives of this thesis were to evaluate whether metabolic features in the faeces of children genetically predisposed to T1D through ^{1}H NMR spectroscopy could serve as biomarkers for increased risk of paediatric T1D.

Hypothesis:

Children at genetic predisposition for developing T1D exhibit distinct faecal ^{1}H NMR metabolic profiles compared to healthy children.

3 Literature research

3.1 Pathogenesis of T1D

T1D develops through a complex pathogenic process, initiated by an abnormal immune response that leads to the destruction of β-cells. This process is characterised by the development of AAbs that target proteins in the pancreatic β-cells (Piya & Michels 2012).

Etiologically, T1D is classified into two main types. Immune-mediated diabetes predominantly occurs in genetically susceptible individuals, with viral infection or exposure to various environmental factors likely serving as precipitating factors for this immune response (Morran et al. 2015). The autoimmune origin of the disease is indicated by the presence of circulating antibodies, collectively known as islet cell AAbs (Kawasaki 2023). These AAbs specifically target glutamic acid decarboxylase (GADA), insulin and proinsulin (IAA), cells of Islets of Langerhans (ICA), the tyrosine phosphatase-related protein (IA- 2 and IA-2ꝺ) and the zinc transporter 8 (ZnT8) (Pihoker et al. 2005). AAbs emerge during the preclinical stage of the disease and serve as key indicators of β-cell destruction (Kawasaki2023). Another, idiopathic diabetes is an atypical form with an unknown aetiology (Piñero-Piloña & Raskin 2001). It includes the absence of β-cell autoimmune markers and the inability to detect antibody responses to common antigens (Redondo et al. 2018).

T1D progresses through distinct stages before symptoms become evident. In Stage 1, autoimmunity is initiated against pancreatic β-cells, primarily involving cytotoxic T cells. This immune response leads to early-stage destruction of β-cells, known as insulitis (Pugliese 2017). During this stage, blood glucose levels remain within the normal range (normoglycemia) and overt T1D symptoms are not yet apparent (Akil et al. 2021). Two or more islet AAbs characterise this stage, with the five-year and ten-year risk of T1D development being 44 % and 70 %, respectively. The lifetime risk of developing T1D reaches 100 %, underscoring the importance of identifying multiple AAbs as a strong predictor of progressing to symptomatic T1D (Kawasaki 2023). In Stage 2, two or more islet AAbs are identified, and β-cell destruction progresses, leading to glucose intolerance or dysglycemia. The risk of T1D development is approximately 75 % within five years. Stage 3 is marked by the manifestation of clinical symptoms and signs of T1D (Akil et al. 2021). These symptoms include sudden weight loss, lack of energy, frequent urination, extreme thirst, constant hunger, blurred vision, fatigue, and weakness (Simmons 2015). In this end stage, only about 10 % to 20 % of functioning insulin-producing β-cells remain (Lebastchi & Herold 2012).

AAbs often emerge as early as 9 months of age and peaking before 2 years of age (Ziegler & Bonifacio 2012). In children, the onset of T1D commonly begins with the emergence of the initial AAbs, typically IAA, followed by the appearance of other AAbs within a span of 3 years, with GADA being the second most commonly detected. The sequence of AAbs appearance is likely influenced by genetic factors such as an individual's HLA genotype (Bonifacio & Achenbach 2019).

Genes play a pivotal role in antigen presentation to T cells, triggering autoimmunity (Morran et al. 2015). As the disease progresses, T cells invade the pancreas, causing inflammation and the subsequent destruction of β-cells, resulting in insulin loss and hyperglycemia. T cells initiate β-cell destruction and prompt the production of insulin AAbs (Rodriguez-Calvo et al. 2021). Once β-cell destruction commences, the immune system targets additional antigens, a phenomenon known as epitope spreading. Following insulin, glucose-like phosphatase protein becomes a secondary target for the immune response (Piya & Michels 2012). Despite ongoing uncertainties surrounding the aetiology and pathogenesis of T1D, it is recognized as a multifactorial disease influenced by both genetic and exposomic factors, collectively contributing to its onset and progression (Ikegami et al. 2011).

3.2 Incidence of T1D

T1D has historically been recognized as a disease predominantly affecting children and young adults, often referred to as juvenile diabetes due to its highest occurrence in children aged 10–14 (Atkinson & Eisenbarth 2001). However, recent studies indicate that children can be affected by the disease at any age (Atkinson & Eisenbarth 2001). Globally, both new (incident) and existing (prevalent) cases of T1D are on the rise each year, attributed to increasing incidence and improved survival rates in various countries (Ogle et al. 2022).

Based on the 2022 estimates from the International Diabetes Federation Atlas Reports, there were 8.75 million individuals worldwide living with T1D, including 1.52 million individuals under the age of 20. This represents an escalating trend compared to 1.21 million individuals in 2021 (Ogle et al. 2022).

In 2021, T1D incidence among children aged 0–14 was highest in Europe (24 700 cases), Middle East Africa and North Africa (20 500 cases) and North America and the Caribbean (18 700 cases) (Graham et al. 2022). In this age group, Nordic countries exhibited the highest age-standardised incidence rates. Specifically, Finland reported rates of 52.2 per 100 000 per year, followed by 44.1 per 100 000 per year in Sweden, and 33.6 per 100 000 per year in Norway (Ogle et al. 2022).

In 2022, T1D was associated with approximately 182 000 deaths globally, with the highest mortality observed in the South-East Asia region, accounting for 42 000 deaths. Africa recorded 38 000 deaths, followed by Europe with 34 000. North America and the Caribbean, each reported 20 000 deaths (Ogle et al. 2022). Additionally, in 2022, an estimated 35 000 deaths related to T1D were observed in individuals under 25 years of age who had not been previously diagnosed, succumbing within 12 months of symptom onset (Ogle et al. 2022).

3.3 Functions of insulin

Insulin, an anabolic endocrine hormone produced by the Islets of Langerhans in the pancreas, performs diverse metabolic functions in the body (Dimitriadis et al. 2011). Effects of insulin vary depending on the specific tissue it targets. In muscle tissue, insulin enhances the uptake of glucose, promotes glycolysis, stimulates glycogen synthesis, and inhibits insulin breakdown (Dimitriadis et al. 2011). In adipose tissue, insulin decreases non-esterified fatty acids (NEFA) in the blood by suppressing lipolytic processes and reduces NEFA oxidation in skeletal muscles and the liver (Dimitriadis et al. 2011). In lipocytes, insulin facilitates glucose transport to the cell membrane through the GLUT4 transporter, thus aiding glucose uptake (Petersen & Shulman 2018).

Insulin also plays a crucial role in protein metabolism by promoting protein synthesis and inhibiting protein breakdown across various tissues and organs (Dimitriadis et al. 2011). In the brain, insulin regulates energy balance within the hypothalamus by reducing food intake and increasing energy expenditure by elevating body temperature and activating the sympathetic nervous system (Dodd & Tiganis 2017). Furthermore, insulin, binding to specific receptors in the hypothalamus, inhibits the expression of neuropeptides involved in hunger regulation (Kleinridders et al. 2014). Additionally, in the hypothalamus, insulin influences glucose metabolism by signalling AgEP-expressing neurons and by reducing glucose production in the liver through nerve impulses (Yung & Giacca 2020).

3.4 Pathology of β-cells

In the early stages of T1D development, the immune system becomes activated in response to signals associated with danger or viral infections affecting β-cells (Lernmark 2021). This leads to the production of type I interferon (e.g. IFNa) cytokines by β-cells and the involvement of immune cells. Macrophages are critical in initiating a pro-apoptotic response in β-cells (Colli et al. 2020).

As T1D progresses, the inflammatory environment allows various immune cells, including CD8+, CD20+ β-cells, CD4+ T cells, and CD68+ macrophages to invade β-cells (Zajec et al. 2022). β-cells respond to cytokines by activating anti-inflammatory pathways and immune checkpoint proteins to prevent excessive immune reactions. However, pro-inflammatory cytokines interfere with β-cell functions, leading to increased production of reactive oxygen species and activation of enzymes (caspases) that induce cell death (Zajec et al. 2022). Both pro-inflammatory and anti-inflammatory responses persist throughout the disease (Colli et al. 2020).

Pathogenesis of T1D involves complex interactions between pancreatic β-cells and immune cells, emphasising their crucial crosstalk (Yi et al. 2018). β-cells actively engage in and potentially exacerbate pathogenic processes (Erdem et al. 2021). In response to immune stress, β-cells utilise compensatory mechanisms to restore cellular homeostasis by modulating endoplasmic reticulum and mitochondrial functions through unfolded protein response (UPR) (Vig et al. 2021). Increased protein production can result in misfolded proteins (Zajec et al. 2022) (inactive proteins which are unable to fold into their native structure and function normally) (Moreno-Gonzalez & Soto, 2011). These changes coincide with a decline in pathways essential for insulin release and β-cell function maintenance, resulting in their reduced activity and a subsequent decrease in insulin production (Zajec et al. 2022).

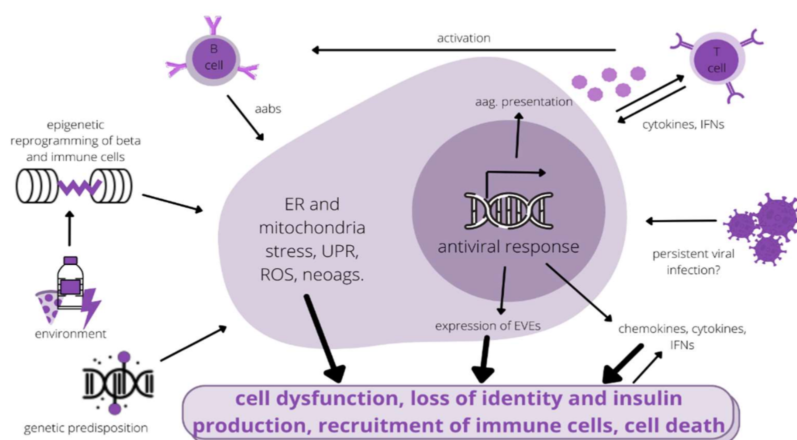

Figure 1 Simplified illustration of the effects on β-cell pathology in T1D (Adapted from (Zajec et al. 2022)

aabs – AAbs, aag – autoantigen, ER – endoplasmic reticulum, EVEs – endogenous viral elements, IFNs – interferons, ROS – reactive oxygen species, UPR – unfolded protein response

3.5 Genetics of T1D

T1D is considered the most prevalent polygenic disorder. Genetic factors such as mutations of key genes, are believed to play a significant role in the disease's onset (Barrett et al. 2009). Among these genetic factors, the HLA (human leukocyte antigen) complex, located on chromosome 6, contributes to over 50 % of the genetic risk, associated with T1D (Redondo et al. 2018). This complex houses genes responsible for encoding proteins crucial for distinguishing between self and foreign antigens and presenting them to immune cells, particularly T cells and non-self-antigens. These proteins are important in presenting antigens to immune cells – T cells (Simmons 2015). Within the HLA complex, class II genes, notably the DQ and DR alleles, are the primary determinants of T1D susceptibility (Simmons 2015). Individuals carrying DR4/DQ8 haplotype, for example, are significantly more prone to developing T1D, with around 90 % of affected individuals possessing either DR4/DQ8 or DR3/DQ2 haplotypes (Simmons 2015). The heritability of T1D within families is estimated to be approximately 40-50 % attributed to the HLA complex (Noble & Valdes 2011). While other genes like insulin and protein tyrosine phosphatase non-receptor type 22 (PTPN22) also contribute to T1D risk, their influence is comparatively weaker than that of HLA genes (Tandon 2015). PTPN22, involved in regulating antigen receptor signalling and T cell activation, and insulin gene polymorphisms, particularly those affecting insulin expression in the thymus (Paschou et al. 2018), contribute to about 10 % of the genetic predisposition for T1D (Simmons, 2015). Additionally, mutations in the Autoimmune Regulator (AIRE) gene, crucial for establishing immunological self-tolerance, can lead to the production of antibodies against self-antigens, contributing to conditions like Polyglandular Syndrome 1 (APS1), which often includes T1D among its symptoms (Paschou et al. 2018). Furthermore, mutations in the Foxp3 gene, associated with an X-linked syndrome, disrupt the function and development of T cells essential for maintaining self-tolerance, adding another layer of complexity to the genetic landscape of T1D (Redondo et al., 2018). These diverse genetic factors collectively shape the intricate aetiology of T1D (Barrett et al., 2009).

3.6 Exposomic factors in T1D

T1D is also acknowledged as a multifactorial disorder. Various environmental exposures likely play a crucial role in T1D development influencing genetic alterations through factors such as diet, infections, vitamin D intake, gut microbiota and toxins potentially contributing to aberrant immune responses and islet autoimmunity (Zullo et al. 2017). While the specific environmental triggers for autoimmune processes remain unclear, identifying them could help define T1D risk, allowing for better prediction and prevention (Esposito et al. 2019).

3.6.1 Infections

Viral infections, including enterovirus, rotavirus, cytomegalovirus, parechovirus, influenza, parvovirus, mumps, rubella and human endogenous retrovirus, are implicated in initiating AAbs production and subsequent β-cell destruction, thereby contributing to T1D progression (Isaacs et al. 2021). Among these viruses, enterovirus infections have received the most attention in the context of T1D (van der Werf et al. 2007).

A systematic review and meta-analysis of observational molecular studies revealed a strong association between enterovirus infection and T1D (Yeung et al. 2011). Enteroviruses, particularly coxsackievirus B (a group of six serotypes of coxsackievirus within the enterovirus family) have been frequently detected in T1D patients compared to healthy individuals (Nekoua et al. 2022). Coxsackievirus B, with its single-stranded RNA genome, typically spreads through contact with faeces, often via contaminated food or water. Studies indicated that individuals often have enteric infection and ongoing gastrointestinal inflammation before T1D onset (Morse et al. 2023). Honkanen et al. (2017) investigated the association between the presence of enterovirus in the stool of AAbs-positive children (n = 129) and healthy controls (n = 282) and the onset of islet autoimmunity. They found that AAbs-positive children had a higher frequency of enterovirus infections compared to their healthy controls (Honkanen et al. 2017).

Genetic factors also play a role, with certain alleles impacting the immune response to enteroviruses and increasing T1D risk (Esposito et al. 2019). Additionally, a higher number of T1D diagnoses typically occur during cold months, aligning with the seasonal prevalence of enteroviral infections (Glatthaar et al. 1988).

The mechanism by which viruses contribute to T1D progression remain uncertain and vary depending on the type of virus (Jun & Yoon 2003). Firstly, a virus can infect pancreatic β-cells, leading to their lysis. Secondly, viral infection might disrupt self-tolerance due to the

presence of viral antigens, potentially modifying β-cell antigens and/or increasing MHC-antigens, cytokines and chemokines that influence the immune system response (Jun & Yoon 2003). Thirdly, viral infection can activate T cells, including those targeting β-cell antigens through autoreaction (van der Werf et al. 2007).

3.6.2 Breastfeeding

Breastfeeding offers numerous health benefits to infants, providing essential nutrients crucial for growth and development (Vieira Borba et al. 2018). Rich in various bioactive compounds, breast milk acts as a powerful source of lifelong immunity, potentially reducing the risk of various illnesses, including T1D (Yahaya & Shemishere 2020).
Research by Lund-Blix et al. (2017) involving 155 392 children in Norway and Denmark revealed that those children not breastfed had a twofold increased risk of developing T1D compared to breastfed children (Lund-Blix et al. 2017).

While the precise protective mechanisms of breastfeeding against T1D are not fully understood, they likely involve reducing infections associated with T1D development and supporting infantile digestive system and gut microbiota development (Vieira Borba et al. 2018). Breast milk, containing beneficial bacteria and bioactive molecules, plays a crucial role in nurturing a healthy gut microbiota, enhancing diversity, reducing permeability, and strengthening immune and metabolic functions (Pannaraj et al. 2017). Insulin in breast milk aids in gut maturation and reducing permeability, potentially delaying the onset of T1D (Shehadeh et al. 2001). Insulin promotes the diversity of gut microbiota by encouraging the growth of Gammaproteobacteria while inhibiting Streptococcaceae bacteria (Lemas et al. 2016). Additionally, breast milk insulin and leptin influence microbial metabolic pathways, suppressing those associated with intestinal inflammation and promoting beneficial pathways (Lemas et al. 2016). Oligosaccharides found in breast milk, consisting of indigestible sugars, support the growth of beneficial bacteria in the colon, including *Lactobacillus* and *Bifidobacterium*, maintaining gut health (Vieira Borba et al. 2018). Lactoferrin, a glycoprotein in breast milk that binds to iron, limits iron availability to microorganisms, possessing antimicrobial properties (Mehta & Petrova, 2011). Additionally, breast milk's abundance of *Lactobacillus* and *Bifidobacterium* fosters the growth of Firmicutes bacteria (Soto et al. 2014) deficient in individuals with T1D (Giongo et al., 2011). This species is crucial for gut diversity and maturation (Soto et al. 2014).

Despite some studies suggesting a weak link between breastfeeding and T1D risk, advocating breastfeeding remains crucial due to its immunomodulatory components, serving protective, developmental, and maturation benefits (Vieira Borba et al. 2018).

3.6.3 Diet

The influence of diet on T1D remains incompletely understood. Evidence indicates a potential association between cow's milk consumption and damage to pancreatic β-cells due to a protein called bovine serum albumin (BSA) found in milk (Martin et al. 1991). A study by Karjalainen et al. (1992) observed elevated levels of BSA antibodies in children with T1D, specifically targeting a protein called ABBOS found in albumin. This finding suggests a possible interaction involving the β-cell protein p69. This protein plays a crucial role in the immune response triggered by dietary milk intake and its adverse impact on β-cells (Karjalainen et al. 1992). Additionally, the Finnish Diabetes Prediction and Prevention Project (DIPP) study found a direct association between the consumption of fresh cow's milk products and β-cell autoimmunity in children with T1D (Virtanen et al. 2012). Furthermore, conflicting results exist, such as those from Atkinson et al. (1993) who found no immune response to BSA or ABBOS in T1D patients. This suggests these antigens may not significantly contribute to T1D pathogenesis (Atkinson et al. 1993). Furthermore, gluten proteins resist an enzymatic breakdown in the intestine, allowing them to persist in the system for an extended duration. This characteristic foster continuous interactions with the intestinal immune system (Antvorskov et al. 2014). One proposed mechanism is through influencing the proportion of immune cells or altering cytokine patterns toward inflammation. This underscores the significant role of gluten intake in T1D development (Antvorskov et al. 2014).

3.6.4 Vitamin D

The association between vitamin D usage and the incidence of T1D remains a topic of debate. Several studies underscore the positive influence of vitamin D supplementation on specific autoimmune diseases (Yu et al. 2022).

Calcitriol, the active form of vitamin D, plays a crucial role in immune regulation, as it possesses receptors on all immune cells (Infante et al. 2019). This suggests a potential regulatory function for calcitriol within the immune system, impacting both innate immune cells, such as dendritic cells and macrophages, and adaptive immune cells, including B and T cells. Calcitriol has the ability to induce immune tolerance by inhibiting pro-inflammatory

cytokines while enhancing anti-inflammatory cytokines (Infante et al. 2019). Concerning T1D, calcitriol may contribute to reducing antibodies and slowing progression of β-cell damage, especially during the initial stages of the disease. Evidence supporting the protective effect of vitamin D against T1D development stems from a comprehensive case-control study conducted across seven European countries. Analysing 820 diabetic patients and 2335 non-diabetic control children, the study revealed a roughly one-third reduction in T1D risk among supplemented children compared to those not receiving supplementation (Dahlquist 1999).

3.6.5 Gut microbiota

The collection of living microorganisms residing in the human intestine, collectively referred to as gut microbiota, encompasses bacteria, archaea, fungi, algae, and small protists (Berg et al. 2020). Major bacterial phyla include Firmicutes and Bacteroidetes, with lesser amounts of Actinobacteria, Proteobacteria and Verrucomicrobia, alongside methanogenic archaea like *Methanobrevibacter smithii* (Lynch & Pedersen 2016).

