Circulating metabolomic and lipidomic changes in subjects with new-onset type 1 diabetes after optimization of glycemic control

Josep Julve[a,b†], Idoia Genua[a,c,d†], Paola Quifer-Rada[b,e], Óscar Yanes[b,f], Maria Barranco-Altirriba[c,g,h,i], Marta Hernández[j,k], Alexandra Junza[b,f], Jordi Capellades[f], Minerva Granado-Casas[b,k], Núria Alonso[b,d,l], Esmeralda Castelblanco[m,n*], Didac Mauricio[a,b,c,n,o*]

a. Institut d'Investigació Biomèdica Sant Pau (IIB SANT PAU), 08041, Barcelona, Spain
b. CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, Barcelona, Spain
c. Department of Endocrinology & Nutrition, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
d. Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain
e. LactApp Women's Health, Barcelona, Spain
f. Universitat Rovira i Virgili. Department of Electronic Engineering & IISPV, Tarragona, Spain
g. B2SLab, Departament d'Enginyeria de Sistemes, Automàtica i Informàtica Industrial, Universitat Politècnica de Catalunya, Barcelona, Spain
h. Networking Biomedical Research Centre in the subject area of Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain
i. Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain
j. Department of Endocrinology & Nutrition, University Hospital Arnau de Vilanova, Lleida, Spain
k. Lleida Institute for Biomedical Research Dr. Pifarré Foundation (IRBLleida), University of Lleida, 25198, Lleida, Spain
l. Department of Endocrinology & Nutrition, University Hospital Germans Trias i Pujol, 08916 Badalona, Spain
m. Endocrinology, Metabolism and Lipid Research Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
n. Unitat de Suport a la Recerca Barcelona, Institut Universitari d'Investigació en Atenció Primària Jordi Gol i Gurina (IDIAP Jordi Gol), 08007 Barcelona, Spain
o. Department of Medicine, University of Vic - Central University of Catalonia, Vic, Spain

† Josep Julve and Idoia Genua contributed equally to this study.

* Correspondence:

Didac Mauricio: didacmauricio@gmail.com
Postal address: C/ Sant Quintí, 89, 08041, Barcelona, Spain
Esmeralda Castelblanco: esmeraldacas@gmail.com
Postal address: 660 S. Euclid Ave., St. Louis, MO 63110, USA

ABSTRACT

Aims: To uncover novel candidate metabolomic and lipidomic biomarkers in newly-diagnosed type 1 diabetes (T1DM) after achieving optimal glucose control.

Methods: Comprehensive lipidomic and metabolomic analysis was performed in serum of 12 adults with T1DM at onset and after achieving optimal glycemic control (HbA1c <7%) (after 2-6 months).

Results: After intensive therapy, subjects (mean age 25.2 years, 58.3% men) showed decreases in blood glucose ($p<0.001$), HbA1c [11.5% (9.2 - 13.4) to 6.2% (5.2 – 6.7); $p<0.001$] and changes in 51 identified lipids. Among these changes, we found that triglycerides (TG) containing medium chain fatty acids (TG45:0, TG47:1), sphingomyelins (SM) (SM(d18:2/20:0), SM42:4)), and phosphatidylcholines (PC) (PC(O-26:2), PC(O-30:0), PC(O-32:0), PC(O-42:6), PC(O-44:5), PC(O-38:3), PC(O-33:0), PC(O-46:8), PC(O-44:6), PC(O-40:3), PC(O-42:4), PC(O-46:7), PC(O-46:6), PC(O-44:5), PC(O-42:3), PC(O-44:4)) decreased; whereas PC(35:1), PC(37:1) and TG containing longer chain fatty acids (TG(52:1), TG(55:7), TG(51:2), TG(53:3), TG52:2), TG(53:2), TG(57:3), TG(61:3), TG(61:2) increased. Further, dihydro O-acylceramide (18:1/18:0/16:0), diacylglycerophosphoethanolamine (PE(34:1)), diacylglycerophosphoinositol (PI(38:6), and dihydrosphingomyelins (dihydroSM(36:0), dihydroSM(40:0), dihydroSM(41:0), dihydroSM(42:0)) increased. Uric acid, mannitol, and mannitol-1-acetate levels also increased.

Conclusions: Our data uncovered potential favorable changes in the metabolism of glycerophospholipids, glycerolipids, and sphingolipids in new-onset T1DM after achieving optimal glycemic control. Further research on their potential role in developing diabetes-related complications is needed.

