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Metabolomics and lipidomics contributions to type 1 diabetes research

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Abstract

Metabolomics is a "omic" science with a focus on the characterization of metabolome, that is the pool of low molecular weight (< 1.5 kDa) metabolites involved in the metabolism of a biological system. When the targets are lipids, metabolomics is commonly referred to as lipidomics. Since the metabolome composition is influenced by several factors such as environment, disease, drugs, and genetics, metabolomics is extensively used in the biomedical research for the identification of metabolic signatures or novel biomarkers useful in diagnosis, prediction, prognosis, and prevention of disease. In recent years, both metabolomics and lipidomics have been extensively employed in diabetes research to elucidate the molecular mechanisms triggering this disease and to discover biomarkers for its prevention, diagnosis, and treatment. In this review, some of the most significant findings relative to studies performed on type 1 diabetes patients are summarized to provide an overview of the potential of these approaches.

Introduction

The term diabetes mellitus, commonly referred to as diabetes, is used to define a group of metabolic disorders characterized by elevated blood glucose levels due to defects in insulin secretion and/or action[1]. Type 1 diabetes (T1D) is an autoimmune disease that represents 5-10% of all cases of diabetes[2]. It develops at any age but occurs most frequently in children and adolescents[3,4]. T1D is characterized by a progressive destruction of insulin-producing pancreatic β-cells with consequent insulin deficiency. Type 2 diabetes (T2D) is the most common form of diabetes (about 90% of cases) and generally occurs after 30-40 years of age[5]. T2D is characterized by a progressive loss of adequate β-cell insulin secretion frequently on the background of insulin resistance. Gestational diabetes is one of the most common pregnancy complications and usually disappears after giving birth[6]. This condition occurs in about 7% of pregnancies. The definition is valid regardless of the type of treatment (diet, physical exercise or insulin) and the persistence of diabetes even after pregnancy. Both World Health Organization (WHO) and American Diabetes Association (ADA) have identified an intermediate type of diabetes for individuals whose glucose levels do not meet the criteria for diabetes but are too high to be considered normal. This condition of very high risk of evolving towards the diabetes, especially T2D, is referred to as impaired glucose tolerance, also known as pre-diabetes. Finally, there is a form of hybrid diabetes called "latent autoimmune diabetes in adults" (LADA) that includes both autoimmune destruction of pancreatic β-cells and some degrees of insulin resistance[7]. Patients with LADA are treated for a short period after diagnosis without insulin injections.

Epidemiological data indicate that the number of people affected by T1D and T2D is increasing rapidly worldwide. According to the International Diabetes Federation[8], in 2019 approximately 463 million adults (20-79 years) were living with diabetes, and by 2045 this number is estimated to reach 700 million. More than 1.1 million children and adolescents are living with T1D with the highest incidence in Finland (64.2/100.000)[9] followed by the island of Sardinia, Italy (45/100.000)[10]. These data are further aggravated by the fact that the chronic hyperglycemia of diabetes is associated with long-term com-

plications and comorbidities such as cardiovascular disease, hypertension, dyslipidemia, diabetic nephropathy, neuropathy, and retinopathy, becoming one of the top 10 causes of death in adults[11].

The exact etiology of diabetes is unknown. The genetic component has an important weight on the predisposition of diabetes, but it does not have a direct cause-effect relationship with this disease. Indeed, the pathogenetic mechanisms underlying the clinical phenotype of diabetes are by nature complex and arise from a variable interaction between genetic and environmental (non-genetic) risk factors. In case of T2D, one of the most significant factors influencing the development of disease is lifestyle, commonly associated with urbanization. Different studies have established that lifestyle modification together with physical activity and/or healthy diet can delay or prevent the onset of T2D[12]. Differently, at present, T1D cannot be prevented yet since the complex molecular network responsible for the destruction of β-cells is still enigmatic, thus slowing down the identification of parameters with the role of specific T1D indicators[13]. So, prediction of T1D is done based on the risk assessment defined by a combined use of genetic, immunologic, and metabolic parameters, while the diagnosis is done when the symptoms are evident. To reduce the pandemic of T1D and its disastrous social, economic, and health impacts worldwide, there is an urgent need to improve the understanding of the molecular network responsible for the destruction of β-cells to identify strategies useful to the prevention, diagnosis and treatment of this disease.

Metabolomics and Lipidomics

Metabolomics is a "omics science" aimed at identifying and quantifying the set of low molecular weight metabolites (typically < 1500 Da), known as metabolome, present in a biological sample such as biofluids, cells, tissues, and organs[14]. The metabolome consists of both endogenous and exogenous components, including amino acids, peptides, nucleic acids, organic acids, vitamins, and carbohydrates. Since the metabolome composition can be viewed as a mirror that reflects the global assessment of a cellular state, interrogating the metabolome enables to investigate the metabolic status of an organism in normal or disruptive physiologic conditions due to altered environmental factors such as lifestyle, diet, drug consumption, and development of diseases, to name just a few[15]. The main analytical platforms used in metabolomics are the following: nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) combined with gas chromatography (GC-MS), liquid chromatography (LC-MS), high performance liquid chromatography (HPLC-MS), and capillary electrophoresis (CE-MS). Basically, NMR offers a good reproducibility, is not destructive, and requires relatively simple and fast preparations of samples. Nevertheless, the low sensitivity of NMR confines its applications to concentration measurements in the micromolar to millimolar range. MS method is more sensitive than NMR, but it is a destructive technique and requires extensive sample preparation, including the derivatization of compounds in case of GC-MS. No one of these techniques can provide alone a complete picture of metabolome; therefore, a complementary use of both technologies is particularly useful to gain a broader perspective. A detailed description of these techniques, their advantages and drawbacks are the subject of various reviews. The reader is referred to the literature for further information[16-19].

Different types of biofluids can be analyzed in metabolomics such as blood, urine, faecal extracts and saliva. No single biofluid is appropriate for all studies; blood[20] and urine[21] samples can provide significant insight into the dysregulation of metabolism at the organ level, while faecal[22] and saliva[23] metabolome are specifically informative on microbiota.

Over the past decades, metabolomics has been successfully applied in medicine and molecular biology. It has provided useful contributions to the discovery of new biochemical pathways in diseases, the understanding of their role in triggering pathologies, and the discovery of new therapeutic targets and biomarkers for early diagnosis or monitoring therapeutic activities[24]. More recently, lipidomics, a branch of metabolomics which studies the complete set of lipids (lipidome) produced in a given cell or organism, has garnered attention, particularly in relation to diseases characterized by dysregulated lipid metabolism. The development of lipidomics has been largely driven by rapid advances in MS technologies[25].