The growth and survival of gut microbiota depends on the host and are vital for various physiological functions. It plays a pivotal role in modulating energy metabolism and fostering the development and regulation of the immune system. It serves as a frontline defence mechanism against pathogens and influences host-cell proliferation and vascularization (Lynch & Pedersen, 2016). Gut microbiota contribute to the biosynthesis of essential compounds such as vitamin K and specific B-vitamins (Jandhyala 2015) neurotransmitters and also metabolises bile salts. Moreover, gut microbiota is integral to various metabolic pathways, including those involving branched-chain amino acids (BCAAs) and dietary components (Lynch & Pedersen 2016). Throughout food metabolism, gut microbiota generates numerous bioactive substances known as "pharmabiotics," exerting influence over various physiological and metabolic processes within the human body (Patterson et al. 2014). Notably, the isomers of conjugated linoleic acids are recognized for their beneficial effects on both T1D and T2D, atherosclerosis, and lipid metabolism. Simultaneously, short-chain fatty acids play a critical role in disease prevention and treatment, as well as contributing to the host's energy metabolism (Jandhyala 2015; Patterson et al. 2014).

The symbiotic relationship between gut microbiota and its host, formed through mutual coexistence and co-evolution, is crucial for maintaining homeostasis. Any disruption in this balance, known as dysbiosis, can profoundly impact immune responses (Wu & Wu 2012).

The composition of gut microbiota is unique to each individual, shaped by various factors including the diversity of microbial strains, their growth rates, structural variations in microbial genes, specific host genetic material, and exposure to diverse environmental influences (Fan & Pedersen 2021). Gut microbiota begins to form at birth and continues to evolve throughout life, with its composition believed to be influenced by factors such as the type of birth (vaginal delivery or cesarean section) and whether the child is breastfed (Lynch & Pedersen 2016).

Recent research underscores the significant role of gut microbiota in determining the risk of T1D development, especially among individuals genetically predisposed to the disease (Zhou et al. 2020). Gut microbiota plays a critical role in T1D development by affecting the intestinal permeability, intestinal immunity and inducing molecular mimicry in the intestinal epithelium, where microbial antigens may trigger an immune response by resembling self-antigens (Zheng et al. 2018). An increase in intestinal permeability, due to weakened intestinal barrier, allows dietary antigens, immune stimulates (e.g. exogenous antigens) and microbial components to enter the bloodstream (Viggiano et al. 2015). This process can lead to systemic inflammation and autoimmune reactions, ultimately resulting in the destruction of pancreatic β-cells (Zhou et al. 2020).

Numerous studies emphasise the importance of gut microbiota composition in the progression of T1D (Zhou et al. 2020). Kostic et al. (2015) observed a notable reduction in microbial diversity in individuals with T1D, while those who did not develop the condition and those who develop AAbs experienced a continuous increase in microbial diversity within their gut communities (Kostic et al. 2015).

Gut microbiota primarily comprises two phyla, Firmicutes and Bacteroidetes, which collectively make up 90% of its composition. In children, gut microbiota is predominantly composed of Bacteroidetes and Firmicutes, with notable levels of Actinobacteria and Proteobacteria (Mokhtari et al. 2021). However, the abundance of Firmicutes and Bacteroidetes phyla differs between healthy children and those with T1D (Murri et al.2013). Healthy children exhibit a higher abundance of Firmicutes compared to those with T1D, whereas Bacteroidetes increases in abundance in children with T1D and those predisposed to the disease (Giongo et al. 2011). The observed alterations at the phylum level, particularly within Firmicutes and Bacteroidetes, are suggested to be linked to changes in specific genera, such as *Clostridiales* and *Bacteroides*, respectively (Giongo et al. 2011). *Bacteroides*, a gram-negative bacterium known for producing acetate and propionate, has been linked to chronic inflammation. It is hypothesised that *Bacteroides* may initiate dysbiosis, impair the function

of the epithelial cell barrier, and thereby contribute to the development of T1D (Mokhtari et al. 2021). Furthermore, it has been suggested that the genus *Bacteroides dorei*, in particular, produces lipopolysaccharides with immunoinhibitory properties, which may hinder early immune development and contribute to T1D development (Mokhtari et al. 2021).

The abundance of species responsible for producing essential short-chain fatty acids (SCFAs) varies significantly. In a study by de Goffau et al. (2013), there was a negative relationship observed between certain key SCFA-producing species (such as *Bifidobacterium adolescentis*, *Bifidobacterium Peeudocatenulatum*, *Faecalibacterium prausnitzii*, *Clostridium clostridioforme* and *Roseburia faecis*) in children with diabetes-associated AAbs. This suggests that those with four AAbs tend to have fewer species producing SCFAs. Additionally, the study found a reduction in the abundance of two dominant *Bifidobacterium* species, while *Bacteroides* abundance increased in these cases (de Goffau et al. 2013). In another case-control study conducted by Murri et al. (2013), T1D children exhibited increased levels of *Clostridium*, *Bacteroides*, and *Veillonella*, along with decreased levels of *Bifidobacterium* and *Lactobacillus* compared to their healthy controls (Murri et al. 2013). Additionally, children with T1D exhibited an increased abundance of 12 genera including *Escherichia*, *Bacteroides*, *Clostridium*, *Veillonella*, *Ruminococcus*, *Blautia*, *Streptococcus*, *Sutterella, Enterobacter*, *Lactobacillus*, *Lactococcus* and *Bifidobacterium* (Mokhtari et al. 2021). The reduced overall diversity observed in T1D may be associated with the prevalence of specific genera, such as *Ruminococcus*, *Blautia*, and *Streptococcus*. This association is intriguing as these bacterial groups encompass species identified as potential "pathobions" which are members of the normal gut microbiota capable of behaving like harmful pathogens (Kostic et al., 2015).

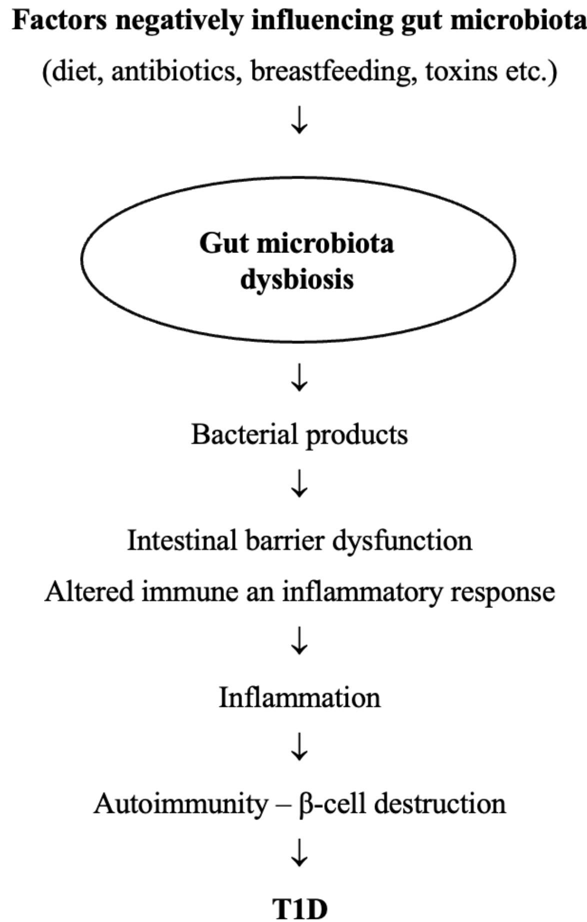

Figure 2 Dysbiosis of the gut microbiota in T1D (Adapted and modified from Mokhtari et al. 2021).

3.6.6 Antibiotics

Antibiotics, commonly used to treat bacterial infections, have a well-documented influence on the composition and diversity of gut microbiota. Their usage is associated with a reduction in microbial variety within the gut, a factor crucial for the optimal functioning of the gut immune system (Korpela et al. 2016). Dysregulation of this system has been implicated in the pathogenesis of T1D, suggesting a potential role of antibiotic-induced alterations in microbial diversity in T1D development (Hviid & Svanstrom 2009). Despite understanding of these effects on microbial communities, the precise implications of antibiotics on T1D development remain unclear (Xu et al., 2022). Bokulich et al. (2016) conducted a comprehensive study investigating the correlation between infant antibiotics exposure and subsequent changes in the microbial diversity. They observed a connection

between antibiotic exposure and reduced levels of Clostridiales and *Ruminococcus* during the initial 3 to 9 months of life, along with delayed maturation of gut microbiota (Bokulich et al. 2016).

In contrast, a nationwide cohort study conducted by Antvorskov et al. (2020), involving over 75 000 infants, revealed no significant association between early antibiotic use (within the first 2 years of life) and an increased risk of T1D (Antvorskov et al. 2020). Similarly, Mikkelsen et al. (2017) reported no overall correlation between antibiotic use and T1D risk in a study comprising 1578 Danish children (Mikkelsen et al. 2017).

Considering the maternal use of antibiotics is crucial, as infants inherit some of their early-life microbiota from their mothers (Cox & Blaser 2015). A study on diabetic children unveiled an elevated risk of T1D when mothers were treated with specific antibiotics before pregnancy (Kilkkinen et al. 2006).

3.6.7 Stress

The stress system comprises a complex network involving both neural and physiological components (Chrousos 1992). Critical hormones, such as glucocorticoids and catecholamines, serve as intermediaries between the brain and specific bodily functions (Sharma et al. 2022). Short-term stress episodes induce an elevation in glucose and insulin levels, aiding in the maintenance of normoglycemia (Ingrosso et al. 2023). However, during long-term stress, known as allostatic stress, the disruption of glucose homeostasis persists, leading to unresolved glucose levels and contributing to chronic insulin resistance. This persistent signalling informs β-cells about insulin resistance, culminating in hyperglycemia, even under normal β-cell function (Ingrosso et al. 2023).

Numerous studies underscore the link between significant childhood life events and their potential role in the initiation of T1D (Ingrosso et al. 2023). Sipetic et al. (2007) conducted a case-control study involving 105 children diagnosed with T1D and 210 controls, matched for age (±1 year), sex, and place of birth (Sipetic et al. 2007). The primary objective was to investigate whether psychological dysfunction and stressful life events could increase the likelihood of T1D onset. The study suggested that stressful life events significantly influence T1D development, and social, psychological and physical triggers may contribute to an increased release of catecholamines and cortisol, thereby elevating insulin requirements and placing additional stress on β-cells, potentially triggering the onset of T1D (Sipetic et al., 2007)

3.6.8 Air pollution and environmental toxins

Elevated exposure to environmental pollutants and particulate matter in the air is considered a potential contributing factor to the increased risk of developing T1D (Howard 2019). These pollutants have the capacity to trigger the autoimmune process or induce β-cell autoimmunity, ultimately leading to subsequent apoptosis (Butalia et al. 2016). Air pollution's involvement in T1D development encompasses its influence on both humoral and cellular immune responses (Choi et al. 2021).

Polluted air initiates oxidative stress and inflammation, recognized risk factors for various metabolic disorders, resulting in adverse effects such as lipid peroxidation, antioxidant depletion, and pro-inflammatory signalling, which contribute to various diseases (Zorena et al. 2022). The robust biological connection between air pollution and an elevated T1D risk involves inhaling pollutants, leading to disruptions in metabolic functions (Zorena et al. 2022). In a study by Hathout et al. (2002) with 61 children having T1D, a connection was established between elevated risk and exposure to ozone and particulate matter. The potential mechanism involves the formation of free radicals, with ozone exposure producing free oxygen radicals, affecting β-cells, and causing their destruction (Hathout et al., 2002)

Nitrates, nitrites and N-nitroso compounds present other potential risks for T1D. They originate from dietary sources such as processed meats and fish and environmental sources including water contaminated by nitrogen-containing fertilisers, sewage, and manure (Butalia et al. 2016). These compounds are thought to induce toxicity either directly on β-cells or through immunological pathways (Butalia et al. 2016). While the exact mechanism of action is not well-understood, one possible pathway involves DNA damage of β-cells leading to their destruction (Virtanen et al. 1994).

Other environmental toxins including persistent organic pollutants (POPs), endocrine disruptors (e.g. bisphenol A, phthalates) or bacterial toxins contribute to β-cell apoptosis through alterations in mitochondrial functions and the initiation of oxidative stress. Alongside apoptosis, additional mechanisms leading to reduced β-cell mass involve disruptions in β-cell replication (Bodin et al., 2015).

3.7 Complications of T1D

Health complications and mortality rates in T1D are closely linked to both microvascular and macrovascular complications (Marcovecchio et al. 2010). Microvascular issues arise from the damage to small blood vessels in vital organs, including the kidneys, retinal tissues of the eye, and nerves (Melendez-Ramirez et al. 2010) driven by chronic

hyperglycaemia and metabolic and functional factors (Banday et al. 2020). Endothelial dysfunction, common in T1D, is linked to coronary artery disease (Bertoluci 2015). These complications significantly increase the risk of cardiovascular disease (CVD) (Zakir et al. 2023) often starting in childhood and escalate during puberty (Chiang et al. 2018).

3.7.1 Microvascular complications

Diabetic Retinopathy is characterised by abnormal blood vessel growth in the retina, leading to vision decline (Aiello et al. 1998). Microaneurysms (swellings in tiny retinal blood vessels) mark the initial stage and progresses to vessel blockages and new vessel growth. If untreated, proliferative retinopathy can cause severe vision impairment or blindness (Aiello et al. 1998).

Diabetic Nephropathy is a leading cause of end-stage renal disease, impacting 20-40 % of diabetic patients after 15–20 years of T1D. It begins with microalbuminuria and progresses to macroalbuminuria (>300 mg/ 24 h) with metabolic, hypertensive, and genetic factors contributing to kidney damage (Dronavalli et al. 2008).

Diabetic Neuropathy refers to nerve damage, primarily impacting small sensory and large motor nerve fibers. Symptoms include numbness, burning, tingling, and pain (Bansal et al. 2006). Hyperglycaemia and abnormal lipid levels disrupt nerve function, triggering inflammation and affecting nerve growth and survival. DSP prevalence varies from 7–57 % in adults and children, with approximately 60–70 % of diabetic adults experiencing progressive neuropathy (Trotta et al. 2004). Currently, no specific treatment is available (Zaheer et al. 2021).

3.7.2 Macrovascular complications

Cardiovascular disease (CVD) is the leading cause of mortality among individuals with T1D, primarily attributed to atherosclerosis – the accumulation of cholesterol and lipid plaques in large blood vessels. This process involves damage to endothelial cells, induced by factors like oxidative stress and inflammation, exacerbated by hyperglycaemia (Schofield et al. 2019). Persistent inflammation, cell death, and plaque formation result in arterial narrowing and increased blood clot risk (H. Sharma et al. 2019). Hyperglycaemia exacerbates cell damage by promoting glucose transport, leading to free radical production and advanced glycation end products (AGEs), resulting in tissue stiffening and impaired heart function (Du et al. 2022). Disruptions in insulin signalling contribute to diabetic cardiomyopathy, affecting various cardiac functions (Frati et al. 2017).

Epidemiological evidence reveals a higher incidence and earlier onset of CVD events among individuals with T1D compared to the general population. However, CVD prevalence in T1D varies based on factors such as disease duration, age, and gender (Colom et al. 2021). A Swedish registry-based study involving 34 000 T1D patients reported significantly increased risks of both total mortality and CVD death rates compared to matched controls. The degree of risk elevation correlated with glycated haemoglobin (HbA1c) levels (Lind et al. 2014).

3.8 Diagnosis of T1D

Biomarkers are crucial in understanding both normal and abnormal physiological processes, aiding in the diagnosis of health conditions, prediction of risks, and monitoring of treatment responses (Bodaghi et al. 2023). However, identifying biomarkers that accurately reflect the state of pancreatic β-cells is challenging due to their small size and limited distribution in the pancreas (Kahn et al. 2021). Pancreatic islets, containing β-cells, constitute only 1 % to 3 % of the pancreas, with approximately half comprising β-cells (Kahn et al. 2021). Established biomarkers for T1D diagnosis include glucose levels, AAbs, C-peptide, cytokines, or nucleic acid-related biomarkers. These biomarkers are particularly useful for predicting the risk of developing T1D (Yi et al. 2018). However, despite advancements, there remains a notable gap in identifying biomarkers that accurately assess β-cell function or predict T1D progression (Nakayasu et al. 2023).

Various factors, genetic and environmental, contribute to the development of T1D, but none are definitive for diagnosis. Current diagnostic indicators primarily rely on hyperglycaemia, with additional markers distinguishing T1D from other forms of diabetes, such as low C-peptide levels or AAbs presence (Yi et al. 2018).

3.8.1 AAbs

Consistent research confirms the significance of AAbs against β-cell proteins and peptides as a standard tool for predicting T1D (Mathieu et al. 2018). The primary AAbs for T1D diagnosis include those targeting glutamic acid decarboxylase (GADA), insulin and proinsulin (IAA), cells of Islets of Langerhans (ICA), protein tyrosine phosphatase-like (IA-2 and IA-2ƛ), and zinc transporter 8 (ZnT8) (Pihoker et al. 2005). Developing multiple AAbs is a critical stage in T1D progression, significantly elevating the risk of developing the disease compared to having only a single AAb (Mathieu et al. 2018).

In 95 % of T1D cases, at least one AAb is present before hyperglycaemia occurs.

However, not all cases of T1D involve the presence of AAbs, making them occasionally inadequate for detecting the disease. This inadequacy may stem from less sensitive testing methods or other forms of autoantigens (AAgs) initiating β-cell destruction (Yi et al. 2018).

Having at least two AAbs in the blood increases the likelihood of developing clinical T1D to over 80 % during childhood or adolescence (Bonifacio 2015). The number of AAbs an individual possesses becomes more crucial than any specific combination when predicting T1D (Winter & Schatz 2011).

Children developing T1D at a very young age often exhibit IAA as the first detected AAb, while GADA is typically present in those developing T1D later in life (Ilonen et al. 2022). Notably, AAbs such as IA-2 and ZnT8 are rarely detected at onset but remain detectable until T1D diagnosis (Yi et al. 2018). IA-2 and ZnT8 are therefore often regarded as substitute markers indicative of β-cell destruction (Kawasaki 2023). Despite uncertainties about the exact role of AAbs in causing T1D, they are thought to be more associated with disease progression or secondary immune responses rather than being the primary cause. The process of seroconversion, when AAbs appear in the blood, is crucial for accurate T1D prediction (Yi et al. 2018). Characteristics such as age at seroconversion, number of AAbs, their titer, affinity, antigenic specificity, and epitope binding enable categorising individuals based on their T1D risk within five years (Achenbach et al. 2004).

While AAbs and genetics are valuable for T1D prediction, limitations exist. Testing for AAbs is typically recommended for individuals with a family history, yet around 85 % developing T1D lack such a history. Moreover, not all AAbs-positive individuals will develop clinical T1D, and if they do, the disease progression varies (Yi et al. 2018). This underscores the necessity to explore novel markers for predicting different T1D developmental phases and determining the optimal timing for treatment (Jin & She 2012).

3.8.2 C-peptide

C-peptide is a widely used method for assessing the function of β-cells (Leighton et al. 2017). When proinsulin, the precursor of insulin, is enzymatically cleaved, both insulin and a 31-amino acid peptide known as C-peptide are released into the bloodstream in equal amounts (Wahren et al. 2012). The choice of measuring C-peptide, as opposed to insulin, is primarily attributed to their distinct rates of degradation in the body. C-peptide has a half-life of approximately 20 to 30 minutes, compared to insulin's half-life of 3 to 5 minutes. This prolonged half-life is a more stable timeframe for monitoring β-cell response (Leighton et al. 2017).

In individuals without T1D, fasting C-peptide levels typically range from 0.3 to 0.6 nmol/l and can rise from 1 to 3 nmol/l after meals (Yosten et al. 2014). C-peptide is minimally affected by hepatic absorption, as opposed to insulin, which is partly absorbed by the liver. This results in consistent elimination of insulin in the bloodstream, posing challenges for accurate measurement (Leighton et al. 2017).

People with T1D often exhibit low plasma C-peptide levels, typically below 0.2-0.4 nmol/l, indicative of severe insulin deficiency. However, in cases of adolescent or adult-onset T1D, low C-peptide levels may not reliably predict severe insulin deficiency within three years of diagnosis. C-peptide levels decline around 30 months before T1D diagnosis, although stability is observed in others (Maddaloni et al. 2022).

Despite these variations, a study by Sosenko et al. (2009) investigated how C-peptide levels respond to oral glucose in T1D progressors. The study revealed a reduced early C-peptide response can be predictive for T1D development in at-risk children, providing a two-year lead in T1D prognosis (Sosenko et al. 2010).