Keywords: translational research, impaired insulin signaling, fatty acids, lipid metabolism, type 1 diabetes

1. Introduction

Type 1 diabetes mellitus (T1DM) is characterized by severe insulin deficiency due to the autoimmune destruction of pancreatic β-cells that results in persistent hyperglycemia and changes in protein and lipid metabolism [1]. If not effectively treated, multiple chronic tissue-specific complications can occur that are associated with increased morbidity and mortality [1]. People with T1DM are treated with insulin with the aim of achieving optimal glycemic control to prevent such complications. However, despite technological advances, achieving and maintaining glycemic control remains challenging [2].

Traditional biomarkers for the diagnosis and monitoring of T1DM are mainly related to glucose metabolism [3]. However, there remains a need to identify novel circulating biomarkers that can improve the accuracy of the diagnosis, therapeutic monitoring, and risk prediction of T1DM (i.e. identifying subjects with T1DM at high risk of developing chronic complications). In this context, interest in the area of lipidomics has been growing in different metabolic conditions [4] [5]. Serum lipidomic profiles may provide a clinically relevant strategy to aid in the management of T1DM including determining the effect of antidiabetic therapies and the progression of adverse pathophysiological outcomes [6,7]. For instance, up to 15 triglyceride (TG) species have been found differentially elevated in T1DM (mainly TG(50:1) and TG(50:3)) compared to subjects without diabetes [5]. In contrast, the relative concentrations of up to 6 phosphatidylcholines (PC), a phospholipid species [8], were aberrantly elevated in the serum of subjects with T1DM [5], with the PC(36:4) and PC(36:5) species being the most reported PC class. However, the favorable changes, if any, in specific serum lipid signatures in response to glycemic optimization still needs to be addressed.

In a study carried out by our group [9], we found significant changes in advanced circulating lipoprotein profile after achieving optimal glycemic control in subjects with newly diagnosed T1DM. We hypothesized that additional lipidomic and metabolomic changes occur with the optimization

89 of glycemic control after the onset of type 1 diabetes, uncovering new potential biomarkers. Thus,
90 in the present study, we aimed at investigating the lipidome and metabolome of newly diagnosed
91 T1DM subjects in serum samples at onset and after up to 6 months of intensive glycemic therapy.
92
93

2. Subjects, material and methods

2.1. Study design, participants, and sample collection

This was a prospective before-and-after observational study conducted under normal clinical practice conditions at the University Hospital Arnau de Vilanova (Lleida) and University Hospital Germans Trias i Pujol (Badalona) in Spain. Patients who were referred at onset of T1DM between February 2016 and January 2018 were invited to participate; 18 subjects agreed to be part of the study. Four did not meet the inclusion criteria (one with age below 18, one had subclinical atherosclerosis, one did not reach the HbA1c<7% goal, and one had concomitant acromegaly). The other 2 subjects were lost to follow up. As they were patients from the diabetes clinics, the ones that did not participate in the study continued with routine diabetes follow-up. Thus, twelve adult subjects (≥18 years old) were included at the clinical onset of T1DM. Subjects did not have carotid atherosclerosis (absence of carotid plaques on ultrasound), and serum sampling was done after full recovery from ketosis. Exclusion criteria were a prior diagnosis of other classic cardiovascular risk factors (including obesity, hypertension, and dyslipidemia), except for hyperglycemia itself and the presence of ketoacidosis or ketosis (defined as ketonemia >0.5 mmol/L) to avoid selecting subjects with advanced decompensation.

The following clinical data were collected: sex, age, tobacco use, weight, height, body mass index (BMI), waist circumference, blood pressure and heart rate. Standard biochemical analysis included glucose and glycated hemoglobin (HbA1c), lipid profile and estimated glomerular filtration rate calculated according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [10]. Data were collected at T1DM onset, and after normalization of glycemic control (defined as HbA1c <7% (53 mmol/mol)) at 2-6 months after the treatment. All subjects were treated with a basal-bolus regimen of insulin.

The Ethics Committees of both participating centers (CEIC-1571 and PI-15-147) approved the study following the principles of the Declaration of Helsinki. All participants were informed and provided written informed consent before inclusion.

2.2. Lipidomic and metabolomic analysis

Serum samples for lipidomic and metabolomic analysis were collected at the start of treatment and after normalization of glycemic control (after a variable period of 2-6 months) and stored at -80ºC until analysis.