Both metabolomics and lipidomics have emerged as promising approaches also in diabetes research. According to a statistics obtained by searching "metabolomics, metabolome, lipidomics, or lipidome and diabetes" in the "article title, abstract

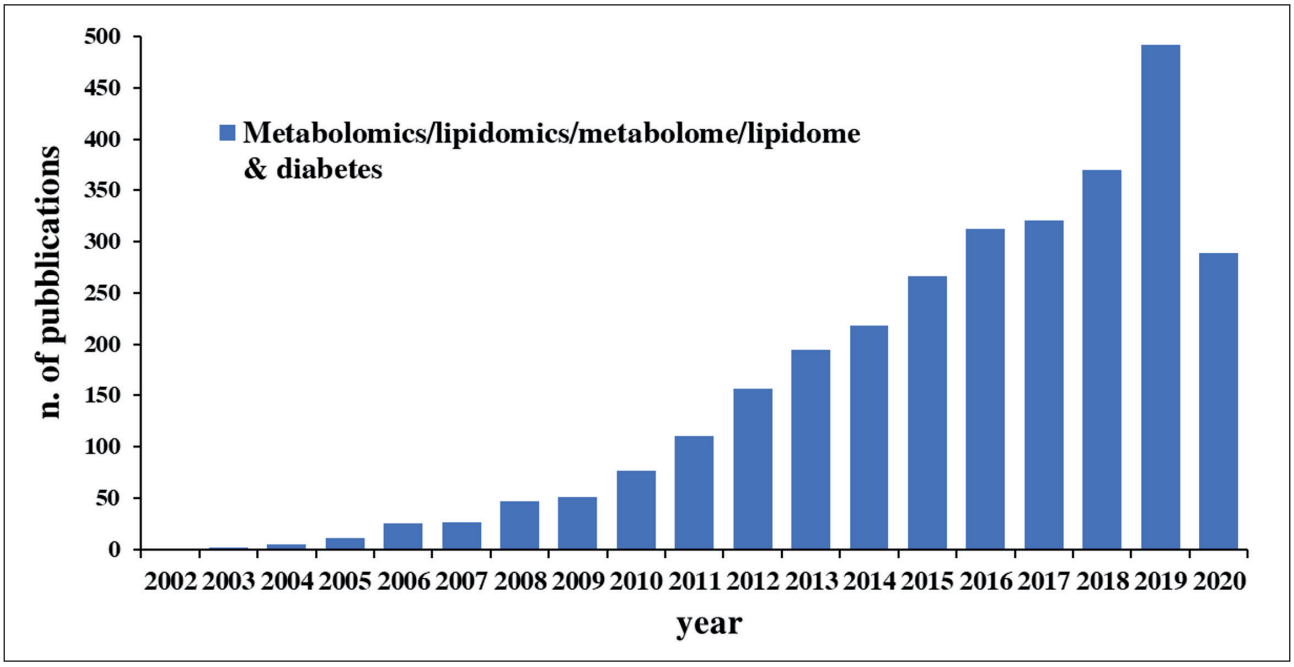

Figure 1. Research outputs of metabolomics and lipidomics studies in diabetes research. Data were gathered by Scopus26 searching for the terms "metabolomics" or "metabolome" or "lipidomics" or "lipidome" and "diabetes" in "article title, abstract and keywords" field.

and keywords" in SCOPUS database[26], 2976 contributions (articles, reviews, books, book chapters, editorials, notes, and letters) have been published since 2002 (Figure 1). Among these, 139 have been focused exclusively on T1D. Exhaustive reviews on these topics have been published so far[27-35]. The aim of the current overview is to illustrate the findings of some of the most representative human studies performed in different areas of T1D research, providing the reader an outline of the metabolomics and lipidomics contributions to diabetes biomarker discovery and the relative experimental designs.

T1D in children: pre- and post-diagnosis

Different long-term studies worldwide have followed up children from birth to investigate the natural history of islet autoimmunity (IA) and set up strategies to predict, delay, and prevent the T1D onset. Among these, we remind the Finnish Type 1 Diabetes Prediction and Prevention (DIPP)[36], the Norwegian Environmental Triggers of Type 1 Diabetes Study (MIDIA)[37], the German BABYDIAB[38] and BABYDIET[39], and the Colorado Diabetes Autoimmunity Study in the Young (DAISY)[40]. Within these studies, important contributions to the understanding of T1D pathogenesis have been obtained by applying metabolomics and lipidomics on subjects starting from birth until clinical diagnosis. In 2008, an integrated lipidomics and metabolomics longitudinal study was performed on the serum specimens of 56 children of the DIPP cohort who progressed to T1D (progressors) with age at time of diagnosis between 6 and 162 months, and 73 healthy and autoantibody negative matched controls (non-progressors)[41]. Additionally, the cord blood samples of 39 DIPP children, 15 of which progressed to diabetes before the age of 12 years, were analyzed. The lipidome of 515 samples collected from progressors, among which 112 taken before seroconversion to autoantibody positivity, was investigated by ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS), while the metabolome of a subset of 419 samples was characterized by two-dimensional gas chromatography coupled to time of flight mass spectrometry (GCxGC-TOF/MS). Compared to non-progressors, the serum lipidome of progressors exhibited decreased phosphatidylcholine (PC) at birth, a reduction of triglycerides (TGs) and antioxidant ether phospholipids during the follow

up, and an increase of proinflammatory lysophosphatidylcholine (LysoPC) in the period before seroconversion to autoantibody positivity. Before IA emergence and T1D diagnosis, the metabolome of progressors showed lower levels of ketoleucine and higher contents of branched chain amino acids (BCAA) and glutamic acid compared to controls. The metabolic profile was partially normalized after the seroconversion.

A possible role of phospholipids in cord blood as predictors of development of autoimmunity was further supported by other two studies performed on a larger DIPP cohort[42,43] and one conducted in children of the Diabetes Prediction in Skåne (DiPiS) study group[44]. In the first two investigations, the population of T1D children was divided into two subgroups: cases that developed T1D-associated autoantibodies and progressed to T1D during the follow-up; cases that developed antibodies (one, two or three/four) but were clinically unaffected. The differences in cord lipidome of cases when compared to controls confirmed that T1D progressors have a characteristic lipidomic profile already present at birth. In particular, choline-containing phospholipids, mainly sphingomyelins and PC were present at lower concentration in T1D progressors than in non-progressors and controls. Since maternal choline intake during pregnancy can affect metabolic and physiologic function of child, a possible link of the abovementioned findings with the intrauterine environment and the pregnancy, such as the mother's nutritional status, was proposed. Corroborating the hypothesis of an association between choline-containing phospholipids and progression to T1D, PCs and phosphatidylethanolamines in cord blood have been found to be significantly low also in Swedish children with a T1D diagnosis before 4 years of age, while TGs were low in children diagnosed before 2 years of age[44].

Additional information on the dysregulation of lipid metabolism prior to IA and T1D onset has been recently obtained by the longitudinal lipidome analysis of plasma samples from three groups of children matched by diabetes risk associated to human leukocyte antigen (HLA), gender, and period of birth: patients who progressed to T1D (n = 40); patients who developed at least a single islet autoantibody but did not progress to T1D during the follow-up (n = 40); healthy non diabetic controls (n = 40)[45]. Samples were taken at 3, 6, 12, 18, 24, and 36 months from birth. The distinct lipidomics profile associated with the progression to T1D appeared to be most pronounced in very young children, i.e. at 3 months of age. Consistent with the literature,[41,42] sphingomyelins were found to be persistently downregulated in progressors when compared to the other two groups, while triacylglycerols and PCs were mainly downregulated in progressors as compared to children who developed at least a single islet autoantibody but did not progress to T1D at the age of 3 months.