3.8.3 Cytokines

Cytokines represent crucial proteins in predicting T1D. Cytokines, low molecular weight molecules, play a central role in mediating the immune response and are integral to complex pathways regulating inflammation processes crucial for managing lesion sites (Gouda et al. 2018). They facilitate communication among immune cells, stimulating and regulating various immune functions (Yi et al. 2018). Pro- and anti-inflammatory cytokines contribute to the pathogenesis of T1D, playing a significant role in the ongoing destruction of β-cells by altering the immuno-regulatory environment of the pancreas (Lu et al. 2020).

Furthermore, cytokines possess the ability to disrupt glucose uptake in response to insulin, initiating the development of insulin resistance (Gouda et al. 2018). Certain cytokines exhibit cytotoxic effects on pancreatic β-cells, while others, along with chemokines, regulate the inflammatory response within these cells (Lu et al. 2020). Maintaining a balance between proinflammatory and anti-inflammatory cytokines is crucial for normal cellular functioning, and variations in cytokine genes can lead to changes in cytokine production, contributing to β-cell inflammation and destruction (M & DR 2014).

Numerous well-known inflammatory-promoting cytokines such as IL-1, IL-6 and tumour necrosis factor (TNF-α) and IFN family members, have been identified in T1D. This discovery has raised significant interest in exploring whether inhibiting these cytokines could offer clinical benefits (Lu et al. 2020). TNF-α, a cytokine with diverse roles in various cellular

events, has been observed in triggering β-cell apoptosis. Blocking TNF-α is believed to contribute to the preservation of β-cell function, particularly in children recently diagnosed with T1D (Lu et al. 2020). Cytokines IFN-α and IFN-γ have been associated with both the onset and progression of T1D. Elevated levels of IFN-α are observed in the blood of individuals with T1D, triggering over-production and increased expression of HLA molecules, leading to endoplasmic reticulum stress and cell death – an indication of the direct involvement of INF-α in β-cell destruction (Marroqui et al. 2017). In a case-control study involving 67 children, screening 65 cytokines and chemokines revealed that 15 cytokines and growth factors were higher in children positive for AAbs. Furthermore, two specific cytokines, IL-10 and IL-21, demonstrated the capability to distinguish two groups with enterovirus infection (Yeung et al. 2012)

While cytokines hold potential as biomarkers for T1D, it is important to note their limitation in specificity for T1D. Cytokine levels can vary in response to injuries and damage in different tissues, as well as in the presence of various inflammatory and immune-related conditions, not restricted to T1D (Yi et al. 2018).

3.8.4 Glucose-related biomarkers

T1D may be diagnosed in patients exhibiting common symptoms of hyperglycaemia, even when the glucose level is below 200 mg/dL. Another indicator of T1D development can be observed in patients with consistent positive results from multiple glucose-related tests, even in the absence of hyperglycaemia (Yi et al. 2018). These diagnostic tests include haemoglobin A1c (HbA1c), fasting plasma glucose (FPG), and oral glucose tolerance test (OGTT), each possessing distinct criteria, sensitivity, and specificity (Yi et al. 2018).

Confirmation of T1D is based on specific threshold values: HbA1C >6.5, FPG >126 mg/dL, and a 2-hour plasma glucose during OGTT >200 mg/dL (Kahanovitz et al. 2017). HbA1c serves as a valuable biomarker for measuring the average blood glucose level over the preceding 2 to 3 months. As blood glucose rises, it binds to haemoglobin in a manner dependent on its concentration. HbA1c is directly related to current blood glucose levels, reflecting chronic hyperglycaemia (Sherwani et al. 2016). However, its precision is relatively lower compared to FPG or OGTT. FPG is effective for assessing immediate glucose levels but lacks detailed insight into the temporal trends (Sherwani et al. 2016). The results of FPG and OGTT may exhibit increased variability when monitoring is conducted frequently to ensure accuracy (Yi et al. 2018).

3.8.5 Nucleic acid biomarkers

Small fragments of DNA are detectable in the bloodstream, likely stemming from cell death or other processes not yet fully understood (van der Vaart & Pretorius 2007). These DNA fragments harbour genetic variations associated with disease onset and progression, serving as valuable biomarkers reflecting pancreatic β-cell activity (Yi et al. 2018).

In the context of T1D, the focus is placed on unmethylated insulin DNA for the early detection of β-cell death. Unique cytosine-guanine sites within the insulin gene remain unmethylated in β-cells but are methylated in other tissues (Zhang et al. 2017). Following β-cell death in T1D, detectable fragments of unmethylated insulin DNA are released into the bloodstream, indicating the extent of β-cell loss (Zhang et al. 2017). The method involves a non-invasive liquid biopsy detecting circulating free DNA (cfDNA) before hepatic or renal clearance (Speake et al. 2020). In T1D, β-cell destruction by T cells releases unmethylated insulin DNA into the bloodstream, detectable through this non-invasive method (Zhang et al. 2017). Herold et al. (2015) observed a notably elevated ratio of unmethylated to methylated insulin DNA in T1D patients at disease onset compared to healthy controls. Elevated levels of unmethylated insulin DNA correlated with reduced insulin secretion, indicating potential β-cell destruction and death (Herold et al. 2015)

Other nucleic acid biomarkers reflecting β-cell health include microRNA (miRNA) molecules (Yi et al. 2018). MiRNAs are small, non-coding RNA molecules with crucial roles in gene expression regulation and various biological processes (Syed & Evans-Molina 2016). Among these processes is glucose metabolism, closely linked to the stability of pancreatic β-cells responsible for insulin synthesis (Margaritis et al. 2021). Numerous studies aim to identify specific miRNA molecules that interfere with the normal functioning of pancreatic β-cells, leading to their apoptosis. This interference is believed to significantly impact pancreas homeostasis and maintenance (Margaritis et al. 2021).

3.9 Metabolomic biomarkers

T1D exerts a profound influence on various biochemical pathways, particularly those involving metabolic substrates. Metabolomics, a discipline within the broader field of “-omics” sciences (such as genomics, proteomics, and transcriptomics) is applied across diverse natural science domains (Pinu et al. 2019). Metabolomics is relatively a new technology (Wu et al. 2022) focused on the identification and characterization of low-molecular weight molecules known as metabolites (biochemicals like sugars, amino acids, organic acids, nucleotides, and lipids) within biological samples (Qiu et al. 2023). The complete set of (Jin

et al. 2019) identified metabolites in a system is commonly referred to as the "metabolome" (Jin et al. 2019). The metabolome provides a comprehensive record of the specific biochemical processes occurring within a sample (Suhre 2014) serving as a direct reflection of cellular activity (Kim et al. 2018).

Metabolomes can be categorised based on their origin. Endometabolomes are associated with intracellular metabolism, while exometabolomes, also known as the metabolic footprint or secretome, are associated with extracellular metabolism. In mammals, metabolic analysis encompasses samples such as serum (or plasma), urine, cerebrospinal fluid, breath, tears, saliva, faecal matter, and various other body tissues (Dunn et al. 2011).

Metabolomics is highly sensitive and focuses on analysing a relatively small set of metabolites compared to the vast number of genes or mRNA molecules addressed by other methodologies (Friedrich 2012). It captures metabolites representing the end-products of the interplay between genes and factors like the environment, lifestyle, or medications. Consequently, metabolite levels provide accurate indications of metabolic pathway activity which greatly aids in identifying both short-term and long-term changes in cells, tissues, and bodily fluids (Friedrich 2012).

Metabolomics is a comprehensive study of metabolism, avoiding the adherence to a specific experiment (Liu & Locasale 2017). It encompasses untargeted screening, which involves profiling numerous unknown features to measure differences between two samples or populations, without quantifying specific metabolites (Hatzakis 2019). This approach is valuable for discovering new, previously unknown metabolites (Liu & Locasale 2017). Conversely, targeted experiments provide deeper insights into specific hypotheses. They aim to quantify the maximum number of metabolites with statistical analyses based on the measurement of absolute concentrations of molecules. Semi-targeted metabolomics combines aspects of both approaches, identifying and quantifying numerous molecules without a predetermined hypothesis (Liu & Locasale 2017). Nonetheless, identifying and validating the properties of metabolites remains challenging due to the physiochemical diversity of the metabolome (Kim et al. 2018).

Given the comprehensive complexity of metabolomics, diverse analytical techniques are utilised for analysis. Two well-known techniques used for analysing data from large sets of metabolomic samples are NMR spectroscopy and mass spectroscopy (MS) which can be further complemented by chromatography or electrophoretic separation before detection (Dunn et al. 2011). While both techniques enable exploration of metabolic profiles, they each possess distinct advantages and limitations (Suhre 2014).

NMR spectroscopy is non-destructive, cost-effective, and reliable, providing quick results with minimal preparation time (Suhre 2014). However, its sensitivity is limited to detecting a narrow range of metabolites with higher abundance (Pinu et al. 2019). In contrast, MS offers high sensitivity and requires only a small sample volume (Jin et al. 2019). Despite its ability to qualify numerous molecules, MS is a destructive technique, preventing the analysis of live samples and the reuse of previously analysed samples (Emwas et al. 2019).

In recent years, metabolomics has revealed new mechanisms and biomarkers in the fields of foods and nutrition, providing valuable insights into cellular processes within the human body. These discoveries have promising implications for healthcare applications (Damiani et al. 2020). Metabolomics has the potential to advance healthcare by improving the understanding of underlying mechanisms in diseases like T1D. It could serve as a powerful tool for identifying new pathways or early biomarkers that indicate metabolic changes contributing to T1D development. Using metabolomics in the study of metabolic disorders holds significant promise due to the direct relationship between metabolic issues and the disease's primary consequences. This capability of metabolomics is crucial for discovering biomarkers (Bervoets et al. 2017).

3.9.1 Metabolomic biomarkers in relation to T1D

Metabolomic research has unveiled potential metabolic disturbances preceding the onset of T1D. Despite advancements, the exact contribution of these metabolic disruptions in initiating the disease remains incompletely understood. Nevertheless, various amino acid and lipid-based metabolites have been implicated in the progression of T1D (Yi et al. 2018).

Methionine, a crucial amino acid involved in DNA methylation, has been associated with the timing of AAbs development. Pflueger et al. (2011) observed that children who developed AAbs before the age of 2 had approximately half the levels of methionine and hydroxyproline compared to those who developed AAbs later or those without AAbs (Pflueger et al. 2011). Jørgenrud et al. (2017) also noted lower methionine levels in infants with two or three AAbs or T1D compared to healthy individuals, although this difference did not reach statistical significance. The authors suggested that the age of the children and the duration of breastfeeding could potentially contribute to these observed differences. Moreover, the decrease in tyrosine and ornithine levels was not statistically significant after adjusting for multiple comparisons (Jørgenrud et al. 2017). Sen et al. (2020) found amino acids alanine, aspartate and glutamate were more prevalent in the peripheral blood mononuclear cells (PBMCs) of those later developing T1D (Sen et al. 2020). Additionally,

Orešič et al. (2008) reported increased levels of glutamic acid and BCAAs such as leucine and isoleucine along with reduced levels of ketoleucine before seroconversion. Reduced levels of succinic acid in PT1D were also observed (Orešič et al. 2008).

Analysing the lipidomic profile has proven to be a valuable predictive method for T1D development. Orešič et al. (2008) noted a consistent decrease in triglycerides and several phospholipids, including phosphatidylcholine and sphingomyelin in children progressing to T1D compared to those remaining negative for AAbs. As phosphatidylcholine functions as the primary source of choline in the body, it indicates a deficiency in choline among T1D individuals since birth (Orešič et al. 2008). Furthermore, Orešič et al. (2013) reported lower levels of essential phospholipids, specifically phosphatidylcholine, phosphatidylethanolamines, and sphingomyelins, in children who later developed T1D before the age of 4 (Orešič et al. 2013). This finding aligns with the understanding that methionine plays a crucial role in breaking down choline, a significant building block of phospholipids. This correlation supports the notion that inadequacies in methionine during early childhood may result in diverse consequences (Overgaard et al. 2016).

Sen et al. (2020) aimed to identify specific lipids and polar metabolites in PBMCs of children who would later develop autoimmunity against β-cells or T1D. Findings showed lower levels of most lipids and polar metabolites in PBMCs of children developing T1D or seroconverting to at least one AAb compared to a control group of children without AAbs. Both groups of children were carefully matched based on similar HLA-associated risk factors, age, and sex (Sen et al. 2020). Furthermore, Lamichhane et al. (2018) indicated that plasma lipid levels including sphingomyelins, triacylglycerols and phosphatidylcholines were predominantly reduced in infants who later developed T1D compared to those with a single AAb and healthy controls (Lamichhane et al. 2018).

Table 1 Protein and lipid biomarkers associated with progression to T1D.

Authors	Year	Type of participants	Sample type	Protein and lipid biomarkers in progressors to T1D
Orešič et al.	2008	PT1D (n = 56); NPT1D (n = 73)	Cord blood, serum	↑ -glutamic acid ↑ leucine, isoleucine ↓ ketoleucine ↓ succinic acid ↓ triglycerides, phospholipids, phosphatidylcholines
Pflueger et al.	2011	AAbs+ (n = 35); AAbs– (n = 35)	Serum	↓ methionine and hydroxyproline ↑ polyunsaturated fatty acid-containing phosphatidylcholines, specific triacylglycerols
Orešič et al.	2013	PT1D (n = 33); 3-4 AAbs (n = 31); 2 AAbs (n = 31); 1 AAb (n = 48); CTR (n = 143)	Cord blood	↓ choline-containing phospholipids
Jørgenrud et al.	2017	PT1D (n = 29); CTR (n = 29)	Plasma	↓ tyrosine, ornithine, methionine (not statistically significant)
Lamichhnane et al.	2018	PT1D (n = 40); P1Ab (n = 40); CTR (n = 40)	Plasma	↓ sphingomyelins, triacylglycerols and phosphatidylcholines
Lamichhane et al.	2019	PT1D (n = 40); P1Ab (n = 40); CTR (n = 40)	Plasma	↓ amino acids, sugar derivatives, fatty acids, catabolites of microbial origin (1,5-anhydrohexitol, 11-eicosenoic acid, 1-monopalmitin, 4-hydroxyphenyllactic acid, Arachidonic acid, aspartic acid, D-(-)-Lyxofuranose, D-arabinose, glutamic acid, glyceric acid, L-5-oxoproline, L-hydroxyproline, linoleic acid, L-threonic acid, oleic acid, palmitic acid, pentadecanoic acid, pyroglutamic acid, ribonic acid, stearic acid, succinic acid, tryptophan)

Lamichhane et al.	2019	PT1D (n = 30); P1Ab (n = 33); CTR (n = 38)	Cord blood	↓ phospholipids (sphingomyelins) ↑ cholesterol esters
Sen et al.	2020	PT1D (n = 34); P1Ab (n = 27); CTR (N = 10)	PBMCs	↓ phosphatidylcholines, sphingomyelins, ceramides, cholesterol esters, lysophosphatidylcholines, phosphatidylethanolamines and triacylglycerols
Abbreviations PT1D = progressors to T1D NPT1D = non-progressors to T1D P1Ab = progressors to developing a single AAb CTR = control (group)				

3.10 Treatment of T1D

Insulin replacement is the primary therapy for T1D, involving daily injections or continuous subcutaneous insulin infusion (Janež et al. 2020). Achieving optimal glycemic control requires tailored insulin therapy, regular glucose monitoring via self-checks or Continuous Glucose Monitoring (CGM), and patient education on carbohydrate counting (Janež et al. 2020). While absence of insulin is a key factor in T1D, other pancreatic abnormalities, particularly issues in glucagon secretion, pose challenges to glucose regulation. The increase in glucagon may negatively impact glycemic control, complicating implementation of intensive insulin therapy (McCall & Farhy 2013).

Current T1D treatment combines strict dietary regimes with exogenous insulin administration via daily injections or pumps. Novel genetically modified insulin analogs like aspart, lispro, and Degludec offer faster action and extended duration, more closely mimicking the endogenous human insulin action (Pathak et al. 2019).

Despite improvements in insulin purity and reduced adverse reactions, animal and human insulins still have practical limitations due to their dissimilar timing in lowering glucose compared to endogenous insulin patterns (McCall & Farhy 2013). Newer insulin analogs, with slight structural and functional variations from human insulin, are gaining

popularity despite higher costs and are increasingly used in treating both T1D and T2D (McCall & Farhy 2013).

Rapid-acting insulin, such as insulin aspart, insulin lispro, and glulisine, act quickly and have shorter duration compared to endogenous insulin. These analogs undergo minor modifications, typically involving changes in one or two amino acids, prevent clumping and improve absorption, all while maintaining their intended physiological effects (Hartman 2008). Long-acting insulin analogs, insulin glargine and insulin detemir, provide continuous basal insulin release by undergoing specific molecular modifications that extend their activity duration. Changes in a specific amino acid of insulin glargine affect its solubility, resulting in stable structures and prolonged absorption. Similarly, detemir's fatty acid side chain slows absorption by creating reversible bonds with albumin in the blood, ensuring consistent plasma insulin levels (Hartman 2008).

The Artificial Pancreas (AP) combines continuous glucose monitoring with automated insulin delivery, marking a significant advancement in managing T1D (Dermawan & Kenichi Purbayanto 2022). This closed-loop system continuously monitors glucose levels and adjusts insulin delivery in real-time, improving glycemic control and reducing the need for manual interventions (Hovorka 2006).

Immunotherapy holds promise for modifying the T1D process (Allen & Dayan 2021). Current T1D immunotherapies include antigen-independent (non-specific) strategies, which modulate the overall immune response (Cabello-Olmo et al. 2019) and antigen-dependent strategies that target specific immune dysfunction responsible for β-cell destruction (Zhang et al. 2022). Despite notable progress, achieving long-term control remains challenging, prompting exploration of combination therapies to address the complex nature of T1D pathogenesis (Huang et al. 2023).

3.11 NMR spectroscopy

NMR spectroscopy stands as a fundamental analytical technique in metabolomics, complemented by gas chromatography with mass spectroscopy (GC-MS) and liquid chromatography with mass spectroscopy (LC-MS). Integration of multiple platforms is often necessary due to the lack of a single method capable of fully identifying and quantifying all metabolites (Emwas et al. 2019). Being 10 to 100 times less sensitive than MS, NMR spectroscopy boasts superior reproducibility and the ability to provide detailed structural and quantitative insights across diverse chemical classes (Emwas et al. 2019).

NMR spectroscopy utilises the magnetic properties of atomic nuclei, including ^{1}H, ^{13}C, ^{15}N, or ^{31}P. The behaviour of NMR-active nuclei within a magnetic field offers valuable molecular insights into substances. Among these nuclei, ^{1}H is the most sensitive and is commonly used in metabolomics via one-dimensional ^{1}H NMR analysis (Friedrich 2012). ^{1}H NMR spectroscopy was initially employed for analysing plasma, urine, and tissues due to the abundance of ^{1}H nuclei. Each compound manifests as a distinct signal in a ^{1}H NMR spectrum (Friedrich 2012). However, this technique possesses significant challenges in sample quantification, particularly for metabolites with similar chemical structures, resulting in spectral overlap (Crook & Powers 2020). Additionally, NMR spectroscopy serves for identifying unique metabolites, especially protein-bound metabolites and for measuring certain inorganic metabolites or ions (such as metal ions and hydrogen cations via pH), which may not be measurable by LC-MS and GC-MS (Emwas et al. 2019) Due to stable isotopes, NMR spectroscopy enables a thorough exploration of metabolic pathways, elucidating the dynamics and mechanisms involved in the transformation of individual metabolites (Markley et al. 2017).