The evidence of differences between the autoimmunity characteristics in the neonatal and puberty period has suggested age-dependent differences in the events that lead to IA or in the immune response to the event[46]. In light of this hypothesis, a possible age effect differentiating also the lipidomic profiles of early developers of islet autoantibodies in comparison with late developers has been investigated by UPLC-MS[47]. In this study, serum samples were collected from children of the BABYDIAB cohort: 35 cases who developed T1D before 2 years of age (n=13) or after 8 years of age (n=22) and matched controls who remained islet autoantibody-negative (n=35). Amino acids (AA) and lipid profiles were assessed in the first antibody-positive serum samples from children who seroconverted to islet autoantibody-positive and in the serum samples of age-matched controls. A second serum sample was analyzed after 1 year of follow-up. Additionally, only for 13 early autoantibody-positive children and matched controls a pre-seroconversion sample was available and analyzed for the AA profile. In good agreement with the literature, seroconverters had higher levels of odd-chain TGs and polyunsaturated fatty acid–containing phospholipids than controls. Furthermore, compared with children who developed autoantibodies in late childhood or remained autoantibody-negative, those who developed autoantibodies by the age of 2 years had two-fold lower concentration of methionine, a metabolite involved in glucose metabolism, insulin resistance, and β-cell dysfunction[48].

In Poland, the first metabolomics study on T1D paediatric patients was performed in 2013[49]. The study population included 30 T1D children and teenagers aged 4–19 years and 12 healthy controls aged 9 years. The case group was divided into two subgroups: patients with low (L-T1D) and high (H-T1D) level of glycated hemoglobin (HbA1c). Urine of children was analyzed by ^{1}H NMR spectroscopy. The findings were in agreement with those obtained by plasma metabolomics[35], pointing

out a metabolic shift induced by T1D development which altered the urinary levels of glucose, ketone bodies, and other metabolites such as amino acids and organic acids, although with different trends for the two subgroups of cases. For instance, for L-T1D all measured AA were down regulated, while in the case of H-T1D the glucogenic amino acid levels were increased, especially glycine, alanine, and valine. Besides a dysregulation at the levels of the two endogenous pathways of glucose production in human organism (gluconeogenesis–lactate pathway and proteins catabolism), a possible role of intestinal microbiota was suggested by the lower urinary levels of hippurate (a host-bacterial co-metabolite)[50] in cases compared to controls.

Another notable example of metabolomics application in T1D research is the study performed in a cohort of 49 Spanish children, ranging from 6 to 11 years old (34 with T1D and 15 controls)[51]. Plasma and urine samples were analyzed by LC-MS and CE-MS, respectively. The plasma specimens were taken from 26 cases and 14 controls, while urine was provided by 16 cases and 15 controls. Urinary AA, their metabolites and derivatives were excreted in higher amount in diabetic children than controls, suggesting alterations in glomerular filtration rate and/or proteins and amino acid metabolism. The higher levels of nonesterified fatty acids, lysophospholipids, and other derivatives of fatty acids in plasma of cases compared to controls further suggested an altered lipid metabolism. Furthermore, in good agreement with other studies performed on animal[52] and human[53,54] models, alteration in bile acids and *p*-cresol sulfate, two well-known metabolites linked to gut microbiota activity, supported the hypothesis of a role for the gut microbiota in the development of β-cell autoimmunity and T1D. Similar conclusions were also reached in a previous urine-based metabolomics study performed on Sardinian children's (29 T1D cases and 90 controls)[55], revealing a significant role of hippurate, *p*-cresol sulfate and phenylacetylglycine in classifying the groups of cases and controls.

The potential of urine-based metabolomics to monitor metabolic dysregulation in T1D children has been recently supported also by an Italian study[56]. An excess of urinary glucocorticoids and mineralocorticoids, phenylalanine and tryptophan catabolites, small peptides, glycerophospholipids, fatty acids, and gut bacterial products have been observed in 56 T1D children (average age of 11.4 ± 2.2 years) compared to 30 healthy controls (average age of 10.7 ± 2.8 years). In addition to a dysregulation of lipid metabolism pathways and changes in gut microbiota, the findings of this study suggested an alteration of the steroid metabolism and a possible impairment of tryptophan catabolism in pediatric T1D patients, in good agreement with the literature[57].

In a study involving T1D infants from the Norwegian MIDIA project, the impact of age, sex, breastfeeding, and development of IA or T1D on the plasma metabolic profile were explored[58]. The study population included 29 subjects with two or three autoantibodies or overt T1D and 29 controls negative for islet autoantibodies matched for sex, date of birth, and area of residence with cases. GC×GC-TOFMS was employed for a longitudinal analysis of samples collected at 3 month-intervals (up to 1 year of life) during infancy, prior to and close to the development of IA. Although some differences were observed between the metabolic profiles of cases and controls, overall a clear association between the metabolic profiles and later development of islet autoimmunity was not observed.

Recently, in the DAISY study, an integrated analysis of genetic, immunologic, metabolomics, and proteomic data has been used for modelling the development of IA and progression to T1D in a cohort of 42 children which developed IA (among which 20 progressed to diabetes) matched by sex and age-matched with healthy subjects[59]. Biomarkers were assessed at four time points: earliest available sample, just prior to IA, just after IA, and just prior to diabetes onset. The analysis of serum metabolome was carried out by UPLC-MS/MS. The top selected feature among the best predictors of progression to IA was found to be ascorbic acid (vitamin C). In particular, the levels of this antioxidant agent in children who developed IA at the earliest time point were lower than that of controls. This finding is in line with the recent observations of an inverse association between the plasma level of vitamin C and the risk of IA in children with increased genetic risk of T1D[60], corroborating the hypothesis of a possible role of genetic variation in vitamin C in T1D development. In the same study, glucose was found to be the top metabolite for progression from IA to diabetes[59]. Other two promising metabolites for prediction of IA outcome were α-ketoisovaleric acid, a valine degradation product[61] as well as precursor of leucine and valine synthesis[62], and 4-hydroxyhippurate, a gut microbial fermentation product.