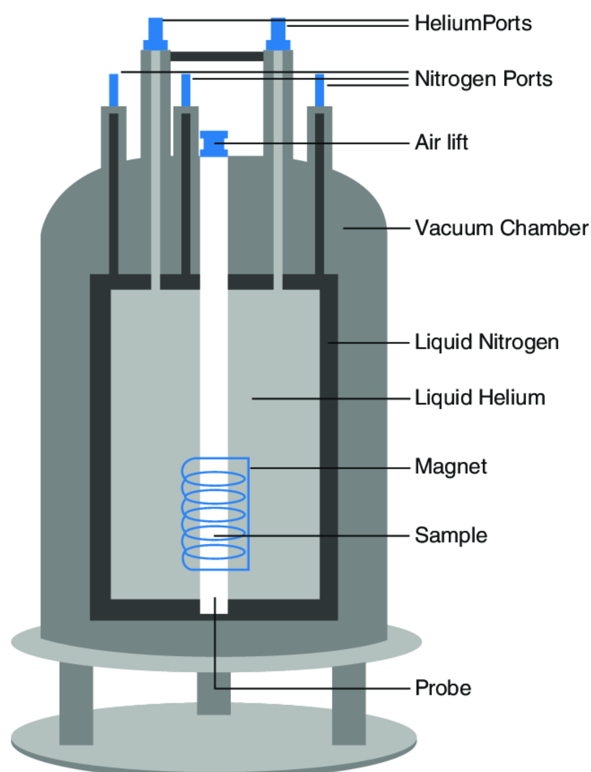

Figure 3 Schematic illustration of the principal components of a NMR spectrometer (Adapted from Antcliffe & Gordon 2016).

4 Methods

4.1 Participants and sample collection

This is a case-control study investigating the differences in the stool metabolome between 24 children aged 4.8 years ± 1.2 years genetically predisposed to T1D and their matched controls.

Stool samples for metabolite analysis were collected between 2015 and 2016 from children enrolled in the Finnish Type 1 Diabetes Prevention and Prediction Study (DIPP) as part of the ADELE project. Inclusion criteria were applied during participant selection, considering factors such as T1D related AAbs, onset of T1D, dietary factors, antibiotic usage, HLA-DQ genotype, age of sampling and date of sampling. A similar set of criteria were employed in the matching of T1D-predisposed children (1) with healthy controls (0). Additionally, each subject was instructed to provide one sample from the winter and summer months. Not all subjects were able to provide both samples. A total of 87 samples (43 samples from T1D-predisposed subjects and 44 samples from control subjects) were available for ^{1}H NMR analysis. Samples were provided by Tampere University.

Stool sample collection was carried out at participants' homes, frozen in-home freezers (-20°C) and subsequently transported to Tampere University Hospital in a frozen state. The samples were then stored for the long term in tanks cooled with liquid nitrogen at -80°C. Two stool aliquots were collected at each timepoint. In 2022, the samples were transported to the Czech University of Life Sciences in a thermobox filled by dry ice at -80°C and stored within the freezer at -80°C until the analysis.

4.2 DIPP Study

DIPP study, an observational birth cohort study, was initiated in 1994 and is currently ongoing across three Finnish universities – Oulu, Tampere, and Turku (Lamichhane et al. 2023). The primary aim of the DIPP study was to refine methods for preventing T1D and develop innovative approaches (Haller & Schatz 2016). Children were included in the study based on various risk factors, including a positive T1D family history among first-degree relatives and HLA-DRB1-DQB1 genotyping (Veijola 2020).The study employed genetic typing through HLA-DQ to identify children with DR3-DQ2 and/or DR4-DQ8 risk haplotypes while excluding those with protective haplotypes (Orešič et al. 2008). The study follows participants from the age of 3 months to 15 years of age or until a T1D diagnosis.

Screening involves 11 000 children annually, with approximately 600–700 children with higher genetic susceptibility initiating the follow-up process each year (Veijola 2020).

In a broader context, the DIPP study is integrated into the Human Exposomic Determinants of Immune-Mediated Diseases (HEDIMED) project, which is a component of the HORIZON 2020 programme in Europe. HEDIMED represents the most extensive study to date in investigating the exposome's role in the onset of immune-mediated diseases (Laiho et al. 2022). The study aims to comprehensively analyse the intricate interactions between external and internal exposomes, along with the pivotal molecules and pathways active in early life. By doing so, HEDIMED seeks to identify disease mechanisms, as well as exposures that pose risks or provide protection, crucial for the development of T1D, celiac disease, allergies, and asthma (Laiho et al. 2022).

4.3 Faecal metabolome

4.3.1 Chemicals

K_2HPO_4 (potassium dihydrogen phosphate), NaH_2PO_4 (sodium dihydrogen phosphate), TSP (((3-(trimethylsilyl)-propionate-2,2,3,3-d4)), D_2O (deuterium oxide), NaN_3 (sodium azide).

4.3.2 NMR buffer

The NMR buffer was prepared by combining 200 mg NaN_3 and 86.135 mg TSP in beaker 1. Simultaneously, salts for pH adjustment were prepared as follows:

For 1.5 M K_2HPO_4, 22.21 g K_2HPO_4 was dissolved in 85 mL D_2O in beaker 2.
For 1.5 M NaH_2PO_4, 4.91 g NaH_2PO_4 was dissolved in 31 mL D_2O in beaker 3.

The salt solutions were mixed until a pH of 7.4 was achieved, and then used to dissolve the contents of beaker 1. Maintaining a pH of 7.4 is crucial to replicate the pH of a stool sample. The buffer, containing TSP as a reference for NMR spectra (see below) and NaN3 to halt microbiota activity and stabilise the sample, is subsequently added to the sample.

4.3.3 Sample preparation for NMR analysis

Prior to analysis, the samples underwent a thawing process at room temperature, afterwards 1000 µL of ultra-pure water was added, and the mixture was vortexed for 10 seconds, followed by centrifugation (4°C, 15,000 rpm, 10 min) to facilitate the separation of solid and liquid phases. Following centrifugation, 630 µL of the supernatant was collected into another Eppendorf microtube. To this, 70 µL of the NMR buffer was added, followed by vortexing for 10 seconds. The centrifugation process was repeated at 15,000 rpm for 10 minutes. If the final solution was not deemed clean, an additional round of centrifugation at 15,000 rpm for 10 minutes was executed. The clear supernatant (550 µL) was transferred into the NMR tube. During the transfer, the NMR tube was held at an approximate 45-degree angle to facilitate the smooth sliding of the drop, and the remaining solution was slowly added.

^{1}H NMR spectra were acquired using a Bruker Avance III spectrometer equipped with a broadband fluorine observation SmartProbeTM (BBFO) featuring Z-axis gradients (Bruker Biospin GmbH, Rheinstetten, DE), operating at a proton frequency of 500.18 MHz. The measurements were conducted at a temperature of 298 K (25°C). To eliminate water molecule signals, a standard Bruker 1D NEOSY sequence at 4.704 ppm was employed. Each sample underwent a one-dimensional proton experiment with the following parameters: 128 NS scans, 32K data points, a spectral width of 16 ppm, an acquisition time of 4 s, a mixing time of 0.1 s, and an interscan relaxation delay of 5 s. All samples were acquired using a 1D NEOSY pulse sequence with presaturation, calibrated against the internal standard TSP at 0.0 ppm. Manual phasing was conducted using TopSpin 3.6.4 (Bruker Biospin GmbH, Rheinstetten, DE). Subsequent processing took place in the online software NMRProcFlow, involving baseline correction and bucketing procedures (Jacob et al. 2017). Bucket annotation was performed after an initial subset annotation in Chenomx version 9.02, utilising the software's built-in spectral library and the in-house database (Ellinger et al. 2012). The final concentration (mg/dL) of each metabolite was determined based on the under-curve area of the peak obtained in the NMRProcFlow software and calculated via regression equations defined by 10 randomly chosen spectra manually fitted in Chenomx software and obtained under-the-curve areas.

4.3.4 NMR statistical data analysis

The ^{1}H NMR data underwent statistical analysis, which was conducted on the normalised bins using principal component analysis (PCA) in MetaboAnalyst 6.0, employing log data transformation and auto-scaling. For univariate comparisons, t-test (2-tailed, equal variances assumed) was applied within the same software. Additionally, MetaboAnalyst 6.0 was employed to generate the heatmap (Figure 10). For the hierarchical clustering, we utilised the Ward algorithm and Pearson's correlations on log-transformed data (Ewald et al. 2024; Lu et al. 2023).

4.3.5 Freeze-Drying

For each sample designated for ^{1}H NMR analysis, a duplicate weighing approximately 100 mg was prepared and lyophilized for 2 days at -55°C using a laboratory lyophilizer (L4-55, Gregor Instruments, Sazava, CZ). Freeze-drying, also referred to as lyophilization, is a process that involves the direct transformation of water from its solid (ice) state to vapour, bypassing the liquid phase, followed by the removal of water from the resulting "dry" layer (Nowak & Jakubczyk 2020). The final dataset underwent normalisation based on the dry weight of the samples. The dry matter contains all components found in the original stool sample, excluding water. This enables the assessment of dilution or concentration of the measured metabolites, allowing for the adjustment of the final value for each specific metabolite.

5 Results and discussion

In total, 74 metabolites were identified, of which 43 are illustrated in Figure 5, whereas the less abundant are omitted for clarity. Some metabolites are stated in the spectrum more than once due to multiple peaks of given compound in different spectral regions. Additionally, the water region (4.5 to 5.2 ppm) was excluded from the visualisation of the spectrum (Figure 5). The profile of identified metabolites through the ^{1}H NMR analysis is similar to that in other metabolomics studies (Orešič et al. 2008; Jørgenrud et al. 2017; Sen et al. 2020).

Among the identified metabolites, SCFAs including acetate, butyrate, and propionate were the most abundant group of metabolites, accounting for 40 (% w) of all identified metabolites in the spectrum in terms of abundance (Figure 4). The second largest group of metabolites comprises amino acids. Glutamate was the most abundant amino acid in the T1D-predisposed group, comprising 7 (% w) (Figure 4).

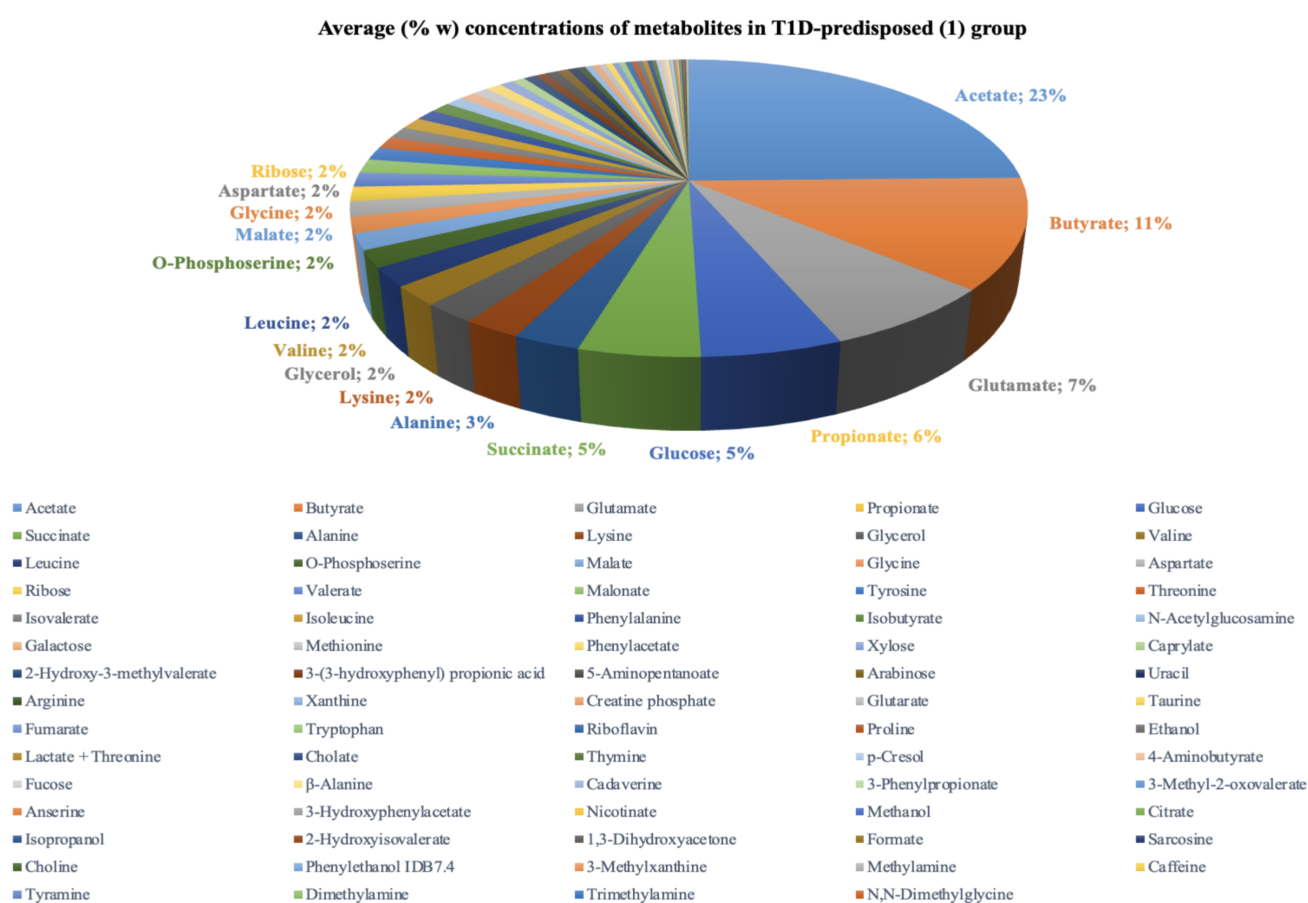

Figure 4 Average concentrations of metabolites in T1D-predisposed (1) group.

The presented pie chart (Figure 4) illustrates the average metabolite concentrations presented as percentages within the T1D-predisposed group. For visual clarity, the names and percentage values are only listed for metabolites that have reached or exceeded 2 %; units are % w.

In our study, both the T1D-predisposed group and the control group showed a distinct SCFAs molar ratio of 9 : 3 : 2 (acetate: butyrate: propionate) which is similar to what has been previously postulated by other authors. Previously, 9 : 3: 3 (acetate: butyrate: propionate) has been published in healthy subjects (Müller et al. 2021). At individual level, SCFAs tend to oscillate in time (Schwiertz et al. 2010).

The observed discrepancy in the SCFAs ratio within our study, particularly the notable difference between butyrate and propionate levels prompts exploration into potential influencing factors, particularly dietary practices, and gut microbiota composition in T1D. Dietary management plays a significant role in the care of individuals with T1D, alongside insulin therapy, serving as a cornerstone of care. Dietary recommendations for T1D emphasise fiber-rich foods such as vegetables, legumes, and grains as preferred carbohydrate intake, contributing to greater glycaemic stability (Pelikánová & Bartoš 2018). Notably, fiber intake and its type significantly influence the production of SCFAs (den Besten et al. 2013). O'Keefe et al. (2015) observed in a dietary intervention study that African-Americans provided with a low-fat, high-fiber diet had increased butyrate production compared to those following high-fat, low-fiber feeding (O'Keefe et al. 2015). Furthermore, the observed abundance of SCFAs may be attributed to variations in gut microbiota composition and/or its function. Acetate and propionate are mainly produced by the phylum Bacteroidetes, while butyrate is predominantly produced by the phylum Firmicutes (den Besten et al. 2013). *Bacteroides* have shown increased levels in numerous studies investigating alterations in T1D gut microbiota (Giongo et al. 2011; de Goffau et al. 2013; Murri et al. 2013; Mokhtari et al. 2021). The influence of *Bacteroides* on T1D may be attributed to its impact on immune system development, potentially through the production of glutamate decarboxylase, a factor implicated in GADA autoimmunity (Elhag et al. 2020). While previous research has suggested diminished levels of butyrate-producing microbes in T1D, our findings indicate elevated butyrate levels, suggesting a nuanced microbiota-metabolite relationship in this context. Additionally, various types of Firmicutes and Bacteroidetes species are capable of producing butyrate from certain peptides and amino acids (Rowland et al. 2018). In particular,

glutamate, the most abundant amino acid in the T1D-predisposed group (Figure 4) may play a contributory role in butyrate synthesis (Louis & Flint 2017).

While our findings are intriguing, it is important to acknowledge their limitations. Insights into SCFAs occurrence and ratio should be approached cautiously due to their speculative nature. A primary limitation is the absence of detailed dietary records of the participating children, particularly regarding adherence to T1D dietary recommendations, which emphasise carbohydrate regulation and increased fiber intake. Leveraging our findings for longitudinal research, integrating individuals' dietary data, and investigating the influence of diet on T1D metabolic profiles could be valuable for future T1D diagnosis. Moreover, speculation also arises regarding whether alterations in gut microbiota and dysbiosis in T1D may affect SCFAs production. In particular to butyrate synthesis, the process involves the collaborative effort of various bacterial species, employing multi-step mechanisms (Del Chierico et al. 2022). Utilising these findings for longitudinal research may offer intriguing insights, potentially enhancing our understanding of the impact of gut microbiota on metabolite profile that could serve as potential T1D biomarkers. Therefore, considering the ongoing T1D research within the DIPP study, our findings could potentially contribute to and be further enriched by investigations involving T1D microbiota using advanced analytical techniques such as GC and LC metabolomics (Zeki et al. 2020) or transcriptomics within the DIPP study (Maan et al. 2023).

In our study, elevated levels of certain BCAAs such as valine and leucine, along with their relative amino acid metabolites like glutamate, were observed (Figure 4). Amino acids found in faeces are often results from catabolism of non-digestible proteins (Rose et al. 2015). Additionally, there is evidence suggesting that amino acids, particularly BCAAs such as valine, leucine, and isoleucine, may impair insulin signalling and induce insulin resistance in β-cells through the activation of protein kinase mTOR, which facilitates insulin receptor activation (Arneth et al. 2019). Orešič et al. (2008) also reported increased levels of BCAAs, particularly leucine and isoleucine, in individuals who later developed T1D (Orešič et al. 2008). Although our study did not show a significant difference in BCAAs levels between the two groups (0; 1), it is plausible to assume that BCAAs might be involved in the pathogenesis of T1D through the mechanism of insulin signalling impairment (Zhenyukh et al. 2017). Despite the complexity of this mechanism and the need for further investigation, specific BCAAs may hold promise as potential metabolite predictors of the disease in children who eventually develop T1D (Sen et al. 2020).

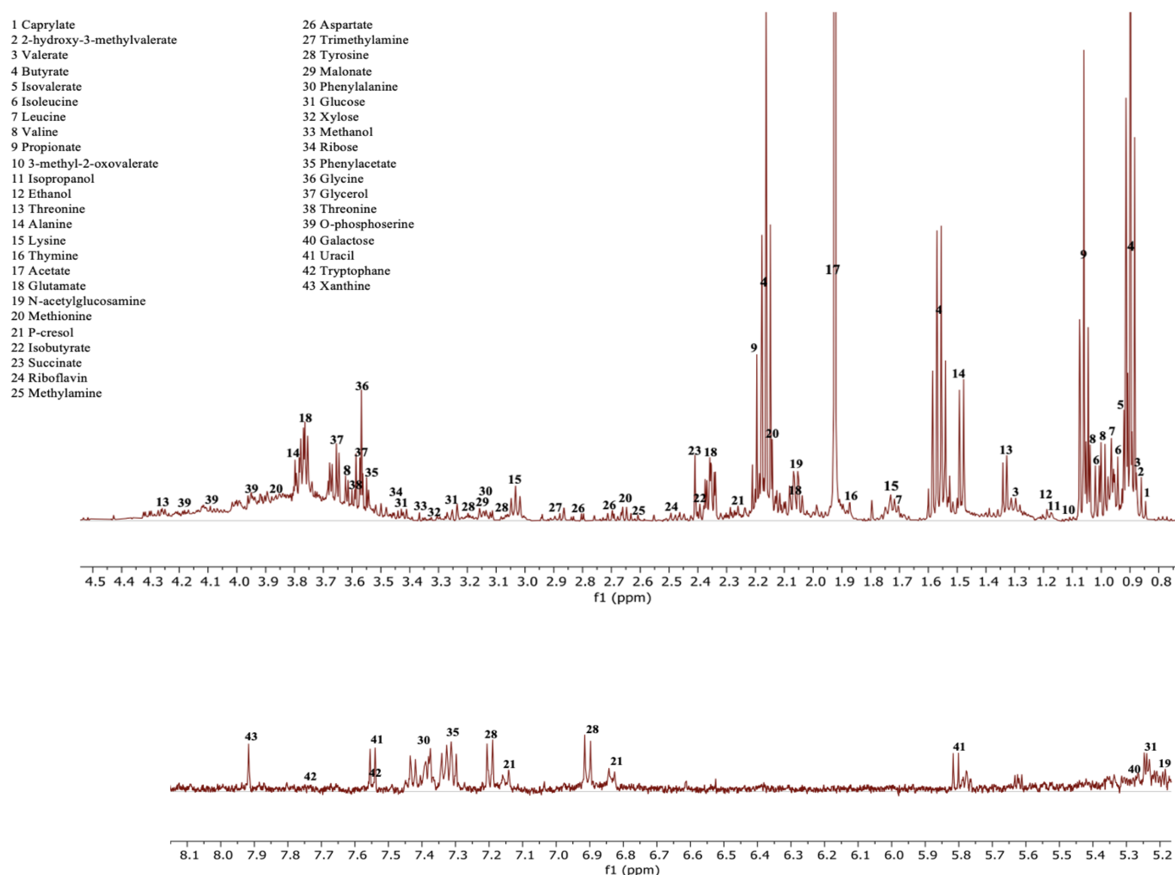

Figure 5 Representative ^{1}H NMR spectrum.