An accurate characterization of gut metabolic composition in combination with the analysis of serum metabolome and gut microbiota has provided information on the dynamics and stability of the developing microbiome in at-risk T1D infants[63]. This prospective and longitudinal study was performed on stool samples of infants from Finland (n = 27) and Estonia (n = 6) recruited at birth based on a positive cord blood testing for HLA: 7 patients were positive for at least two of the five autoantibodies but they did not develop T1D; 4 subjects developed T1D; 22 were controls matched for gender, HLA genotype, and country with seroconverters. Serum metabolomics (by GCxGC-TOFMS) and lipidomics (by UPLC-MS) measurements were performed on samples at 0 (cord blood), 3, 6, 12, 18, 24, and 36 months from birth. Each sample was paired with the closest stool specimen before the serum collection time that was analyzed by LC-MS to measure polar metabolites and lipids. The analysis of gut microbiota showed a significant shift of inter- and intra-individual taxonomic composition with age during the first 3 years of life before becoming more stable. Nevertheless, the stool metabolomics profile remained approximately unchanged over time and across individuals. Additionally, as compared to non-converters and seroconverters, a drop in α-diversity in progressors was observed in the temporal window between the seroconversion and the diagnosis of clinical disease. Correlation analysis between absolute abundances of metabolites and microbial relative abundances highlighted several metabolite-microbe clusters, among which one of the most significant was between TGs, BCAA and a number of microbes[63].

T1D in adults

The influence of insulin therapy and deficiency on human metabolome has been investigated by analyzing plasma samples of seven T1D subjects (31.16 ± 2.9 years) during insulin treatment (I^+) and acute insulin deprivation (I^-) and matched controls (30.26 ± 3.4 years)[64]. AA and AA metabolites were profiled by LC-MS/MS, while ^{1}H NMR spectroscopy was used to assess the profile of the pool of water-soluble low-molecular weight metabolites. Multivariate statistical analysis of NMR data allowed I^- subjects to be clearly differentiated from I^+ and controls. In particular, as compared to I^+, the NMR spectra of I^- were richer in lactate, acetate, allantoin, and ketones, while MS spectra exhibited higher amount of AA and AA metabolites. Furthermore, results of the correlation analysis among metabolites measured in common by both techniques showed opposite trends for I^+ and I^-. For instance, ketone levels (acetoacetate, 3-hydroxybutyrate, acetone) were found to be positively correlated with lactate and several AA during insulin deprivation, but negatively correlated with these metabolites during insulin treatment. Also, citrate was negatively correlated with glutamate during insulin deprivation but the relationship between these two metabolites became positive with insulin treatment. Taken together, the compositional changes in plasma of T1D subjects induced by short-term insulin deprivation provided evidence for known altered physiological processes such as mitochondrial dysfunction, oxidative stress, protein synthesis, degradation, and oxidation, gluconeogenesis, and ketogenesis[64]. In an extension study, these findings were further confirmed by using UPLC-TOFMS, which allowed the detection and identification of a larger pool of metabolites (i.e. 330) in all three study groups (I^+, I^-, controls)[65]. In addition to the abovementioned pathways, the findings pointed toward a different regulation of other metabolites including prostaglandin, arachidonic acid, leukotrienes, neurotransmitters, nucleotides, and anti-inflammatory responses. Consistent changes were also observed when the metabolite plasma levels of I^+ subjects were compared with those of controls, evidencing that insulin treatment in T1D does not completely restore the metabolic alterations[65].

The presence of a metabolic fingerprint associated with good glycemic control in T1D subjects has been evaluated by comparing plasma profiles of 14 patients with poor glycemic control (T1D(-), HbA1c ≥ 8.5 %), 14 patients with good glycemic control (T1D(+), HbA1c ≤ 6.5 %), and matched non-diabetic controls[66]. Compared to controls, T1D(-) showed an elevation of carbohydrate metabolites, various AA (such as BCAA, lysine, proline, serine, N,N-dimethyl histidine, methionine), short-chain fatty acids, and lipid inflammatory mediators, like eicosanoids, and metabolites associated with the vitamin D pathway, accompanied by lower levels of glycolytic metabolites and TCA (tricarboxylic acid) cycle metabolites, metabolites in purine metabolism, alanine and homoarginine. Although many of these alterations were normal-

ized by good glycemic control, several abnormalities persisted even in presence of long-term good glycemic control[66].

A combined use of ^{1}H-NMR and GC-MS has been applied in an exercise-based investigation of the metabolic changes induced by a short-term session of acute aerobic exercise in ten T1D patients (30 min exercise on a cycle ergometer at an intensity of 80% of maximal oxygen uptake, VO_2max)[67]. A serum sample was obtained by cases and matched healthy controls at rest and after a short period of intense exercise. Findings revealed similar metabolic events in the two groups of subjects such as increased concentrations of gluconeogenic precursors and TCA cycle intermediates, although the overall metabolic response after exercise was attenuated in T1D patients. In particular, compared to controls, a reduced activation of glycogenolysis and glycolysis, a less significant accumulation of TCA cycle intermediates, an attenuated lipolytic action and a decrease in protein catabolism were observed in the T1D group after exercise[67].

In a German case-control study, the plasma metabolome profiles of T1D (n = 127) and T2D (n = 244) adults with disease duration less than 1 year have been compared to those of non-diabetic controls (n = 129) to explore the metabolic alterations occurring in early onset diabetes[68]. Similar altered metabolite patterns in both T1D and T2D were observed when compared with healthy subjects, suggesting common mechanisms responsible for the deregulation of metabolic control. In particular, 28 and 49 metabolites were found to be significantly altered in T1D and T2D, respectively. For both diabetes types, the levels of various phosphatidylcholine species were reduced compared to controls, while those of BAA, aromatic AA and short-chain fatty acids were higher. Additionally, diabetes type-specific differences were observed for free fatty acids, which were more abundant in T2D compared to controls and T1D, as a likely consequence of a higher degree of systemic inflammation in T2D[68].

Diabetic complications

Patients with diabetes are at increased risk of microvascular complications such as diabetic kidney disease (DKD), neuropathy, and retinopathy. Among these diseases, DKD (also called diabetic nephropathy) is the one most studied by metabolomics and lipomics. DKD is the leading cause of end-stage renal disease (ESRD)[69]. Moderately increased albuminuria, known as microalbuminuria and defined as urine albumin excretion rate (AER) ranging from 30 to 300 mg/day, is considered an early marker of renal dysfunction, and, especially for T1D patients, it may be indicative of an early clinical manifestation of diabetic nephropathy. Severely increased albuminuria, also known as macroalbuminuria (defined as urine AER $\geq$ 300 mg/day) has long been regarded as the stage of irreversible kidney damage[70]. The only therapeutic options available for ESRD are dialysis or kidney transplantation. Since in adult DKD contributes to significant morbidity and mortality, an early detection of diabetic patients (especially with T1D) who are at risk of micro- or macroalbuminuria may represent a great opportunity to prevent or delay the incidence of ESRD.

Different NMR-based investigations have been performed on patients enrolled in the Finnish Diabetic Nephropathy (FinnDiane) study, pointing out clear distinct metabolic characteristics of T1D related to DKD[71-74]. In 2006, the ^{1}H NMR profiles of serum samples from 182 T1D subjects (73 with normoalbuminuria, 16 with microalbuminuria, and 93 with macroalbuminuria) were compared to those from 21 non-diabetic controls[71]. Samples were analyzed without any pretreatment and two types of ^{1}H NMR experiments were performed on each specimen in order to explore separately two different molecular classes: lipoprotein lipids (LIPO) and low-molecular-weight metabolites (LMWM). The NMR spectrum of serum recorded in the LIPO window was dominated by broad bands arising from fatty acids in TGs, cholesterol compounds, phospholipids in various lipoprotein particles, and albumin. The spectrum of serum recorded in the LMWM window exhibited peaks from BCAA, some organic acids (citric, acetic and lactic acids), glucose, creatinine, choline, and *N*-acetyl protons of mobile *N*-acetylated carbohydrate side-chains of glycoproteins. Spectroscopic and biochemical data were analyzed at three different levels. First, a pairwise association between the two type of data was explored. Second, the quantitative nature of NMR data was investigated. Finally, models aiming to separate non-diabetic controls from T1D patients were constructed. Overall, the good correlation between biochemical variables and NMR spectra (us-

ing Spearman correlation coefficient) and the high predictive values of models built with NMR data highlighted the diagnostic potential of the NMR metabolomics for diabetic nephropathy[71].

An extension of the abovementioned NMR-based study was later performed on a population of 613 T1D subjects, among which 251 with normoalbuminuria, 137 with microalbuminuria, and 225 with macroalbuminuria[72]. To structure and categorize the metabolic features within these NMR data, a self-organizing map (SOM)-based analysis was carried out, pointing out complex interactions between DKD, insulin resistance, diabetic retinal disease (DRD), macrovascular diseases (MVDs), and metabolic syndrome (MetS). These pathophysiological processes were found to share many features of the same biochemical basis. For instance, plasma metabolic profiles of patients with a detectable loss in kidney function (i.e., elevated creatinine and urea, decreased serum albumin) partially overlapped with those of subjects with insulin resistance and related impairment in glucose metabolism (dyslipidemia, high insulin dose, high HbA1c, elevated lactate and acetate and increased fasting glucose). Nevertheless, as compared to patients with normoalbuminuria, patient with DKD exhibited higher contents of TGs, creatinine, and urea and lower levels of high-density lipoproteins (HDL) cholesterol and albumin in cases, while the MetS profile was richer in TGs, lactate, and acetate[72].

A similar strategy has been used in a more recent study to assess the composition of serum from 637 Danish T1D adults (297 with normoalbuminuria, 158 with microalbuminuria and 182 with macroalbuminuria) by GC×GC-TOFMS[75]. Cross-sectional associations between single metabolites and longitudinal outcomes data at follow-up (median 5.5 years) on renal events (including estimated glomerular filtration rate and albuminuria slopes, ESRD and all-cause mortality) provided evidence for an association between polyols, AA and hydroxybutyrates with renal endpoints, delineating a potential link of diabetes with the pentose phosphate pathway and gut dysbiosis[75].

A comprehensive metabolomics approach has been applied to associate the serum metabolite profiles to progression of albuminuria, taking into account three different phases, namely: i) from normal AER to micro- or macroalbuminuria; ii) from microalbuminuria to macroalbuminuria; iii) from macroalbuminuria to ESRD[73]. In total, 325 T1D Finnish patients were enrolled. In this study, the NMR analysis of serum was focused on three molecular windows: lipoprotein lipids, low-molecular-weight metabolites and lipid extracts. Correlation-based network analysis evidenced connection of many of the clinical variables with each other and to the biochemical measures, while no strong connections to lipids or other metabolites were observed, except via urinary albumin. Furthermore, the SOM analysis of data highlighted the presence of different phenotypes according to the phases of disease: the early sub-clinical phase was characterized by an increase of phospholipids, intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and unsaturated fatty acids; the accelerated progression exhibited metabolic diversity for saturated fatty acids, inflammation, and HDL metabolism; a possible role of sphingolipid pathway was proposed for the ESRD and/or premature death[73]. The latter hypothesis was further supported by the results of another blood-based metabolomics study[74]. More recently, a sophisticated serum metabolomics approach combining UPLC/MS/MS, GC/MS and random forest analysis, has evidenced the presence of 111 metabolites significantly different between progressors and non-progressors, among which erythritol, 3-phenylpropionate, and *N*-trimethyl-5-aminovalerate were found to be the best set of variables to predict early-phase diabetic nephropathy[76].

Also, urine metabolomics has exhibited its potential to asses metabolic alteration linked to DKD. LC- and GC-MS have been employed to characterize the urine metabolome of 52 T1D patients clinically defined as having a normal AER[77]. Patients were followed for a median of 5.5 years. Half of this group progressed to microalbuminuria or DKD (progressors); the other half did not show a progression in albumin excretion (non-progressors). By using logistic regression models, a distinction between progressors and non-progressors was obtained with an accuracy of 75% and a precision of 73% from LC-MS data, and an accuracy of 65% and a precision of 64% from GC-MS data. Novel biomarkers including acylcarnitines, acylglycines and metabolites related to tryptophan metabolism were found, supporting new biochemical events associated with DKD[77].

Metabolomic profiles of T1D patients who progress to ESRD have been analyzed by LC- and GC-MS[68]. One hundred fifty-eight subjects enrolled in

the Joslin Proteinuria Cohort Study were included in this study with a median follow-up of 11.5 years. Ninety-nine patients developed ESRD. The global serum metabolomic profiling revealed 9 candidate metabolite biomarkers as potential risk factors for progression to ESRD in T1D: C-glycosyltryptophan, pseudouridine, *O*-sulfotyrosine, *N*-acetylthreonine, *N*-acetylserine, N6-carbamoylthreonyladenosine, N6-acetyllysine, *N*-acetylalanine, and phenol sulfate. These metabolites correlated with one another and with the indices of tubular injury[78].

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes[79]. It is a multifaced disease with progression and clinical manifestations varying between patients, and its pathophysiological mechanisms are still unknown. Vitreous samples from 22 T1D patients (age: 46.1 ± 9.2 years; diabetes duration: 14.2 ± 6.7 years) with DR and 22 non-diabetic age-matched controls with macular hole (MH) taken as control group (age: 45.3 ± 11.5 years) have been analyzed by ^{1}H NMR. Multivariate statistical analysis of the NMR dataset showed that the main differences in the spectra of MH and DR samples were higher levels of lactate and glucose, and lower levels of galactitol and ascorbic acid in DR compared to MH. Although this study has the limitation of the absence of healthy non-diabetic controls (justified by the nature of the sample under investigation), the results provided evidence for a metabolic signature of DR likely associated with a metabolic shift due to anaerobic glycolysis and/or inflammation (lactate), polyol pathway (galactitol), ketone body formation by β-oxidation (acetate), and oxidative stress (ascorbic acid)[79].

Very recently, a novel study has been performed to profile the metabolic perturbations associated with T1D and progression of cardiovascular autonomic neuropathy (CAN), a complication characterized by impaired autonomic control of the cardiovascular system[80]. Plasma samples were drawn from 47 T1D subjects and 10 age- and sex-matched healthy controls followed for 3 years. Three main pathways were found to be correlated with CAN progression: gluconeogenesis, ornithine synthesis, and TCA cycle.