Furthermore, PCA scores plot illustrated in Figure 6 provides insight into the differences in metabolite profiles between the T1D-predisposed group and the control group. The x-axis (PC1) captures a significant proportion (23.2 %) of the data variance, while the y-axis (PC2) contributes to this variation to a lesser extent (11.6 %). The distance between points representing the two groups (0; 1) indicates the degree of difference in their metabolic profiles. Samples that are closer together suggest greater similarity within each group in terms of their metabolite profiles. Samples that are farther apart may exhibit distinct metabolite profiles, indicating potential differences between the two groups. Based on the provided PCA, the observation suggests that there is limited discrimination between the T1D-predisposed group and the control group based on their metabolite profiles, indicating a lack of clear distinction between the two groups based on their metabolite profiles.

The loadings plot (Figure 7) illustrates the contribution of metabolites to the observed differences in the metabolomics data. Each point on the plot represents a metabolite, with its position and direction indicating its influence on Loading 1 and Loading 2, the principal components.

Metabolites contributing to the separation between the T1D-predisposed group and the control group were identified: Metabolites with the highest positive loading values on Loading 1 include methionine (0.147), glutamate (0.144), lactate + threonine (0.143), and thymine (0.143). Metabolites with the highest negative loading values on Loading 1 include succinate (-0.099), glucose (-0.075), N,N-Dimethylglycine (-0.058), and butyrate (-0.052) Examining Loading 2, metabolites like glycine (0.167), valine (0.158), phenylalanine (0.152), and tyrosine (0.148) exhibit the highest positive loadings. In contrast, metabolites 2-Hydroxy-3-methylvalerate (-0.171), caprylate (-0.145), isovalerate (-0.145), and valerate (-0.141), exhibit highest negative loadings on Loading 2.

The spatial distribution of metabolites on the loading plot suggests clustering based on shared metabolic pathways, especially those related to proteolytic and saccharolytic processes, which could potentially contribute to this group differentiation. Metabolites on the right side of the graph are predominantly associated with proteolytic action. Methionine, glutamate, and threonine are among the 20 proteinogenic amino acids that are incorporated biosynthetically into proteins (Ayon 2020). Thus, they arise as a result of protein catabolism, wherein proteins are broken down into their constituent amino acids (Moran 2016). Conversely, metabolites on the left side such as glucose, succinate, and butyrate (excluding N,N-dimethylglycine) are predominantly linked with saccharolytic action. They function as end products of carbohydrate digestion (glucose) (Wong & Jenkins 2007) or microbial saccharolytic fermentation (succinate, butyrate) (Hernández et al. 2021). All of these metabolites play significant roles in energy metabolism (Remesar & Alemany 2020; Zhang & Lang 2023; Singh et al. 2023). Additionally, metabolites positioned toward the upper side are all proteinogenic amino acids (glycine, valine, phenylalanine, tyrosine), thus emerging during proteolysis. Consequently, metabolites toward the lower side (valerate, isovalerate, caprylate, 2-hydroxy-3-methylvalerate) comprise a diverse group of fatty acids. They occur naturally and originate from various dietary sources, including caprylate from breastmilk or coconuts and isovalerate from plant and essential oils. Valerate, commonly found in human faeces, is produced by gut microbiota, primarily by Clostridia species. Similarly, 2-hydroxy-3methylvalerate is present in urine and blood of healthy individuals (Wishart et al. 2007; Wishart et al. 2009). Therefore, accurately categorising these metabolites solely into proteolytic or saccharolytic pathways presents significant challenges, as they overlap in both pathways (Hernández et al. 2021).

In conclusion, metabolites illustrated in this PCA Scores plot that are involved in proteolytic and saccharolytic pathways tend to group together, thereby contributing slightly to

the difference of 95% confidence intervals of the T1D-predisposed group and the control group.

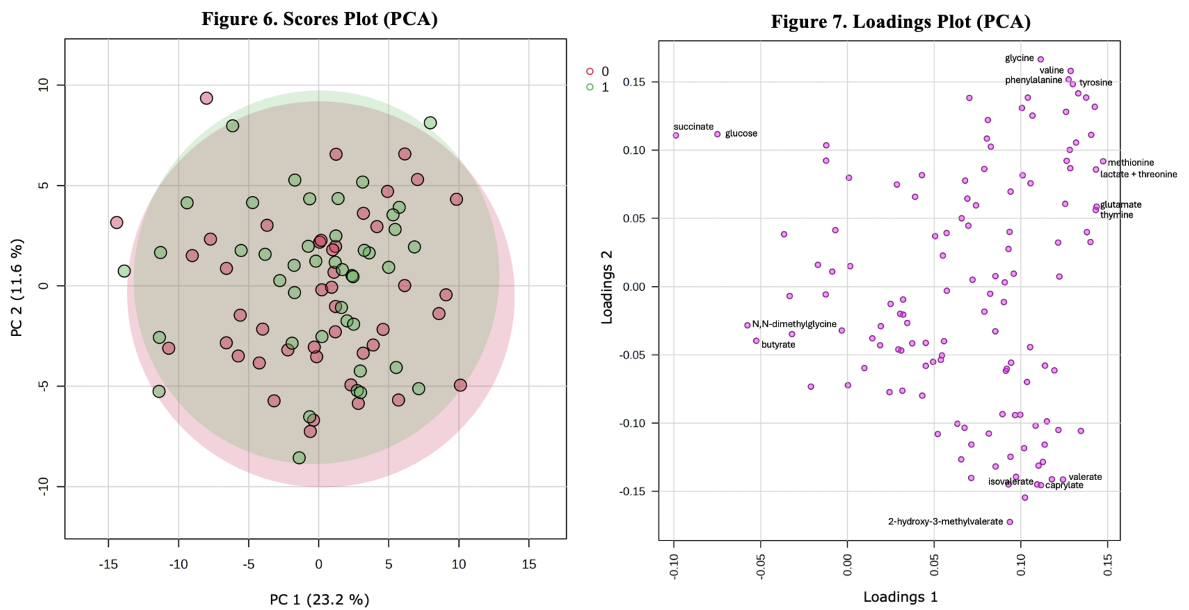

Figure 6 Scores plot of Principal component analysis (PCA) for faecal metabolomic profile of T1D-predisposed group (1; red) vs. Control group (0; green).

Figure 7 Metabolite loadings plot of principal component analysis for faecal metabolomic profile of T1D-predisposed group vs. control group. Each dot represents one metabolite, notable metabolites are annotated.

After the comparison of all metabolites, 3-(3-hydroxyphenyl)propionic acid showed statistically different abundance between the T1D-predisposed group (1; green) and the control group (0; red). Statistical significance ($p < 0.05$) of this metabolite´s abundance was determined using raw data within t-test. The box-plot (Figure 8) illustrates the distribution of 3-(3-hydroxyphenyl)propionic acid concentrations (mg/g) in these two groups (0; 1). Each box represents the interquartile range (IQR) where the majority of the metabolite abundance falls, with the median concentration denoted by the central line. 3-(3-hydroxyphenyl)propionic acid concentration in the T1D-predisposed group was found to be higher than that in the control group. This suggests that a greater proportion of samples in the control group has the concentration of the metabolite that closely resemble each other, contributing to reduced variability within this group. Conversely, the control group displays a

slightly narrower distribution of metabolite abundance, centred around a lower median and average concentrations than the T1D-predisposed group, which exhibits a wider spread of abundance values and has an elevated median. In contrast, the T1D-predisposed group displayed greater variability in metabolite concentrations, as depicted by the wider box-plot. 3-(3-hydroxyphenyl)propionic acid belongs to the phenol class, characterised by a benzene ring with a hydroxyl group (Figure 9). Interestingly, some of the structurally similar compounds, such as 4-OH phenylacetic acid, phenylacetic or phenylpropionic acid can also be formed by the degradation of aromatic acids, such as of phenylacetate and tyrosine (Russell et al. 2013). However, this is not the case of 3-OH derivatives, which are known to originate from 3-hydroxy precursors, such as plant phenolics (Havlik et al. 2020). Moreover, 3-(3-hydroxyphenyl)propionic acid is probably formed through the degradation of caffeic acid (Konishi & Kobayashi 2004) and other phenolic compounds, primarily by gut microbiota Manso et al. 2009). Specific gut bacteria contribute to its production, associated with the consumption of whole grains (Koistinen 2019). The observed significance of this metabolite may be hypothetically attributed, at least in part, to variations in dietary patterns within the T1D-predisposed group. 3-(3-hydroxyphenyl)propionic acid originates from phenylpropanoic acid, derived from the breakdown of lignin and other plant-based compounds (Manso et al. 2009). Lignin, found abundantly in whole-grain products and cereals, is a major component of insoluble fiber (P. & Joye 2020). However, most high-fiber foods contain varying amounts of both soluble and insoluble fiber (Barber et al. 2020). Furthermore, the metabolic pathway leading to the formation of 3-(3-hydroxyphenyl)propionic acid involves bacterial activity (Rowland et al. 2018). Key bacterial genera such as *Clostridium*, *Escherichia*, and *Eubacterium* play crucial roles in breaking down dietary fiber and complex carbohydrates, ultimately resulting in the production of this metabolite (Rowland et al. 2018). Moreover, various studies, such as a study by Mokhatari et al. (2021) have highlighted an increased presence of certain microbial genera, including *Clostridium* and *Escherichia*, in T1D individuals (Mokhtari et al. 2021). Hence, the influence of gut microbiota on 3-(3-hydroxyphenyl)propionic acid levels in the T1D-predisposed group remains a subject of debate. Nevertheless, as previously mentioned, additional research is needed to clarify a direct association between gut microbiota composition, this metabolite, and its potential implications for T1D.

Figure 8. 3-(3-hydroxyphenyl)propionic acid (p-value 0.027817)

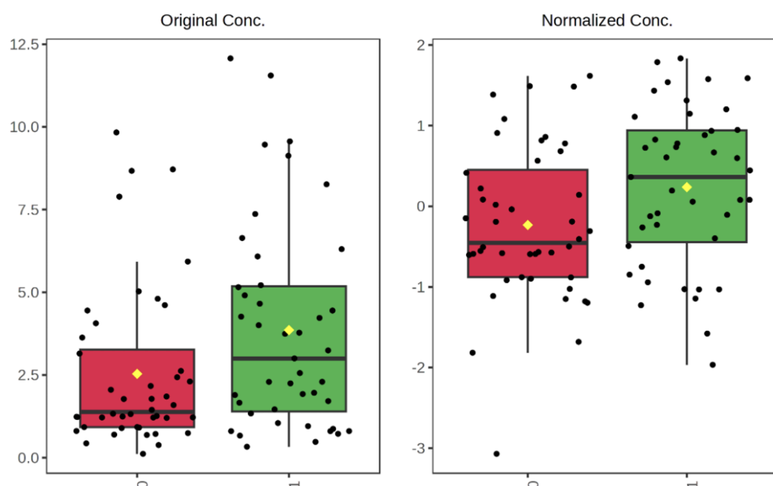

Figure 9. 3-(3-hydroxyphenyl)propionic acid skeletal formula

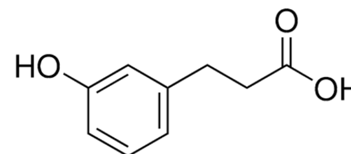

Figure 8 3-(3-hydroxyphenyl)propionic acid concentration (mg/g) box-plot in T1D-predisposed group (1; green) and the control group (0; red) using raw and normalised data.

Figure 9 3-(3-hydroxyphenyl)propionic acid skeletal formula.

The heatmap (Figure 10) illustrates correlations among samples within both the T1D-predisposed group (1; green) and the control group (0; purple) indicating metabolite abundance. Each column represents a sample, while each row represents a metabolite. The colour scale indicates metabolite abundance, with warmer colours indicating higher abundance (positive correlations) and cooler colours indicating lower abundance (negative correlations). Distinct patterns and clusters of metabolite abundance emerge across different samples within both groups (0; 1), potentially suggesting the involvement of these metabolites in different biological pathways. Notably, some samples exhibit increased abundance (cooler colours) of several proteinogenic amino acids (alanine, phenylalanine, tyrosine, isoleucine, leucine, valine, glycine, threonine, glutamate, methionine), suggesting protein catabolism (proteolytic actions) (Moran 2016), while other samples show their decreased abundance. Additionally, some samples collected from the same participant in different seasons (winter and summer months) cluster together, indicating individual consistency. However, no significant seasonal differences in metabolite abundance are observed. Here, speculations arise regarding potential variations in metabolite abundance within the same samples collected in different seasons, assuming data on participants' dietary intake were available. Differences in metabolite abundance are evident in samples from both groups (0; 1)

suggesting that metabolite abundance is not solely influenced by the group to which a participant belongs. These observations may be potentially attributed to shared metabolic pathways or processes that these metabolites are involved in, reflecting the complex nature of biological systems.

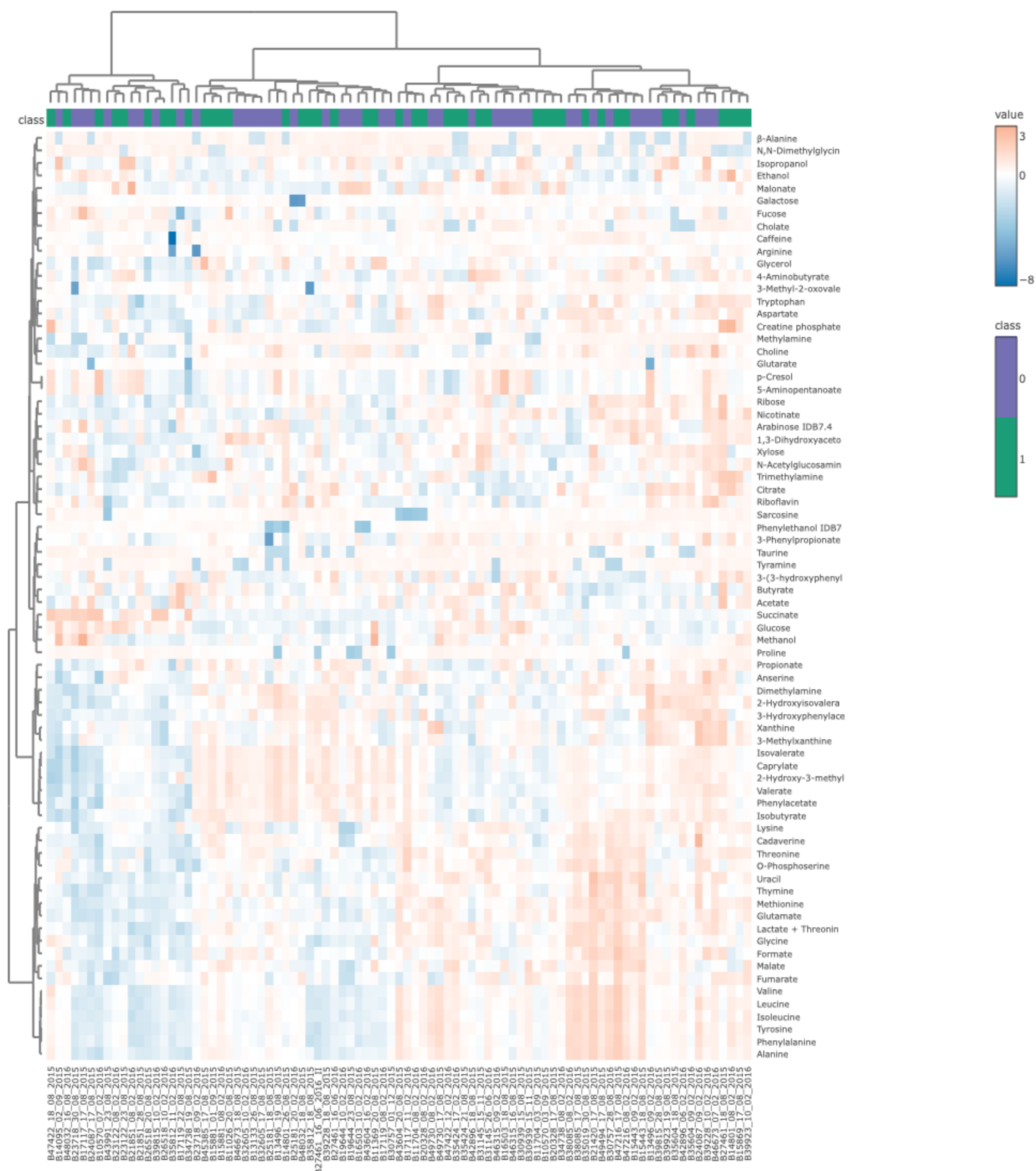

Figure 10 Heatmap based on metabolite % relative abundance.

6 Conclusion

Our case-control study has effectively demonstrated the potential of ^{1}H NMR spectroscopy in differentiating faecal metabolic profiles between children genetically predisposed to T1D and healthy controls. Among the 74 identified features, the overall metabolic profile showed marginal differences between the T1D-predisposed group and the control group. A significant finding was the elevated levels of 3-(3-hydroxyphenyl)propionic acid in the T1D-predisposed group, suggesting distinct metabolic pathway activities potentially influenced by variations in gut microbiota composition and dietary patterns. This study underscores the utility of ^{1}H NMR spectral analysis as a powerful tool in biological research for exploring metabolic alterations associated with susceptibility to T1D. The identification of specific faecal metabolites highlights their potential as early biomarkers for T1D. These biomarkers could significantly assist in the early detection and formulation of intervention strategies for paediatric populations at risk of developing T1D. Moving forward, future research should focus on integrating comprehensive dietary data and detailed microbiota analyses to deepen our understanding of the interrelationships between diet, microbiota composition, and metabolic profiles in T1D. Such thorough investigations are critical as they could expand our knowledge of the pathophysiological mechanisms underlying T1D, providing vital insights that enhance early diagnosis and proactive management of T1D in young individuals.