Among the various disorders associated with diabetes mellitus, there are also different oral complications such as gingivitis, periodontitis, dental caries, salivary gland dysfunction, oral infections, and oral mucosal diseases[81]. Due to its role in the oral cavity, saliva is a biofluid that can be used for disease diagnosis and monitoring, for the study of the pathogenesis of several diseases, as well as for the investigation of oral host–microbiome interactions. The salivary metabolome of 34 T1D children under six years of age has been compared to that of 34 healthy controls[82]. The case group included 20% of uncontrolled diabetic subjects according to the postprandial glucose level (> 200 mg/dL) at the time of saliva collection. Levels of different organic acids were significantly different between cases and controls. In particular, saliva of uncontrolled T1D children was characterized by high contents of lactate and acetate, suggesting their possible implication in the metabolic acidosis observed in patients with diabetic ketoacidosis. In addition, uncontrolled T1D subjects exhibited low salivary succinate levels. Since this metabolite is an intermediate of the Krebs cycle in mitochondria as well as an insulinotropic compound[83], this result could be linked to an impaired metabolism and insulin secretion produced by hyperglycaemia and diabetes[84].

Conclusions

The multifactorial nature of T1D makes the pathogenesis of this disease in many respects still obscure in the eyes of the scientific community. Indeed, although in the recent years the knowledge of the metabolic imbalances due to insulin deficiency has increased significantly, the complex relationship between the T1D-related metabolic disorders and the interplay of trigger factors (genetic and environmental factors) is not fully explained yet.

Several metabolomics and lipidomics studies have been performed in this field, providing contributions useful to generate and support hypotheses on the molecular mechanisms of T1D and to identify potential biomarkers for early detection, monitoring, and prevention of these disease. In addition to the changes in carbohydrate metabolism, the analysis of metabolome and lipidome in case-control investigations provided evidence for metabolic dysregulations at the level of other metabolites including lipids, branched-chain amino acids, aromatic amino acids, markers of oxidative stress, tryptophan, tricarboxylic acid cycle intermediates, and gut microbiota-related metabolites. Different are the variables that hindered, in some cases, the achievement of results with a high degree of agreement, such as the size of study population, age at

the time of disease onset, gender, width of the temporal window between seroconversion and T1D onset, environmental factors, to name just a few. Due to the complexity of the molecular network behind this disease and in light of the rapid technological advances in the field of systems biology over the past decades, a multi-omic data integration applied to larger cohort of patients is certainly the future direction toward progresses in the identification and validation of novel biomarkers for early detection, prevention, and treatment of T1D.

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References

1. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes. Diabetes Care 2019; 42: S13-S28
2. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet 2014; 383: 69-82.
3. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am 2010; 39: 481-497.
4. Infante M, Ricordi C. Editorial - Moving forward on the pathway of targeted immunotherapies for type 1 diabetes: the importance of disease heterogeneity. Eur Rev Med Pharmacol Sci 2019; 23: 8702-8704.
5 Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017; 389: 2239-2251.
6 American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 2004; 27: s88-s90.
7. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC. Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 2005; 48: 2206-2212.
8. www.idf.org, accessed July 30, 2020.
9 Harjutsalo V, Sund R, Knip M, Groop PH. Incidence of Type 1 Diabetes in Finland. JAMA 2013; 310: 427-428.
10 Bruno G, Maule M, Biggeri A, Ledda A, Mannu C, Merletti F, Songini M. More Than 20 Years of Registration of Type 1 Diabetes in Sardinian Children Temporal Variations of Incidence With Age, Period of Diagnosis, and Year of Birth. Diabetes 2013; 62: 3542-3546.
11. www.who.int, accessed July 30, 2020.
12. Uusitupa M, Khan T, Viguiliouk E, Kahleova H. Prevention of Type 2 Diabetes by Lifestyle Changes. Nutrients 2019; 11: 1-22.
13. Skyler JS. Prediction and prevention of type 1 diabetes: Progress, problems, and prospects. Clin Pharmacol Ther 2007; 81: 768-771.
14. Shulaev V. Metabolomics technology and bioinformatics. Brief Bioinform 2006; 7: 128-139.
15. Wishart DS. Quantitative metabolomics using NMR. Trends Anal Chem 2008; 27: 228-237.
16. Lindon JC, Nicholson JK. Spectroscopic and Statistical Techniques for Information Recovery in Metabonomics and Metabolomics. Annu Rev Anal Chem 2008; 1: 45-69.
17. Lenz EM, Wilson ID. Analytical strategies in metabonomics. J Proteome Res 2007; 6: 443-458.
18. Smolinska A, Blanchet L, Buydens LMC, Wijmenga SS. NMR and pattern recognition methods in metabolomics: From data acquisition to biomarker discovery: A review. Anal Chim Acta 2012; 750: 82-97.
19. Scalbert A, Brennan L, Fiehn O, Hankemeier T, Kristal BS, van Ommen B, Pujos-Guillot E, Verheij E, Wishart D, Wopereis S. Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. Metabolomics 2009; 5: 435-458.
20. Denery JR, Nunes AAK, Dickerson TJ. Characterization of Differences between Blood Sample Matrices. Anal Chem 2011; 83: 1040-1047.
21. Zhang A, Sun H, Wu X, Wang X. Urine metabolomics. Clin Chim Acta 2012; 414: 65-69.
22. Karu N, Deng L, Slae M, Chi A, Sajed T, Huynh H, Wine E, Wishart DS. A review on human fecal metabolomics: Methods, applications and the human fecal metabolome database. Anal Chim Acta 2018; 1030: 1-24.
23. Gardner A, Carpenter G. Salivary Metabolomics : From Diagnostic Biomarker Discovery to Investigating Biological Function. Metabolites 2020; 10: 1-23.
24. Beger RD, Dunn W, Schmidt MA, Gross SS, Kirwan JA, Cascante M, Brennan L, Wishart DS, Fiehn O. Metabolomics enables precision medicine: "A White Paper, Community Perspective". Metabolomics 2016; 12: 1-15.
25. Hu T, Zhang JL. Mass-spectrometry-based lipidomics. J Sep Sci 2018; 41: 351-372.
26. www.scopus.com, accessed July 30, 2020.
27. Arneth B, Arneth R, Shams M. Metabolomics of Type 1 and Type 2 Diabetes. Int J Mol Sci 2019; 20: 1-14.
28. Pallares-Méndez R, Aguilar-Salinas CA, Cruz-Cautista I, del Bosque-Plata L. Metabolomics in diabetes. Ann Med 2016; 48: 89-102.
29. Lu J, Xie G, Jia W, Jia W. Metabolomics in human type 2 diabetes research. Front Med 2013; 7: 4-13.
30. Sas KM, Karnovsky A, Michailidis G, Pennathur S. Metabolomics and Diabetes: Analytical and Computational Approaches. Diabetes 2015; 64: 718-732.
31. Zhang A, Qiu S, Xu H, Sun H, Wang X. Metabolomics in diabetes. Clin Chim Acta 2014; 429: 106-110.
32. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics Applied to Diabetes Research Moving From Information to Knowledge . Diabetes 2009; 58: 2429-2443.
33. Klein MS, Shearer J. Metabolomics and Type 2 Diabetes: Translating Basic Research into Clinical Application. J Diabetes Res 2016; 3898502: 1-10.
34. Oresic M. Metabolomics in the Studies of Islet Autoimmunity and Type 1 Diabetes. Rev Diabet Stud 2012; 9: 236-247.
35. Frohnert BI, Rewers MJ. Metabolomics in childhood diabetes. Pediatr Diabetes 2016; 17: 3-14.

36. Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hämäläinen AM, Korhonen S, Kimpimäki T, Sjöroos M, Ilonen J, Knip M, Simell O. Feasibility of genetic and immunological prediction of Type I diabetes in a population-based birth cohort. Diabetologia 2001; 44: 290-297.
37. Stene LC, Witsø E, Torjesen PA, Rasmussen T, Magnus P, Cinek O, Wetlesen T, Rønningen KS. Islet autoantibody development during follow-up of high-risk children from the general Norwegian population from three months of age: design and early results from the MIDIA study. J Autoimmun 2007; 29: 44-51.
38. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: The 2-year analysis of the German BABYDIAB Study. Diabetes 1999; 48: 460-468.
39. Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes. Diabetes Care 2011; 34: 1301-1305.
40. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, McDuffie RS, Hamman RF, Klingensmith G, Eisenbarth GS, Elrich HA. Newborn screening for HLA markers associated with IDDM: Diabetes autoimmunity study in the young (DAISY). Diabetologia 1996; 39: 807-812.
41. 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, Lä hde J, Suortti T , Hakalax J, Simell T , Hyöty H , Veijola R, Ilonen J , Lahesmaa R, Knip M, Simell O. Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. J Exp Med 2008; 205: 2975-2984.
42. 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. Cord serum lipidome in prediction of islet autoimmunity and type 1 diabetes. Diabetes 2013; 62: 3268-3274.
43. Lamichhane S, Ahonen L, Dyrlund TS, Dickens, AM, Siljander H, Hyöty H, Ilonen J, Toppari J, Veijola R, Hyötyläinen T, Knip M, Orešič O. Cord-blood lipidome in progression to islet autoimmunity and type 1 diabetes. Biomolecules 2019; 9: 1-9.
44. La Torre D, Seppänen-Laakso T, Larsson HE, Hyötyläinen T, Ivarsson SA, Lernmark Å, Orešič M. Decreased cord-blood phospholipids in young age-at-onset type 1 diabetes. Diabetes 2013; 62: 3951-3956.
45. Lamichhane S, Ahonen L, Dyrlund TS, Kemppainen E, Siljander H, Hyöty H, Ilonen J, Toppari J, Veijola R, Hyötyläinen T, Knip M, Orešič O. Dynamics of Plasma Lipidome in Progression to Islet Autoimmunity and Type 1 Diabetes-Type 1 Diabetes Prediction and Prevention Study (DIPP). Sci Rep 2018; 8: 1-12.
46. Ziegler A, Nepom GT. Prediction and Pathogenesis in Type 1. Immunity 2010; 332: 468-478.
47. Pflueger M, Seppänen-Laakso T, Suortti T, Hyötyläinen T, Achenbach P, Bonifacio E, Orešič M, Ziegler AG. Age- and islet autoimmunity-associated differences in amino acid and lipid metabolites in children at risk for type 1 diabetes. Diabetes 2011; 60: 2740-2747.
48. Yin J, Ren W, Chen S, Li Y, Han H, Gao J, Liu G, Wu X, Li T, Kim SW, Yin Y. Metabolic Regulation of Methionine Restriction in Diabetes. Mol Nutr Food Res 2018; 62: 1-9.
49. Deja S, BargE, MłynarzP, Basiak A, Willak-Janc E. 1H NMR-based metabolomics studies of urine reveal differences between type 1 diabetic patients with high and low HbAc1 values. J Pharm Biomed Anal 2013; 83: 43-48.
50. Nicholls AW, Mortishire-Smith RJ, Nicholson JK. NMR Spectroscopic-Based Metabonomic Studies of Urinary Metabolite Variation in Acclimatizing Germ-Free Rats. Chem Res Toxicol 2003; 16: 1395-1404.
51. Balderas C, Rupérez F J, Ibañez E, Señorans J, Guerrero-Fernández J, Casado IG, Gracia-Bouthelier R, García A, Barbas C. Plasma and urine metabolic fingerprinting of type 1 diabetic children. Electrophoresis 2013; 34: 2882-2890.
52. Wen L, Ley RE, Volchkov PV, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Jeffrey AB, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 2009; 455: 1109-1113.
53. Goffau MC De, Luopajärvi K, Knip M, Ilonen J, Ruohtula T, HärkönenT, Orivuori L, Hakala S, Welling GW, Harmsen HJ. Fecal Microbiota Composition Differs Between Children With b -Cell Autoimmunity and Those Without. Diabetes 2013; 62: 1238-1244.
54. Soyucen E, Gulcan A, Aktuglu-Zeybek A C, Onal H, Kiylim E, Aydin A. Differences in the gut microbiota of healthy children and those with type 1 diabetes. Pediatr Int 2014; 56: 336-343.
55. Culeddu N, Chessa M, Porcu M C, Fresu P, Tonolo G, Virgilio G, Migaleddu V. NMR-based metabolomic study of type 1 diabetes. Metabolomics 2012; 8: 1162-1169.
56. Galderisi A, Pirillo P, Moret V, Stocchero M, Gucciardi A, Perilongo G, Moretti C, Monciotti C, Giordano G, Baraldi E. Metabolomics reveals new metabolic perturbations in children with type 1 diabetes. Pediatr Diabetes 2018; 19: 59-67.
57. Herrera R, Manjarrez G, Nishimura E, Hernandez J. Serotonin-related tryptophan in children with insulin-dependent diabetes. Pediatr Neurol 2003; 28: 20-23.
58. Jørgenrud B, Stene LC, Tapia G, Bøås H, Pepaj M, Berg J P, Thorsby PM, Orešič M, Hyötyläinen T, Rønningen KS. Longitudinal plasma metabolic profiles, infant feeding, and islet autoimmunity in the MIDIA study. Pediatr Diabetes 2017; 18: 111-119.
59. Frohnert BI, Webb-Robertson B J, Bramer LM, Reehl SM, Waugh K, Steck AK, Norris JM, Rewers M. Predictive modeling of Type 1 diabetes stages using disparate data sources. Diabetes 2020; 69: 238-248.
60. Mattila M, Erlund I, Lee H, Niinisto S, Uusitalo U, Andrén Aronsson C, Hummel S, Parikh H, Rich SS, Hagopian W, Toppari J, Lernmark A, Ziegler AG, Rewers M, Krischer JP, Norris JM, Virtanen SV. Plasma ascorbic acid and the risk of islet autoimmunity and type 1 diabetes: the TEDDY study. Diabetologia 2020; 63: 278-286.