7 Bibliography

Achenbach, P., Warncke, K., Reiter, J., Naserke, H. E., Williams, A. J. K., Bingley, P. J., Bonifacio, E., & Ziegler, A.-G. (2004). Stratification of Type 1 Diabetes Risk on the Basis of Islet Autoantibody Characteristics. *Diabetes*, *53*(2), 384–392. https://doi.org/10.2337/diabetes.53.2.384

Aiello, L. P., Gardner, T. W., King, G. L., Blankenship, G., Cavallerano, J. D., Ferris, F. L., & Klein, R. (1998). Diabetic Retinopathy. *Diabetes Care*, *21*(1), 143–156. https://doi.org/10.2337/diacare.21.1.143

Akil, A. A.-S., Yassin, E., Al-Maraghi, A., Aliyev, E., Al-Malki, K., & Fakhro, K. A. (2021). Diagnosis and treatment of type 1 diabetes at the dawn of the personalized medicine era. *Journal of Translational Medicine*, *19*(1), 137. https://doi.org/10.1186/s12967-021-02778-6

Allen, L. A., & Dayan, C. M. (2021). Immunotherapy for type 1 diabetes. *British Medical Bulletin*, *140*(1), 76–90. https://doi.org/10.1093/bmb/ldab027

Antcliffe, D., & Gordon, A. C. (2016). Metabonomics and intensive care. *Critical Care*, *20*(1), 68. https://doi.org/10.1186/s13054-016-1222-8

Antvorskov, J. C., Josefsen, K., Engkilde, K., Funda, D. P., & Buschard, K. (2014). Dietary gluten and the development of type 1 diabetes. *Diabetologia*, *57*(9), 1770–1780. https://doi.org/10.1007/s00125-014-3265-1

Antvorskov, J. C., Morgen, C. S., Buschard, K., Jess, T., Allin, K. H., & Josefsen, K. (2020). Antibiotic treatment during early childhood and risk of type 1 diabetes in children: A national birth cohort study. *Pediatric Diabetes*, *21*(8), 1457–1464. https://doi.org/10.1111/pedi.13111

Arneth, B., Arneth, R., & Shams, M. (2019). Metabolomics of Type 1 and Type 2 Diabetes. *International Journal of Molecular Sciences*, *20*(10), 2467. https://doi.org/10.3390/ijms20102467

Atkinson, M. A., Bowman, M. A., Kao, K.-J., Campbell, L., Dush, P. J., Shah, S. C., Simell, O., & Maclaren, N. K. (1993). Lack of Immune Responsiveness to Bovine Serum Albumin in Insulin-Dependent Diabetes. *New England Journal of Medicine*, *329*(25), 1853–1858. https://doi.org/10.1056/NEJM199312163292505

Atkinson, M. A., & Eisenbarth, G. S. (2001). Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *The Lancet*, *358*(9277), 221–229. https://doi.org/10.1016/S0140-6736(01)05415-0

Banday, M. Z., Sameer, A. S., & Nissar, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*, *10*(04), 174–188. https://doi.org/10.4103/ajm.ajm_53_20

Bansal, V., Kalita, J., & Misra, U. K. (2006). Diabetic neuropathy. *Postgraduate Medical Journal*, *82*(964), 95–100. https://doi.org/10.1136/pgmj.2005.036137

Barber, T. M., Kabisch, S., Pfeiffer, A. F. H., & Weickert, M. O. (2020). The Health Benefits of Dietary Fibre. *Nutrients*, *12*(10), 3209. https://doi.org/10.3390/nu12103209

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Nierras, C., Plagnol, V., Pociot, F., Schuilenburg, H., Smyth, D. J., Stevens, H., Todd, J. A., Walker, N. M., & Rich, S. S. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nature Genetics*, *41*(6), 703–707. https://doi.org/10.1038/ng.381

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0

Bertoluci, M. C. (2015). Endothelial dysfunction as a predictor of cardiovascular disease in type 1 diabetes. *World Journal of Diabetes*, *6*(5), 679. https://doi.org/10.4239/wjd.v6.i5.679

Bervoets, L., Massa, G., Guedens, W., Louis, E., Noben, J.-P., & Adriaensens, P. (2017). Metabolic profiling of type 1 diabetes mellitus in children and adolescents: a case–control study. *Diabetology & Metabolic Syndrome*, *9*(1), 48. https://doi.org/10.1186/s13098-017-0246-9

Bodaghi, A., Fattahi, N., & Ramazani, A. (2023). Biomarkers: Promising and valuable tools towards diagnosis, prognosis and treatment of Covid-19 and other diseases. *Heliyon*, *9*(2), e13323. https://doi.org/10.1016/j.heliyon.2023.e13323

Bodin, J., Stene, L. C., & Nygaard, U. C. (2015). Can Exposure to Environmental Chemicals Increase the Risk of Diabetes Type 1 Development? *BioMed Research International*, *2015*, 1–19. https://doi.org/10.1155/2015/208947

Bokulich, N. A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., D. Lieber, A., Wu, F., Perez-Perez, G. I., Chen, Y., Schweizer, W., Zheng, X., Contreras, M., Dominguez-Bello, M. G., & Blaser, M. J. (2016). Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science Translational Medicine*, *8*(343). https://doi.org/10.1126/scitranslmed.aad7121

Bonifacio, E. (2015). Predicting Type 1 Diabetes Using Biomarkers. *Diabetes Care*, *38*(6), 989–996. https://doi.org/10.2337/dc15-0101

Bonifacio, E., & Achenbach, P. (2019). Birth and coming of age of islet autoantibodies. *Clinical and Experimental Immunology*, *198*(3), 294–305. https://doi.org/10.1111/cei.13360

Butalia, S., Kaplan, G. G., Khokhar, B., & Rabi, D. M. (2016). Environmental Risk Factors and Type 1 Diabetes: Past, Present, and Future. *Canadian Journal of Diabetes*, *40*(6), 586–593. https://doi.org/10.1016/j.jcjd.2016.05.002

Cabello-Olmo, M., Araña, M., Radichev, I., Smith, P., Huarte, E., & Barajas, M. (2019). New Insights into Immunotherapy Strategies for Treating Autoimmune Diabetes. *International Journal of Molecular Sciences*, *20*(19), 4789. https://doi.org/10.3390/ijms20194789

Chai, J., Sun, Z., & Xu, J. (2022). A Contemporary Insight of Metabolomics Approach for Type 1 Diabetes: Potential for Novel Diagnostic Targets. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *Volume 15*, 1605–1625. https://doi.org/10.2147/DMSO.S357007

Chiang, J. L., Maahs, D. M., Garvey, K. C., Hood, K. K., Laffel, L. M., Weinzimer, S. A., Wolfsdorf, J. I., & Schatz, D. (2018). Type 1 Diabetes in Children and Adolescents: A Position Statement by the American Diabetes Association. *Diabetes Care*, *41*(9), 2026–2044. https://doi.org/10.2337/dci18-0023

Choi, H. S., Kim, J. T., Seo, J.-Y., Linkov, F., Shubnikov, E., & Lee, H. K. (2021). Correlation between total air pollutant emissions and incidence of type 1 diabetes in the Russian Federation. *Clinical and Experimental Pediatrics*, *64*(10), 525–530. https://doi.org/10.3345/cep.2020.01501

Chrousos, G. P. (1992). The Concepts of Stress and Stress System Disorders. *JAMA*, *267*(9), 1244. https://doi.org/10.1001/jama.1992.03480090092034

Colli, M. L., Szymczak, F., & Eizirik, D. L. (2020). Molecular Footprints of the Immune Assault on Pancreatic Beta Cells in Type 1 Diabetes. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/fendo.2020.568446

Colom, C., Rull, A., Sanchez-Quesada, J. L., & Pérez, A. (2021). Cardiovascular Disease in Type 1 Diabetes Mellitus: Epidemiology and Management of Cardiovascular Risk. *Journal of Clinical Medicine*, *10*(8), 1798. https://doi.org/10.3390/jcm10081798

Cox, L. M., & Blaser, M. J. (2015). Antibiotics in early life and obesity. *Nature Reviews Endocrinology*, *11*(3), 182–190. https://doi.org/10.1038/nrendo.2014.210

Crook, A. A., & Powers, R. (2020). Quantitative NMR-Based Biomedical Metabolomics: Current Status and Applications. *Molecules*, *25*(21), 5128. https://doi.org/10.3390/molecules25215128

Dahlquist. (1999). Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. *Diabetologia*, *42*(1), 51–54. https://doi.org/10.1007/s001250051112

Damiani, C., Gaglio, D., Sacco, E., Alberghina, L., & Vanoni, M. (2020). Systems metabolomics: from metabolomic snapshots to design principles. *Current Opinion in Biotechnology*, *63*, 190–199. https://doi.org/10.1016/j.copbio.2020.02.013

de Goffau, M. C., Luopajärvi, K., Knip, M., Ilonen, J., Ruohtula, T., Härkönen, T., Orivuori, L., Hakala, S., Welling, G. W., Harmsen, H. J., & Vaarala, O. (2013). Fecal Microbiota Composition Differs Between Children With β-Cell Autoimmunity and Those Without. *Diabetes*, *62*(4), 1238–1244. https://doi.org/10.2337/db12-0526

Dedrick, S., Sundaresh, B., Huang, Q., Brady, C., Yoo, T., Cronin, C., Rudnicki, C., Flood, M., Momeni, B., Ludvigsson, J., & Altindis, E. (2020). The Role of Gut Microbiota and Environmental Factors in Type 1 Diabetes Pathogenesis. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/fendo.2020.00078

Del Chierico, F., Rapini, N., Deodati, A., Matteoli, M. C., Cianfarani, S., & Putignani, L. (2022). Pathophysiology of Type 1 Diabetes and Gut Microbiota Role. *International Journal of Molecular Sciences*, *23*(23), 14650. https://doi.org/10.3390/ijms232314650

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340. https://doi.org/10.1194/jlr.R036012

Dermawan, D., & Kenichi Purbayanto, M. A. (2022). An overview of advancements in closed-loop artificial pancreas system. *Heliyon*, *8*(11), e11648. https://doi.org/10.1016/j.heliyon.2022.e11648

Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., & Raptis, S. A. (2011). Insulin effects in muscle and adipose tissue. *Diabetes Research and Clinical Practice*, *93*, S52–S59. https://doi.org/10.1016/S0168-8227(11)70014-6

Dodd, G. T., & Tiganis, T. (2017). Insulin action in the brain: Roles in energy and glucose homeostasis. *Journal of Neuroendocrinology*, *29*(10). https://doi.org/10.1111/jne.12513

Dronavalli, S., Duka, I., & Bakris, G. L. (2008). The pathogenesis of diabetic nephropathy. *Nature Clinical Practice Endocrinology & Metabolism*, *4*(8), 444–452. https://doi.org/10.1038/ncpendmet0894

Du, C., Whiddett, R. O., Buckle, I., Chen, C., Forbes, J. M., & Fotheringham, A. K. (2022). Advanced Glycation End Products and Inflammation in Type 1 Diabetes Development. *Cells*, *11*(21), 3503. https://doi.org/10.3390/cells11213503

Dunn, W. B., Broadhurst, D. I., Atherton, H. J., Goodacre, R., & Griffin, J. L. (2011). Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem. Soc. Rev.*, *40*(1), 387–426. https://doi.org/10.1039/B906712B

Elhag, D. A., Kumar, M., & Al Khodor, S. (2020). Exploring the Triple Interaction between the Host Genome, the Epigenome, and the Gut Microbiome in Type 1 Diabetes. *International Journal of Molecular Sciences*, *22*(1), 125. https://doi.org/10.3390/ijms22010125

Ellinger, J., A. Chylla, R., L. Ulrich, E., & L. Markley, J. (2012). Databases and Software for NMR-Based Metabolomics. *Current Metabolomics*, *1*(1), 28–40. https://doi.org/10.2174/2213235X11301010028

Emwas, A.-H., Roy, R., McKay, R. T., Tenori, L., Saccenti, E., Gowda, G. A. N., Raftery, D., Alahmari, F., Jaremko, L., Jaremko, M., & Wishart, D. S. (2019). NMR Spectroscopy for Metabolomics Research. *Metabolites*, *9*(7), 123. https://doi.org/10.3390/metabo9070123

Erdem, N., Montero, E., & Roep, B. O. (2021). Breaking and restoring immune tolerance to pancreatic beta-cells in type 1 diabetes. *Current Opinion in Endocrinology, Diabetes & Obesity*, *28*(4), 397–403. https://doi.org/10.1097/MED.0000000000000646

Esposito, S., Toni, G., Tascini, G., Santi, E., Berioli, M. G., & Principi, N. (2019). Environmental Factors Associated With Type 1 Diabetes. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00592

Ewald, J. D., Zhou, G., Lu, Y., Kolic, J., Ellis, C., Johnson, J. D., Macdonald, P. E., & Xia, J. (2024). Web-based multi-omics integration using the Analyst software suite. *Nature Protocols*. https://doi.org/10.1038/s41596-023-00950-4

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology*, *19*(1), 55–71. https://doi.org/10.1038/s41579-020-0433-9

Frati, G., Schirone, L., Chimenti, I., Yee, D., Biondi-Zoccai, G., Volpe, M., & Sciarretta, S. (2017). An overview of the inflammatory signalling mechanisms in the myocardium underlying the development of diabetic cardiomyopathy. *Cardiovascular Research*, *113*(4), 378–388. https://doi.org/10.1093/cvr/cvx011

Friedrich, N. (2012). Metabolomics in diabetes research. *Journal of Endocrinology*, *215*(1), 29–42. https://doi.org/10.1530/JOE-12-0120

Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., Novelo, L. L., Casella, G., Drew, J. C., Ilonen, J., Knip, M., Hyöty, H., Veijola, R., Simell, T., Simell, O., Neu, J., Wasserfall, C. H., Schatz, D., Atkinson, M. A., & Triplett, E. W. (2011). Toward defining the autoimmune microbiome for type 1 diabetes. *The ISME Journal*, *5*(1), 82–91. https://doi.org/10.1038/ismej.2010.92

Glatthaar, C., Whittall, D. E., Welborn, T. A., Gibson, M. J., Brooks, B. H., Ryan, M. M. P., & Byrne, G. C. (1988). Diabetes in Western Australian children: descriptive epidemiology. *Medical Journal of Australia*, *148*(3), 117–123. https://doi.org/10.5694/j.1326-5377.1988.tb112770.x

Gong, B., Yang, W., Xing, Y., Lai, Y., & Shan, Z. (2024). Global, regional, and national burden of type 1 diabetes in adolescents and young adults. *Pediatric Research*. https://doi.org/10.1038/s41390-024-03107-5

Gouda, W., Mageed, L., Abd El Dayem, S. M., Ashour, E., & Afify, M. (2018). Evaluation of pro-inflammatory and anti-inflammatory cytokines in type 1 diabetes mellitus. *Bulletin of the National Research Centre*, *42*(1), 14. https://doi.org/10.1186/s42269-018-0016-3

Graham D. O, Wang F, Gregory G. A, Maniam J. (2022). International Diabetes Federation: Type 1 diabetes estimates in children and adults. Available from https://diabetesatlas.org (accessed March 18).

Guasch-Ferré, M., Hruby, A., Toledo, E., Clish, C. B., Martínez-González, M. A., Salas-Salvadó, J., & Hu, F. B. (2016). Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis. *Diabetes Care*, *39*(5), 833–846. https://doi.org/10.2337/dc15-2251

Haller, M. J., & Schatz, D. A. (2016). The DIPP project: 20 years of discovery in type 1 diabetes. *Pediatric Diabetes*, *17*, 5–7. https://doi.org/10.1111/pedi.12398
Hartman, I. (2008). Insulin Analogs: Impact on Treatment Success, Satisfaction, Quality of Life, and Adherence. *Clinical Medicine & Research*, *6*(2), 54–67. https://doi.org/10.3121/cmr.2008.793
Hathout, E. H., Beeson, W. L., Nahab, F., Rabadi, A., Thomas, W., & Mace, J. W. (2002). Role of exposure to air pollutants in the development of type 1 diabetes before and after 5 yr of age. *Pediatric Diabetes*, *3*(4), 184–188. https://doi.org/10.1034/j.1399-5448.2002.30403.x
Hatzakis, E. (2019). Nuclear Magnetic Resonance (NMR) Spectroscopy in Food Science: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 189–220. https://doi.org/10.1111/1541-4337.12408
Havlik, J., Marinello, V., Gardyne, A., Hou, M., Mullen, W., Morrison, D. J., Preston, T., Combet, E., & Edwards, C. A. (2020). Dietary Fibres Differentially Impact on the Production of Phenolic Acids from Rutin in an In Vitro Fermentation Model of the Human Gut Microbiota. *Nutrients*, *12*(6), 1577. https://doi.org/10.3390/nu12061577
Hernández, M. A. G., Canfora, E. E., & Blaak, E. E. (2021). Faecal microbial metabolites of proteolytic and saccharolytic fermentation in relation to degree of insulin resistance in adult individuals. *Beneficial Microbes*, *12*(3), 259–266. https://doi.org/10.3920/BM2020.0179
Herold, K. C., Usmani-Brown, S., Ghazi, T., Lebastchi, J., Beam, C. A., Bellin, M. D., Ledizet, M., Sosenko, J. M., Krischer, J. P., & Palmer, J. P. (2015). β Cell death and dysfunction during type 1 diabetes development in at-risk individuals. *Journal of Clinical Investigation*, *125*(3), 1163–1173. https://doi.org/10.1172/JCI78142
Honkanen, H., Oikarinen, S., Nurminen, N., Laitinen, O. H., Huhtala, H., Lehtonen, J., Ruokoranta, T., Hankaniemi, M. M., Lecouturier, V., Almond, J. W., Tauriainen, S., Simell, O., Ilonen, J., Veijola, R., Viskari, H., Knip, M., & Hyöty, H. (2017). Detection of enteroviruses in stools precedes islet autoimmunity by several months: possible evidence for slowly operating mechanisms in virus-induced autoimmunity. *Diabetologia*, *60*(3), 424–431. https://doi.org/10.1007/s00125-016-4177-z
Hovorka, R. (2006). Continuous glucose monitoring and closed-loop systems. *Diabetic Medicine*, *23*(1), 1–12. https://doi.org/10.1111/j.1464-5491.2005.01672.x
Howard, S. G. (2019). Exposure to environmental chemicals and type 1 diabetes: an update. *Journal of Epidemiology and Community Health*, *73*(6), 483–488. https://doi.org/10.1136/jech-2018-210627
Huang, M., Chen, W., Wang, M., Huang, Y., Liu, H., Ming, Y., Chen, Y., Tang, Z., & Jia, B. (2023). Advanced Delivery Strategies for Immunotherapy in Type I Diabetes Mellitus. *BioDrugs*, *37*(3), 331–352. https://doi.org/10.1007/s40259-023-00594-6
Hviid, A., & Svanstrom, H. (2009). Antibiotic Use and Type 1 Diabetes in Childhood. *American Journal of Epidemiology*, *169*(9), 1079–1084. https://doi.org/10.1093/aje/kwp038
Ikegami, H., Noso, S., Babaya, N., & Kawabata, Y. (2011). Genetics and pathogenesis of type 1 diabetes: prospects for prevention and intervention. *Journal of Diabetes Investigation*, *2*(6), 415–420. https://doi.org/10.1111/j.2040-1124.2011.00176.x
Ilonen, J., Laine, A., Kiviniemi, M., Härkönen, T., Lempainen, J., & Knip, M. (2022). Associations between deduced first islet specific autoantibody with sex, age at diagnosis and genetic risk factors in young children with type 1 diabetes. *Pediatric Diabetes*, *23*(6), 693–702. https://doi.org/10.1111/pedi.13340
Infante, M., Ricordi, C., Sanchez, J., Clare-Salzler, M. J., Padilla, N., Fuenmayor, V., Chavez, C., Alvarez, A., Baidal, D., Alejandro, R., Caprio, M., & Fabbri, A. (2019). Influence of

Vitamin D on Islet Autoimmunity and Beta-Cell Function in Type 1 Diabetes. *Nutrients*, *11*(9), 2185. https://doi.org/10.3390/nu11092185
Ingrosso, D. M. F., Primavera, M., Samvelyan, S., Tagi, V. M., & Chiarelli, F. (2023). Stress and Diabetes Mellitus: Pathogenetic Mechanisms and Clinical Outcome. *Hormone Research in Paediatrics*, *96*(1), 34–43. https://doi.org/10.1159/000522431
Isaacs, S. R., Foskett, D. B., Maxwell, A. J., Ward, E. J., Faulkner, C. L., Luo, J. Y. X., Rawlinson, W. D., Craig, M. E., & Kim, K. W. (2021). Viruses and Type 1 Diabetes: From Enteroviruses to the Virome. *Microorganisms*, *9*(7), 1519. https://doi.org/10.3390/microorganisms9071519
J. Ayon, N. (2020). Features, roles and chiral analyses of proteinogenic amino acids. *AIMS Molecular Science*, *7*(3), 229–268. https://doi.org/10.3934/molsci.2020011
Jacob, D., Deborde, C., Lefebvre, M., Maucourt, M., & Moing, A. (2017). NMRProcFlow: a graphical and interactive tool dedicated to 1D spectra processing for NMR-based metabolomics. *Metabolomics*, *13*(4), 36. https://doi.org/10.1007/s11306-017-1178-y
Jandhyala, S. M. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*, *21*(29), 8787. https://doi.org/10.3748/wjg.v21.i29.8787
Janež, A., Guja, C., Mitrakou, A., Lalic, N., Tankova, T., Czupryniak, L., Tabák, A. G., Prazny, M., Martinka, E., & Smircic-Duvnjak, L. (2020). Insulin Therapy in Adults with Type 1 Diabetes Mellitus: a Narrative Review. *Diabetes Therapy*, *11*(2), 387–409. https://doi.org/10.1007/s13300-019-00743-7
Jin, Q., Black, A., Kales, S. N., Vattem, D., Ruiz-Canela, M., & Sotos-Prieto, M. (2019). Metabolomics and Microbiomes as Potential Tools to Evaluate the Effects of the Mediterranean Diet. *Nutrients*, *11*(1), 207. https://doi.org/10.3390/nu11010207
Jin, Y., & She, J.-X. (2012). Novel Biomarkers in Type 1 Diabetes. *The Review of Diabetic Studies*, *9*(4), 224–235. https://doi.org/10.1900/RDS.2012.9.224
Jørgenrud, B., Stene, L. C., Tapia, G., Bøås, H., Pepaj, M., Berg, J. P., Thorsby, P. M., Orešič, M., Hyötyläinen, T., & Rønningen, K. S. (2017). Longitudinal plasma metabolic profiles, infant feeding, and islet autoimmunity in the MIDIA study. *Pediatric Diabetes*, *18*(2), 111–119. https://doi.org/10.1111/pedi.12360
Jun, H.-S., & Yoon, J.-W. (2003). A new look at viruses in type 1 diabetes. *Diabetes/Metabolism Research and Reviews*, *19*(1), 8–31. https://doi.org/10.1002/dmrr.337
Kahanovitz, L., Sluss, P. M., & Russell, S. J. (2017). Type 1 Diabetes—A Clinical Perspective. *Point of Care: The Journal of Near-Patient Testing & Technology*, *16*(1), 37–40. https://doi.org/10.1097/POC.0000000000000125
Kahn, S. E., Chen, Y.-C., Esser, N., Taylor, A. J., van Raalte, D. H., Zraika, S., & Verchere, C. B. (2021). The β Cell in Diabetes: Integrating Biomarkers With Functional Measures. *Endocrine Reviews*, *42*(5), 528–583. https://doi.org/10.1210/endrev/bnab021
Karjalainen, J., Martin, J. M., Knip, M., Ilonen, J., Robinson, B. H., Savilahti, E., Akerblom, H. K., & Dosch, H.-M. (1992). A Bovine Albumin Peptide as a Possible Trigger of Insulin-Dependent Diabetes Mellitus. *New England Journal of Medicine*, *327*(5), 302–307. https://doi.org/10.1056/NEJM199207303270502
Kawasaki, E. (2023). Anti-Islet Autoantibodies in Type 1 Diabetes. *International Journal of Molecular Sciences*, *24*(12), 10012. https://doi.org/10.3390/ijms241210012
Kilkkinen, A., Virtanen, S. M., Klaukka, T., Kenward, M. G., Salkinoja-Salonen, M., Gissler, M., Kaila, M., & Reunanen, A. (2006). Use of antimicrobials and risk of type 1 diabetes in a population-based mother–child cohort. *Diabetologia*, *49*(1), 66–70. https://doi.org/10.1007/s00125-005-0078-2
Kim, H. K., Kostidis, S., & Choi, Y. H. (2018). *NMR Analysis of Fecal Samples* (pp. 317–328). https://doi.org/10.1007/978-1-4939-7592-1_24

Kleinridders, A., Ferris, H. A., Cai, W., & Kahn, C. R. (2014). Insulin Action in Brain Regulates Systemic Metabolism and Brain Function. *Diabetes*, *63*(7), 2232–2243. https://doi.org/10.2337/db14-0568

Koistinen V. M. (2019). Effects of food processing and gut microbial metabolism on whole grain phytochemicals: A metabolomics approach. [Dissertations in Health Sciences]. University of Eastern Finland, Kuopio.

Konishi, Y., & Kobayashi, S. (2004). Microbial Metabolites of Ingested Caffeic Acid Are Absorbed by the Monocarboxylic Acid Transporter (MCT) in Intestinal Caco-2 Cell Monolayers. *Journal of Agricultural and Food Chemistry*, *52*(21), 6418–6424. https://doi.org/10.1021/jf049560y

Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., & de Vos, W. M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nature Communications*, *7*(1), 10410. https://doi.org/10.1038/ncomms10410

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A.-M., Peet, A., Tillmann, V., Pöhö, P., Mattila, I., Lähdesmäki, H., Franzosa, E. A., Vaarala, O., de Goffau, M., Harmsen, H., Ilonen, J., Virtanen, S. M., Clish, C. B., Orešič, M., … Xavier, R. J. (2015). The Dynamics of the Human Infant Gut Microbiome in Development and in Progression toward Type 1 Diabetes. *Cell Host & Microbe*, *17*(2), 260–273. https://doi.org/10.1016/j.chom.2015.01.001

Laiho, J. E., Laitinen, O. H., Malkamäki, J., Puustinen, L., Sinkkonen, A., Pärkkä, J., & Hyöty, H. (2022). Exposomic determinants of immune-mediated diseases. *Environmental Epidemiology*, *6*(3), e212. https://doi.org/10.1097/EE9.0000000000000212

Lamichhane, S., Ahonen, L., Dyrlund, T. S., Siljander, H., Hyöty, H., Ilonen, J., Toppari, J., Veijola, R., Hyötyläinen, T., Knip, M., & Orešič, M. (2018). A longitudinal plasma lipidomics dataset from children who developed islet autoimmunity and type 1 diabetes. *Scientific Data*, *5*(1), 180250. https://doi.org/10.1038/sdata.2018.250

Lamichhane, S., Ahonen, L., Dyrlund, T. S., Dickens, A. M., Siljander, H., Hyöty, H., Ilonen, J., Toppari, J., Veijola, R., Hyötyläinen, T., Knip, M., & Oresic, M. (2019). Cord-Blood Lipidome in Progression to Islet Autoimmunity and Type 1 Diabetes. *Biomolecules*, *9*(1), 33. https://doi.org/10.3390/biom9010033

Lamichhane, S., Kemppainen, E., Trošt, K., Siljander, H., Hyöty, H., Ilonen, J., Toppari, J., Veijola, R., Hyötyläinen, T., Knip, M., & Orešič, M. (2019). Circulating metabolites in progression to islet autoimmunity and type 1 diabetes. *Diabetologia*, *62*(12), 2287–2297. https://doi.org/10.1007/s00125-019-04980-0

Lamichhane, S., Sen, P., Dickens, A. M., Kråkström, M., Ilonen, J., Lempainen, J., Hyöty, H., Lahesmaa, R., Veijola, R., Toppari, J., Hyötyläinen, T., Knip, M., & Orešič, M. (2023). Circulating metabolic signatures of rapid and slow progression to type 1 diabetes in islet autoantibody-positive children. *Frontiers in Endocrinology*, *14*. https://doi.org/10.3389/fendo.2023.1211015

Lebastchi, J., & Herold, K. C. (2012). Immunologic and Metabolic Biomarkers of -Cell Destruction in the Diagnosis of Type 1 Diabetes. *Cold Spring Harbor Perspectives in Medicine*, *2*(6), a007708–a007708. https://doi.org/10.1101/cshperspect.a007708

Leighton, E., Sainsbury, C. A., & Jones, G. C. (2017). A Practical Review of C-Peptide Testing in Diabetes. *Diabetes Therapy*, *8*(3), 475–487. https://doi.org/10.1007/s13300-017-0265-4

Lemas, D. J., Young, B. E., Baker, P. R., Tomczik, A. C., Soderborg, T. K., Hernandez, T. L., de la Houssaye, B. A., Robertson, C. E., Rudolph, M. C., Ir, D., Patinkin, Z. W., Krebs, N. F., Santorico, S. A., Weir, T., Barbour, L. A., Frank, D. N., & Friedman, J. E. (2016).

Alterations in human milk leptin and insulin are associated with early changes in the infant intestinal microbiome. *The American Journal of Clinical Nutrition*, *103*(5), 1291–1300. https://doi.org/10.3945/ajcn.115.126375

Lernmark, Å. (2021). Etiology of Autoimmune Islet Disease: Timing Is Everything. *Diabetes*, *70*(7), 1431–1439. https://doi.org/10.2337/dbi18-0034

Lind, M., Svensson, A.-M., Kosiborod, M., Gudbjörnsdottir, S., Pivodic, A., Wedel, H., Dahlqvist, S., Clements, M., & Rosengren, A. (2014). Glycemic Control and Excess Mortality in Type 1 Diabetes. *New England Journal of Medicine*, *371*(21), 1972–1982. https://doi.org/10.1056/NEJMoa1408214

Liu, X., Liu, R., Zhao, X.-M., & Chen, L. (2013). Detecting early-warning signals of type 1 diabetes and its leading biomolecular networks by dynamical network biomarkers. *BMC Medical Genomics*, *6*(S2), S8. https://doi.org/10.1186/1755-8794-6-S2-S8

Liu, X., & Locasale, J. W. (2017). Metabolomics: A Primer. *Trends in Biochemical Sciences*, *42*(4), 274–284. https://doi.org/10.1016/j.tibs.2017.01.004

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*, *19*(1), 29–41. https://doi.org/10.1111/1462-2920.13589

Lu, J., Liu, J., Li, L., Lan, Y., & Liang, Y. (2020). Cytokines in type 1 diabetes: mechanisms of action and immunotherapeutic targets. *Clinical & Translational Immunology*, *9*(3). https://doi.org/10.1002/cti2.1122

Lu, Y., Pang, Z., & Xia, J. (2023). Comprehensive investigation of pathway enrichment methods for functional interpretation of LC–MS global metabolomics data. *Briefings in Bioinformatics*, *24*(1). https://doi.org/10.1093/bib/bbac553

Lund-Blix, N. A., Dydensborg Sander, S., Størdal, K., Nybo Andersen, A.-M., Rønningen, K. S., Joner, G., Skrivarhaug, T., Njølstad, P. R., Husby, S., & Stene, L. C. (2017). Infant Feeding and Risk of Type 1 Diabetes in Two Large Scandinavian Birth Cohorts. *Diabetes Care*, *40*(7), 920–927. https://doi.org/10.2337/dc17-0016

Lynch, S. V., & Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease. *New England Journal of Medicine*, *375*(24), 2369–2379. https://doi.org/10.1056/NEJMra1600266

M, S., & DR, M. (2014). Inflammation and Diabetes. *Interdisciplinary Journal of Microinflammation*, *01*(01). https://doi.org/10.4172/ijm.1000110

Maan, K., Baghel, R., Dhariwal, S., Sharma, A., Bakhshi, R., & Rana, P. (2023). Metabolomics and transcriptomics based multi-omics integration reveals radiation-induced altered pathway networking and underlying mechanism. *Npj Systems Biology and Applications*, *9*(1), 42. https://doi.org/10.1038/s41540-023-00305-5

Maddaloni, E., Bolli, G. B., Frier, B. M., Little, R. R., Leslie, R. D., Pozzilli, P., & Buzzetti, R. (2022). C-peptide determination in the diagnosis of type of diabetes and its management: A clinical perspective. *Diabetes, Obesity and Metabolism*, *24*(10), 1912–1926. https://doi.org/10.1111/dom.14785

Manso, I., Torres, B., Andreu, J. M., Menéndez, M., Rivas, G., Alfonso, C., Díaz, E., García, J. L., & Galán, B. (2009). 3-Hydroxyphenylpropionate and Phenylpropionate Are Synergistic Activators of the MhpR Transcriptional Regulator from Escherichia coli. *Journal of Biological Chemistry*, *284*(32), 21218–21228. https://doi.org/10.1074/jbc.M109.008243

Marcovecchio, M. L., Tossavainen, P. H., & Dunger, D. B. (2010). Prevention and treatment of microvascular disease in childhood type 1 diabetes. *British Medical Bulletin*, *94*(1), 145–164. https://doi.org/10.1093/bmb/ldp053

Margaritis, K., Margioula-Siarkou, G., Giza, S., Kotanidou, E. P., Tsinopoulou, V. R., Christoforidis, A., & Galli-Tsinopoulou, A. (2021). Micro-RNA Implications in Type-1

Diabetes Mellitus: A Review of Literature. *International Journal of Molecular Sciences*, *22*(22), 12165. https://doi.org/10.3390/ijms222212165
Markley, J. L., Brüschweiler, R., Edison, A. S., Eghbalnia, H. R., Powers, R., Raftery, D., & Wishart, D. S. (2017). The future of NMR-based metabolomics. *Current Opinion in Biotechnology*, *43*, 34–40. https://doi.org/10.1016/j.copbio.2016.08.001
Marroqui, L., Dos Santos, R. S., Op de beeck, A., Coomans de Brachène, A., Marselli, L., Marchetti, P., & Eizirik, D. L. (2017). Interferon-α mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia*, *60*(4), 656–667. https://doi.org/10.1007/s00125-016-4201-3
Martin, J. M., Trink, B., Daneman, D., Dosch, H.-M., & Robinson, B. (1991). Milk Proteins in the Etiology of Insulin-Dependent Diabetes Mellitus (IDDM). *Annals of Medicine*, *23*(4), 447–452. https://doi.org/10.3109/07853899109148088
Mathieu, C., Lahesmaa, R., Bonifacio, E., Achenbach, P., & Tree, T. (2018). Immunological biomarkers for the development and progression of type 1 diabetes. *Diabetologia*, *61*(11), 2252–2258. https://doi.org/10.1007/s00125-018-4726-8
McCall, A. L., & Farhy, L. S. (2013). Treating type 1 diabetes: from strategies for insulin delivery to dual hormonal control. *Minerva Endocrinologica*, *38*(2), 145–163.
Mehta, R., & Petrova, A. (2011). Biologically active breast milk proteins in association with very preterm delivery and stage of lactation. *Journal of Perinatology*, *31*(1), 58–62. https://doi.org/10.1038/jp.2010.68
Melendez-Ramirez, L. Y., Richards, R. J., & Cefalu, W. T. (2010). Complications of Type 1 Diabetes. *Endocrinology and Metabolism Clinics of North America*, *39*(3), 625–640. https://doi.org/10.1016/j.ecl.2010.05.009
Mikkelsen, K. H., Knop, F. K., Vilsbøll, T., Frost, M., Hallas, J., & Pottegård, A. (2017). Use of antibiotics in childhood and risk of Type 1 diabetes: a population-based case–control study. *Diabetic Medicine*, *34*(2), 272–277. https://doi.org/10.1111/dme.13262
Mokhtari, P., Metos, J., & Anandh Babu, P. V. (2021). Impact of type 1 diabetes on the composition and functional potential of gut microbiome in children and adolescents: possible mechanisms, current knowledge, and challenges. *Gut Microbes*, *13*(1). https://doi.org/10.1080/19490976.2021.1926841
Moran, E. T. (2016). Gastric digestion of protein through pancreozyme action optimizes intestinal forms for absorption, mucin formation and villus integrity. *Animal Feed Science and Technology*, *221*, 284–303. https://doi.org/10.1016/j.anifeedsci.2016.05.015
Moreno-Gonzalez, I., & Soto, C. (2011). Misfolded protein aggregates: Mechanisms, structures and potential for disease transmission. *Seminars in Cell & Developmental Biology*, *22*(5), 482–487. https://doi.org/10.1016/j.semcdb.2011.04.002
Morran, M. P., Vonberg, A., Khadra, A., & Pietropaolo, M. (2015). Immunogenetics of type 1 diabetes mellitus. *Molecular Aspects of Medicine*, *42*, 42–60. https://doi.org/10.1016/j.mam.2014.12.004
Morse, Z. J., Simister, R. L., Crowe, S. A., Horwitz, M. S., & Osborne, L. C. (2023). Virus induced dysbiosis promotes type 1 diabetes onset. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1096323
Müller, B., Rasmusson, A. J., Just, D., Jayarathna, S., Moazzami, A., Novicic, Z. K., & Cunningham, J. L. (2021). Fecal Short-Chain Fatty Acid Ratios as Related to Gastrointestinal and Depressive Symptoms in Young Adults. *Psychosomatic Medicine*, *83*(7), 693–699. https://doi.org/10.1097/PSY.0000000000000965
Mureddu, L., & Vuister, G. W. (2019). Simple high-resolution spectroscopy as a tool in molecular biology. *The FEBS Journal*, *286*(11), 2035–2042. https://doi.org/10.1111/febs.14771

Murri, M., Leiva, I., Gomez-Zumaquero, J. M., Tinahones, F. J., Cardona, F., Soriguer, F., & Queipo-Ortuño, M. I. (2013). Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Medicine*, *11*(1), 46. https://doi.org/10.1186/1741-7015-11-46

Nakayasu, E. S., Bramer, L. M., Ansong, C., Schepmoes, A. A., Fillmore, T. L., Gritsenko, M. A., Clauss, T. R., Gao, Y., Piehowski, P. D., Stanfill, B. A., Engel, D. W., Orton, D. J., Moore, R. J., Qian, W.-J., Sechi, S., Frohnert, B. I., Toppari, J., Ziegler, A.-G., Lernmark, Å., … Metz, T. O. (2023). Plasma protein biomarkers predict the development of persistent autoantibodies and type 1 diabetes 6 months prior to the onset of autoimmunity. *Cell Reports Medicine*, *4*(7), 101093. https://doi.org/10.1016/j.xcrm.2023.101093

Nekoua, M. P., Alidjinou, E. K., & Hober, D. (2022). Persistent coxsackievirus B infection and pathogenesis of type 1 diabetes mellitus. *Nature Reviews Endocrinology*, *18*(8), 503–516. https://doi.org/10.1038/s41574-022-00688-1

Noble, J. A., & Valdes, A. M. (2011). Genetics of the HLA Region in the Prediction of Type 1 Diabetes. *Current Diabetes Reports*, *11*(6), 533–542. https://doi.org/10.1007/s11892-011-0223-x

Nowak, D., & Jakubczyk, E. (2020). The Freeze-Drying of Foods—The Characteristic of the Process Course and the Effect of Its Parameters on the Physical Properties of Food Materials. *Foods*, *9*(10), 1488. https://doi.org/10.3390/foods9101488

Ogle, G. D., James, S., Dabelea, D., Pihoker, C., Svennson, J., Maniam, J., Klatman, E. L., & Patterson, C. C. (2022). Global estimates of incidence of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Atlas, 10th edition. *Diabetes Research and Clinical Practice*, *183*, 109083. https://doi.org/10.1016/j.diabres.2021.109083

O'Keefe, S. J. D., Li, J. V., Lahti, L., Ou, J., Carbonero, F., Mohammed, K., Posma, J. M., Kinross, J., Wahl, E., Ruder, E., Vipperla, K., Naidoo, V., Mtshali, L., Tims, S., Puylaert, P. G. B., DeLany, J., Krasinskas, A., Benefiel, A. C., Kaseb, H. O., … Zoetendal, E. G. (2015). Fat, fibre and cancer risk in African Americans and rural Africans. *Nature Communications*, *6*(1), 6342. https://doi.org/10.1038/ncomms7342

Orešič, M., Gopalacharyulu, P., Mykkänen, J., Lietzen, N., Mäkinen, M., Nygren, H., Simell, S., Simell, V., Hyöty, H., Veijola, R., Ilonen, J., Sysi-Aho, M., Knip, M., Hyötyläinen, T., & Simell, O. (2013). Cord Serum Lipidome in Prediction of Islet Autoimmunity and Type 1 Diabetes. *Diabetes*, *62*(9), 3268–3274. https://doi.org/10.2337/db13-0159

Orešič, M., Simell, S., Sysi-Aho, M., Näntö-Salonen, K., Seppänen-Laakso, T., Parikka, V., Katajamaa, M., Hekkala, A., Mattila, I., Keskinen, P., Yetukuri, L., Reinikainen, A., Lähde, J., Suortti, T., Hakalax, J., Simell, T., Hyöty, H., Veijola, R., Ilonen, J., … Simell, O. (2008). Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. *The Journal of Experimental Medicine*, *205*(13), 2975–2984. https://doi.org/10.1084/jem.20081800

Overgaard, A. J., Kaur, S., & Pociot, F. (2016). Metabolomic Biomarkers in the Progression to Type 1 Diabetes. *Current Diabetes Reports*, *16*(12), 127. https://doi.org/10.1007/s11892-016-0820-9

P., N. P. V., & Joye, I. J. (2020). Dietary Fibre from Whole Grains and Their Benefits on Metabolic Health. *Nutrients*, *12*(10), 3045. https://doi.org/10.3390/nu12103045

Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., Adisetiyo, H., Zabih, S., Lincez, P. J., Bittinger, K., Bailey, A., Bushman, F. D., Sleasman, J. W., & Aldrovandi, G. M. (2017). Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatrics*, *171*(7), 647. https://doi.org/10.1001/jamapediatrics.2017.0378

Paschou, S. A., Papadopoulou-Marketou, N., Chrousos, G. P., & Kanaka-Gantenbein, C. (2018). On type 1 diabetes mellitus pathogenesis. *Endocrine Connections*, *7*(1), R38–R46. https://doi.org/10.1530/EC-17-0347