61. Gorissen SHM, Phillips SM. Branched-Chain Amino Acids (Leucine, Isoleucine, and Valine) and Skeletal Muscle. Nutrition and Skeletal Muscle 2019; pp. 283-298.
62. Reitzer L. Amino Acid Synthesis. Encyclopedia of Microbiology 2009; pp. 1-17.
63. Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen AM, Peet A, Tillmann V, Pöhö P, Mattila I, Lähdesmäki H, Franzosa EA, Vaarala O, de Goffau M, Harmsen H, Ilonen J, Virtanen SM, Clish CB, Orešič M, Huttenhower C, Knip M; DIABIMMUNE Study Group. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe 2015; 17: 260-273.
64. Lanza IR, Zhang S, Ward LE, Karakelides H, Raftery D, Nair KS. Quantitative metabolomics by H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. PLoS One 2010; 5: e10538.
65. Dutta T, Chai HS, Ward LE, Ghosh A, Persson X M T, Ford GC, Kudva YC, Sun Z, Asmann YW, Kocher JPA, Nail KS. Concordance of changes in metabolic pathways based on plasma metabolomics and skeletal muscle transcriptomics in type 1 diabetes. Diabetes 2012; 61: 1004-1016.
66. Dutta T, Kudva YC, Persson XMT, Schenck LA, Ford CG, Singh RJ, Carter R, Nair KS. Impact of long-term poor and good glycemic control on metabolomics alterations in type 1 diabetic people. J Clin Endocrinol Metab 2016; 101: 1023-1033.
67. Brugnara L, Vinaixa M, Murillo S, Samino S, Rodriguez M A, Beltran A, Lerin C, Davison G, Correig X, Novials A. Metabolomics approach for analyzing the effects of exercise in subjects with type 1 diabetes mellitus. PLoS One 2012; 7: 2-9.
68. Knebel B, Strassburger K, Szendroedi J, Kotzka J, Scheer M, Nowotny B, Müssig K, Lehr S, Pacini G, Finner H, Klüppelholz B, Giani G, Al-Hasani H, Roden M, for the German Diabetes Study Group. Specific metabolic profiles and their relationship to insulin resistance in recent-onset type 1 and type 2 diabetes. J Clin Endocrinol Metab 2016; 101: 2130-2140.
69. Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. Kidney Int Suppl 2018; 8: 2-7.
70. de Boer IH, Afkarian M, Rue TC, Cleary PA, Lachin JM, Molitch ME, Steffes MW, Sun W, Zinman B. Renal outcomes in patients with type 1 diabetes and macroalbuminuria. J Am Soc Nephrol 2014; 25: 2342-2350.
71. Mäkinen VP, Soininen P, Forsblom C, Parkkonen M, Ingman P, Kaski K, Groop PH, Ala-Korpela M. Diagnosing diabetic nephropathy by 1H NMR metabonomics of serum. Magn Reson Mater Physics, Biol Med 2006; 19: 281-296.
72. Mäkinen VP, Soininen P, Forsblom C, Parkkonen M, Ingman P, Kaski K, Groop PH; FinnDiane Study Group, Ala-Korpela M. 1H NMR metabonomics approach to the disease continuum of diabetic complications and premature death. Mol Syst Biol 2008; 4: 167.
73. Makinen VP, Tynkkynen T, Soininen P, Pelotla T, Kangas A J, Forsblom C, Thorn LM, Kaski K, Laatikainen R. Metabolic Diversity of Progressive Kidney Disease in 325 Patients with Type 1 Diabetes (the FinnDiane Study). J Proteome Res 2012; 11: 1782-1790
74. Mäkinen VP, Tynkkynen T, Soininen P, ForsblomC, Peltola T, Kangas AJ, Groop P H, Ala-Korpela M. Sphingomyelin is associated with kidney disease in type 1 diabetes (The FinnDiane Study). Metabolomics 2012; 8: 369-375.
75. Tofte N, Suvitaival T, Trost K, Mattila IM, Theilade S, Winther SA, Ahluwalia TS, Frimodt-Møller M, Legido-Quigley C, Rossing, P. Metabolomic Assessment Reveals Alteration in Polyols and Branched Chain Amino Acids Associated With Present and Future Renal Impairment in a Discovery Cohort of 637 Persons With Type 1 Diabetes. Front Endocrinol 2019; 10: e818.
76. Haukka JK, Sandholm N, Forsblom C, Cobb JE, Groop P, Ferrannini E. Metabolomic Profile Predicts Development of Microalbuminuria in Individuals with Type 1 Diabetes. Sci Rep 2018; 8: e13853.
77. van der Kloet FMr, Tempels FWA, Ismail N, van Der Heijden R, Kasper PT, Doorn R Van, Spijksma G, Koek M, Forsblom C, Holthoef, Groop PH, Reijemers TH, Hankemeier T. Discovery of early-stage biomarkers for diabetic kidney disease using ms-based metabolomics (FinnDiane study). Metabolomics 2012; 8: 109-119.
78. Niewczas MA, Mathew AV, Croall S, Byun J, Major M, Sabisetti VS, Smiles A, Bonventre J V, Pennathur S. Circulating Modified Metabolites and a Risk of ESRD in Patients With Type 1 Diabetes and Chronic Kidney Disease. Diabetes Care 2017; 40: 383-390.
79. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight 2017; 2: e93751.
80. Mathew AV, Jaiswal M, Ang L, Michailidis G, Pennathur S, Pop-Busui R. Impaired amino acid and TCA metabolism and cardiovascular autonomic neuropathy progression in type 1 diabetes. Diabetes 2019; 6: 2035-2044.
81. Lamster IB, Lalla E, Borgnakke WS, Taylor GW. The relationship between oral health and diabetes mellitus. J Am Dent Assoc 2008; 139 Suppl: 19S-24S.
82. De Oliveira LRP, Martins C, Fidalgo TKS, Freitas-Fernandes LB, De Oliveira Torres R, Soares AL, Almeida FCL, Valente AP, De Souza IPR. Salivary metabolite fingerprint of type 1 diabetes in young children. J Proteome Res 2016; 15: 2491-2499.
83. Attali V, Parnes M, Ariav Y, Cerasi E, Kaiser N, Leibowitz G. Regulation of insulin secretion and proinsulin biosynthesis by succinate. Endocrinology 2006; 147: 5110-5118.
84. Haythorne E, Rohm M, van de Bunt M, Brereton MF,. Tarasov AI, Blacker TS, Sachse G, Silva Dos Santos M, Terron Exposito R, Davis S, Baba O, Fischer R. Duchen MR, Rorsman P. MacRae JI,. Ashcroft FM. Diabetes causes marked inhibition of mitochondrial metabolism in pancreatic beta-cells. Nat Commun 2019; 10: 2474.