Pathak, V., Pathak, N. M., O'Neill, C. L., Guduric-Fuchs, J., & Medina, R. J. (2019). Therapies for Type 1 Diabetes: Current Scenario and Future Perspectives. *Clinical Medicine Insights: Endocrinology and Diabetes*, *12*, 117955141984452. https://doi.org/10.1177/1179551419844521

Patterson, E., Cryan, J. F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society*, *73*(4), 477–489. https://doi.org/10.1017/S0029665114001426

Pelikánová T. & Bartoš V. (2018). Praktická diabetologie. Maxdorf. Česko

Peng, Y., Zhang, Z., He, L., Li, C., & Liu, M. (2024). NMR spectroscopy for metabolomics in the living system: recent progress and future challenges. *Analytical and Bioanalytical Chemistry*, *416*(9), 2319–2334. https://doi.org/10.1007/s00216-024-05137-8

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of Insulin Action and Insulin Resistance. *Physiological Reviews*, *98*(4), 2133–2223. https://doi.org/10.1152/physrev.00063.2017

Pflueger, M., Seppänen-Laakso, T., Suortti, T., Hyötyläinen, T., Achenbach, P., Bonifacio, E., Orešič, M., & Ziegler, A.-G. (2011). Age- and Islet Autoimmunity–Associated Differences in Amino Acid and Lipid Metabolites in Children at Risk for Type 1 Diabetes. *Diabetes*, *60*(11), 2740–2747. https://doi.org/10.2337/db10-1652

Pihoker, C., Gilliam, L. K., Hampe, C. S., & Lernmark, A. (2005). Autoantibodies in Diabetes. *Diabetes*, *54*(suppl_2), S52–S61. https://doi.org/10.2337/diabetes.54.suppl_2.S52

Piñero-Piloña, A., & Raskin, P. (2001). Idiopathic Type 1 diabetes. *Journal of Diabetes and Its Complications*, *15*(6), 328–335. https://doi.org/10.1016/S1056-8727(01)00172-6

Pinu, F. R., Goldansaz, S. A., & Jaine, J. (2019). Translational Metabolomics: Current Challenges and Future Opportunities. *Metabolites*, *9*(6), 108. https://doi.org/10.3390/metabo9060108

Piya, A., & Michels, A. W. (2012). Understanding the Immunology of Type 1 Diabetes— An Overview of Current Knowledge and Perspectives for the Future. *US Endocrinology*, *08*(01), 12. https://doi.org/10.17925/USE.2012.08.01.12

Pugliese, A. (2017). Autoreactive T cells in type 1 diabetes. *Journal of Clinical Investigation*, *127*(8), 2881–2891. https://doi.org/10.1172/JCI94549

Qiu, S., Cai, Y., Yao, H., Lin, C., Xie, Y., Tang, S., & Zhang, A. (2023). Small molecule metabolites: discovery of biomarkers and therapeutic targets. *Signal Transduction and Targeted Therapy*, *8*(1), 132. https://doi.org/10.1038/s41392-023-01399-3

Redondo, M. J., Steck, A. K., & Pugliese, A. (2018). Genetics of type 1 diabetes. *Pediatric Diabetes*, *19*(3), 346–353. https://doi.org/10.1111/pedi.12597

Remesar, X., & Alemany, M. (2020). Dietary Energy Partition: The Central Role of Glucose. *International Journal of Molecular Sciences*, *21*(20), 7729. https://doi.org/10.3390/ijms21207729

Rewers, M., & Ludvigsson, J. (2016). Environmental risk factors for type 1 diabetes. *The Lancet*, *387*(10035), 2340–2348. https://doi.org/10.1016/S0140-6736(16)30507-4

Rodriguez-Calvo, T., Johnson, J. D., Overbergh, L., & Dunne, J. L. (2021). Neoepitopes in Type 1 Diabetes: Etiological Insights, Biomarkers and Therapeutic Targets. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.667989

Rose, C., Parker, A., Jefferson, B., & Cartmell, E. (2015). The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology. *Critical*

Reviews in Environmental Science and Technology, *45*(17), 1827–1879. https://doi.org/10.1080/10643389.2014.1000761
Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *European Journal of Nutrition*, *57*(1), 1–24. https://doi.org/10.1007/s00394-017-1445-8
Russell, W. R., Duncan, S. H., Scobbie, L., Duncan, G., Cantlay, L., Calder, A. G., Anderson, S. E., & Flint, H. J. (2013). Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. *Molecular Nutrition & Food Research*, *57*(3), 523–535. https://doi.org/10.1002/mnfr.201200594
Schofield, J., Ho, J., & Soran, H. (2019). Cardiovascular Risk in Type 1 Diabetes Mellitus. *Diabetes Therapy*, *10*(3), 773–789. https://doi.org/10.1007/s13300-019-0612-8
Schwiertz, A., Taras, D., Schäfer, K., Beijer, S., Bos, N. A., Donus, C., & Hardt, P. D. (2010). Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity*, *18*(1), 190–195. https://doi.org/10.1038/oby.2009.167
Sen, P., Dickens, A. M., López-Bascón, M. A., Lindeman, T., Kemppainen, E., Lamichhane, S., Rönkkö, T., Ilonen, J., Toppari, J., Veijola, R., Hyöty, H., Hyötyläinen, T., Knip, M., & Orešič, M. (2020). Metabolic alterations in immune cells associate with progression to type 1 diabetes. *Diabetologia*, *63*(5), 1017–1031. https://doi.org/10.1007/s00125-020-05107-6
Sharma, H., Lencioni, M., & Narendran, P. (2019). Cardiovascular disease in type 1 diabetes. *Cardiovascular Endocrinology & Metabolism*, *8*(1), 28–34. https://doi.org/10.1097/XCE.0000000000000167
Sharma, K., Akre, S., Chakole, S., & Wanjari, M. B. (2022). Stress-Induced Diabetes: A Review. *Cureus*. https://doi.org/10.7759/cureus.29142
Shehadeh, N., Shamir, R., Berant, M., & Etzioni, A. (2001). Insulin in human milk and the prevention of type 1 diabetes. *Pediatric Diabetes*, *2*(4), 175–177. https://doi.org/10.1034/j.1399-5448.2001.20406.x
Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A., & Sakharkar, M. K. (2016). Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomarker Insights*, *11*, BMI.S38440. https://doi.org/10.4137/BMI.S38440
Simmons, K. M. (2015). Type 1 diabetes: A predictable disease. *World Journal of Diabetes*, *6*(3), 380. https://doi.org/10.4239/wjd.v6.i3.380
Singh, V., Lee, G., Son, H., Koh, H., Kim, E. S., Unno, T., & Shin, J.-H. (2023). Butyrate producers, "The Sentinel of Gut": Their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.1103836
Sipetic, S., Vlajinac, H., Marinkovi, J., Kocev, N., Milan, B., Ratkov, I., & Sajic, S. (2007). Stressful Life Events and Psychological Dysfunctions Before the Onset of Type 1 Diabetes Mellitus. *Journal of Pediatric Endocrinology and Metabolism*, *20*(4). https://doi.org/10.1515/JPEM.2007.20.4.527
Sosenko, J. M., Palmer, J. P., Rafkin, L. E., Krischer, J. P., Cuthbertson, D., Greenbaum, C. J., Eisenbarth, G., & Skyler, J. S. (2010). Trends of Earlier and Later Responses of C-peptide to Oral Glucose Challenges With Progression to Type 1 Diabetes in Diabetes Prevention Trial–Type 1 Participants. *Diabetes Care*, *33*(3), 620–625. https://doi.org/10.2337/dc09-1770
Soto, A., Martín, V., Jiménez, E., Mader, I., Rodríguez, J. M., & Fernández, L. (2014). Lactobacilli and Bifidobacteria in Human Breast Milk. *Journal of Pediatric Gastroenterology and Nutrition*, *59*(1), 78–88. https://doi.org/10.1097/MPG.0000000000000347

Speake, C., Ylescupidez, A., Neiman, D., Shemer, R., Glaser, B., Tersey, S. A., Usmani-Brown, S., Clark, P., Wilhelm, J. J., Bellin, M. D., Herold, K. C., Mirmira, R. G., Dor, Y., & Evans-Molina, C. (2020). Circulating Unmethylated Insulin DNA As a Biomarker of Human Beta Cell Death: A Multi-laboratory Assay Comparison. *The Journal of Clinical Endocrinology & Metabolism*, *105*(3), 781–791. https://doi.org/10.1210/clinem/dgaa008

Suhre, K. (2014). Metabolic profiling in diabetes. *Journal of Endocrinology*, *221*(3), R75–R85. https://doi.org/10.1530/JOE-14-0024

Syed, F., & Evans-Molina, C. (2016). Nucleic acid biomarkers of β cell stress and death in type 1 diabetes. *Current Opinion in Endocrinology, Diabetes & Obesity*, *23*(4), 312–317. https://doi.org/10.1097/MED.0000000000000261

Tandon, N. (2015). Understanding type 1 diabetes through genetics: Advances and prospects. *Indian Journal of Endocrinology and Metabolism*, *19*(7), 39. https://doi.org/10.4103/2230-8210.155391

Trotta, D., Verrotti, A., Salladini, C., & Chiarelli, F. (2004). Diabetic neuropathy in children and adolescents. *Pediatric Diabetes*, *5*(1), 44–57. https://doi.org/10.1111/j.1399-543X.2004.00041.x

van der Vaart, M., & Pretorius, P. J. (2007). The Origin of Circulating Free DNA. *Clinical Chemistry*, *53*(12), 2215–2215. https://doi.org/10.1373/clinchem.2007.092734

van der Werf, N., Kroese, F. G. M., Rozing, J., & Hillebrands, J.-L. (2007). Viral infections as potential triggers of type 1 diabetes. *Diabetes/Metabolism Research and Reviews*, *23*(3), 169–183. https://doi.org/10.1002/dmrr.695

Veijola, R, et al. (2020). DIPP Study. Hedimed kick-off meeting. University of Oulu and University Hospital, Tampere.

Vieira Borba, V., Sharif, K., & Shoenfeld, Y. (2018). Breastfeeding and autoimmunity: Programing health from the beginning. *American Journal of Reproductive Immunology*, *79*(1). https://doi.org/10.1111/aji.12778

Vig, S., Lambooij, J. M., Zaldumbide, A., & Guigas, B. (2021). Endoplasmic Reticulum-Mitochondria Crosstalk and Beta-Cell Destruction in Type 1 Diabetes. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.669492

Viggiano, D., Ianiro, G., Vanella, G., Bibbò, S., Bruno, G., Simeone, G., & Mele, G. (2015). Gut barrier in health and disease: focus on childhood. *European Review for Medical and Pharmacological Sciences*, *19*(6), 1077–1085.

Virtanen, S. M., Jaakkola, L., Räsänen, L., Ylönen, K., Aro, A., Lounamaa, R., Åkerblom, H. K., & Tuomilehto, J. (1994). Nitrate and Nitrite Intake and the Risk for Type 1 Diabetes in Finnish Children. *Diabetic Medicine*, *11*(7), 656–662. https://doi.org/10.1111/j.1464-5491.1994.tb00328.x

Virtanen, S. M., Nevalainen, J., Kronberg-Kippilä, C., Ahonen, S., Tapanainen, H., Uusitalo, L., Takkinen, H.-M., Niinistö, S., Ovaskainen, M.-L., Kenward, M. G., Veijola, R., Ilonen, J., Simell, O., & Knip, M. (2012). Food consumption and advanced β cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes: a nested case-control design. *The American Journal of Clinical Nutrition*, *95*(2), 471–478. https://doi.org/10.3945/ajcn.111.018879

Wahren, J., Kallas, Å., & Sima, A. A. F. (2012). The Clinical Potential of C-Peptide Replacement in Type 1 Diabetes. *Diabetes*, *61*(4), 761–772. https://doi.org/10.2337/db11-1423

White, N. H. (2015). Long-term Outcomes in Youths with Diabetes Mellitus. *Pediatric Clinics of North America*, *62*(4), 889–909. https://doi.org/10.1016/j.pcl.2015.04.004

Winter, W. E., & Schatz, D. A. (2011). Autoimmune Markers in Diabetes. *Clinical Chemistry*, *57*(2), 168–175. https://doi.org/10.1373/clinchem.2010.148205

Wishart, D. S., Knox, C., Guo, A. C., Eisner, R., Young, N., Gautam, B., Hau, D. D., Psychogios, N., Dong, E., Bouatra, S., Mandal, R., Sinelnikov, I., Xia, J., Jia, L., Cruz, J. A., Lim, E., Sobsey, C. A., Shrivastava, S., Huang, P., … Forsythe, I. (2009). HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Research*, *37*(Database), D603–D610. https://doi.org/10.1093/nar/gkn810

Wishart, D. S., Tzur, D., Knox, C., Eisner, R., Guo, A. C., Young, N., Cheng, D., Jewell, K., Arndt, D., Sawhney, S., Fung, C., Nikolai, L., Lewis, M., Coutouly, M.-A., Forsythe, I., Tang, P., Shrivastava, S., Jeroncic, K., Stothard, P., … Querengesser, L. (2007). HMDB: the Human Metabolome Database. *Nucleic Acids Research*, *35*(Database), D521–D526. https://doi.org/10.1093/nar/gkl923

Wong, J. M. W., & Jenkins, D. J. A. (2007). Carbohydrate Digestibility and Metabolic Effects. *The Journal of Nutrition*, *137*(11), 2539S-2546S. https://doi.org/10.1093/jn/137.11.2539S

Wu, H.-J., & Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes*, *3*(1), 4–14. https://doi.org/10.4161/gmic.19320

Wu, W., Zhang, L., Zheng, X., Huang, Q., Farag, M. A., Zhu, R., & Zhao, C. (2022). Emerging applications of metabolomics in food science and future trends. *Food Chemistry: X*, *16*, 100500. https://doi.org/10.1016/j.fochx.2022.100500

Xu, Z.-R., Yuan, X.-X., Chen, R.-M., Wei, H.-Y., Chen, L.-Q., Du, H.-W., Li, G.-M., Yang, Y., Chen, X.-J., Fang, X., & Luo, F.-H. (2022). Association between new onset type 1 diabetes and real-world antibiotics and neonicotinoids' exposure-related gut microbiota perturbation. *World Journal of Pediatrics*, *18*(10), 671–679. https://doi.org/10.1007/s12519-022-00589-3

Yahaya, T., & Shemishere, U. (2020). Association between Bioactive Molecules in Breast Milk and Type 1 Diabetes Mellitus. *Sultan Qaboos University Medical Journal [SQUMJ]*, *20*(1), 5. https://doi.org/10.18295/squmj.2020.20.01.002

Yeung, W.-C. G., Al-Shabeeb, A., Pang, C. N. I., Wilkins, M. R., Catteau, J., Howard, N. J., Rawlinson, W. D., & Craig, M. E. (2012). Children With Islet Autoimmunity and Enterovirus Infection Demonstrate a Distinct Cytokine Profile. *Diabetes*, *61*(6), 1500–1508. https://doi.org/10.2337/db11-0264

Yeung, W.-C. G., Rawlinson, W. D., & Craig, M. E. (2011). Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. *BMJ*, *342*(feb03 1), d35–d35. https://doi.org/10.1136/bmj.d35

Yi, L., Swensen, A. C., & Qian, W.-J. (2018). Serum biomarkers for diagnosis and prediction of type 1 diabetes. *Translational Research*, *201*, 13–25. https://doi.org/10.1016/j.trsl.2018.07.009

Yosten, G. L. C., Maric-Bilkan, C., Luppi, P., & Wahren, J. (2014). Physiological effects and therapeutic potential of proinsulin C-peptide. *American Journal of Physiology-Endocrinology and Metabolism*, *307*(11), E955–E968. https://doi.org/10.1152/ajpendo.00130.2014

Yu, J., Sharma, P., Girgis, C. M., & Gunton, J. E. (2022). Vitamin D and Beta Cells in Type 1 Diabetes: A Systematic Review. *International Journal of Molecular Sciences*, *23*(22), 14434. https://doi.org/10.3390/ijms232214434

Yung, J. H. M., & Giacca, A. (2020). Role of c-Jun N-terminal Kinase (JNK) in Obesity and Type 2 Diabetes. *Cells*, *9*(3), 706. https://doi.org/10.3390/cells9030706

Zaheer, A., Zaheer, F., Saeed, H., Tahir, Z., & Tahir, M. W. (2021). A Review of Alternative Treatment Options in Diabetic Polyneuropathy. *Cureus*. https://doi.org/10.7759/cureus.14600

Zajec, A., Trebušak Podkrajšek, K., Tesovnik, T., Šket, R., Čugalj Kern, B., Jenko Bizjan, B., Šmigoc Schweiger, D., Battelino, T., & Kovač, J. (2022). Pathogenesis of Type 1

Diabetes: Established Facts and New Insights. *Genes*, *13*(4), 706. https://doi.org/10.3390/genes13040706

Zakir, M., Ahuja, N., Surksha, M. A., Sachdev, R., Kalariya, Y., Nasir, M., Kashif, M., Shahzeen, F., Tayyab, A., Khan, M. S. moazzam, Junejo, M., Manoj Kumar, F., Varrassi, G., Kumar, S., Khatri, M., & Mohamad, T. (2023). Cardiovascular Complications of Diabetes: From Microvascular to Macrovascular Pathways. *Cureus*. https://doi.org/10.7759/cureus.45835

Zeki, Ö. C., Eylem, C. C., Reçber, T., Kır, S., & Nemutlu, E. (2020). Integration of GC–MS and LC–MS for untargeted metabolomics profiling. *Journal of Pharmaceutical and Biomedical Analysis*, *190*, 113509. https://doi.org/10.1016/j.jpba.2020.113509

Zhang, K., Lin, G., Han, Y., Xie, J., & Li, J. (2017). Circulating unmethylated insulin DNA as a potential non-invasive biomarker of beta cell death in type 1 Diabetes: a review and future prospect. *Clinical Epigenetics*, *9*(1), 44. https://doi.org/10.1186/s13148-017-0343-5

Zhang, X., Dong, Y., Liu, D., Yang, L., Xu, J., & Wang, Q. (2022). Antigen-specific immunotherapies in type 1 diabetes. *Journal of Trace Elements in Medicine and Biology*, *73*, 127040. https://doi.org/10.1016/j.jtemb.2022.127040

Zhang, W., & Lang, R. (2023). Succinate metabolism: a promising therapeutic target for inflammation, ischemia/reperfusion injury and cancer. *Frontiers in Cell and Developmental Biology*, *11*. https://doi.org/10.3389/fcell.2023.1266973

Zheng, P., Li, Z., & Zhou, Z. (2018). Gut microbiome in type 1 diabetes: A comprehensive review. *Diabetes/Metabolism Research and Reviews*, *34*(7). https://doi.org/10.1002/dmrr.3043

Zhenyukh, O., Civantos, E., Ruiz-Ortega, M., Sánchez, M. S., Vázquez, C., Peiró, C., Egido, J., & Mas, S. (2017). High concentration of branched-chain amino acids promotes oxidative stress, inflammation and migration of human peripheral blood mononuclear cells via mTORC1 activation. *Free Radical Biology and Medicine*, *104*, 165–177. https://doi.org/10.1016/j.freeradbiomed.2017.01.009

Zhou, H., Sun, L., Zhang, S., Zhao, X., Gang, X., & Wang, G. (2020). Evaluating the Causal Role of Gut Microbiota in Type 1 Diabetes and Its Possible Pathogenic Mechanisms. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/fendo.2020.00125

Ziegler, A.-G., & Bonifacio, E. (2012). Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. *Diabetologia*, *55*(7), 1937–1943. https://doi.org/10.1007/s00125-012-2472-x

Zorena, K., Jaskulak, M., Michalska, M., Mrugacz, M., & Vandenbulcke, F. (2022). Air Pollution, Oxidative Stress, and the Risk of Development of Type 1 Diabetes. *Antioxidants*, *11*(10), 1908. https://doi.org/10.3390/antiox11101908

Zullo, A., Sommese, L., Nicoletti, G., Donatelli, F., Mancini, F. P., & Napoli, C. (2017). Epigenetics and type 1 diabetes: mechanisms and translational applications. *Translational Research*, *185*, 85–93. https://doi.org/10.1016/j.trsl.2017.05.002