Sample extraction was performed with a biphasic extraction of 200 µL of serum samples diluted with 300 µL of phosphate buffered saline (PBS) at pH 7.4 and 50 µL of heavy water (deuterium oxide [D_2O]) used for H-NMR analysis [9]. To 140 µL of diluted serum (equivalent to 50 µL non-diluted serum), 220 µL of cold methanol was added. Subsequently, 440 µL of dichloromethane was added, followed by 140 µL of water. After vortexing, samples were kept on ice for 20 minutes (min). To separate the phases, samples were centrifuged for 10 min at 14,500 rpm at 4ºC. 400 µL of the organic phase was evaporated to dryness under a stream of N_2 gas and reconstituted with 150 µL of methanol: toluene (9:1, v:v) before LC-MS analysis.

For lipidomic analysis, liquid chromatography–high resolution mass spectrometry (LC-HRMS) analysis was performed using a UHPLC system (1200 Series, Agilent Technologies) coupled to an MS system (Agilent Technologies series 6550 ESI-QTOF MS system) operating in positive electrospray ionization mode (ESI +). Lipids were separated by reverse phase chromatography using an Acquity UPLC BEH C18 column (2.1×150 mm, 1.7 µm, Waters Corporation) and a mobile gradient phase consisting of 10 mM ammonium formate in acetonitrile: water (60:40, v:v) as mobile phase A, and 10 mM ammonium formate in isopropanol: acetonitrile (90:10, v:v) as a mobile phase B. The chromatographic gradient was the following: from 0 to 2 min, the percentage of B raised from 15%

to 30%, from 2 to 2.5 min %B increased to 48%, until min 11% B kept increasing to 82% until 11.5% B raised to 99% min, isocratic for 30 s at 99% B, from 12 to 12.1 min decreased quickly to 15% B and finally column equilibrated at 15% B until 15 min. The flow of the method was 0.4 mL/min. The column temperature was 65ºC, and the injection volume was 2 µL. All samples were analyzed once. The parameters for the electrospray ionization source (ESI) were as follow: temperature and flow of the drying gas, 150°C and 11 L/min; nebulizer, 35 psig; temperature and flow of sheath gas, 350°C and 11 L/min; capillary voltage, 3,500 V, fragmentor, 120 V; and skimmer, 65 V. The *m/z* acquisition range was set between 50 and 1200, acquiring 3 spectra/s. To identify compounds, MS/MS data were generated in targeted mode and the instrument was set to acquire spectra over the *m/z* range from 50 to 1200, with a narrow width of 1.3 *m/z*. The collision energies used were 10, 20, 30 and 40 V.

For metabolomic analysis, liquid chromatography–high resolution mass spectrometry (LC-HRMS) analysis was performed using a UHPLC system (Vanquish, Thermo Scientific) coupled to an MS system (THermo QExactive Orbitrap MS system) operating in positive and negative electrospray ionization mode (ESI +/ESI -). Metabolites were separated using an Acquity UPLC BEH HILIC column (2.1×150 mm, 1.7 µm, Waters Corporation) and a mobile gradient phase consisting of 50 mM ammonium acetate in water as mobile phase A, and acetonitrile as a mobile phase B. The chromatographic gradient was the following: from 0 to 2 min, the percentage of A was maintained at 2%, from 2 to 9 min % A increased to 40 %, in 30 s % A decreased to 2% and finally column equilibrated at 2 % A until 13 min. The flow of the method was 0.4 mL/min. The column temperature was 25ºC, and the injection volume was 2 µL. All samples were analyzed once.

The parameters for the electrospray ionization source (ESI) were as follow: Spray voltages were 3200 and 3000 V in positive and negative mode, respectively; capillary temperature 300°C; flow rates of sheat and sweep gases were 50 and 1 mL/min, respectively. Temperature and flow of auxiliary gas,

270°C and 20 L/min. The *m/z* acquisition range was set between 100 and 1000. The collision energy (EC) used for identifying compounds was 45 % ECmax.

The metabolite features were identified by looking for their characteristic *m/z* in databases, mainly Lipid Maps, Metlin, and the Human Metabolome database. Lipid Maps gave a list of possible lipids and their respective adducts. To discern the correct identification, the MS2 spectra were comprehensively studied. Elucidation of the lipids was performed by analyzing the pattern of fragmentation of the different lipid families (supplementary Figure 1) and using Chemdraw to draw and assign the structures to each fragment of the MS2 spectra.

2.3. Data processing and statistical analysis

Baseline characteristics of study participants were compared with those excluded using a non-parametric U Mann Whitney test. For lipidomic and metabolomic analysis, raw data acquired in each analytical batch were converted from the instrument-specific format to the mzML file format by applying the open access ProteoWizard (version 3.0.11417) msconvert tool [11]. During this procedure, peak picking and centroiding were achieved using vendor algorithms. Isotopologue Parameter Optimization (IPO - version 1.0.0, using XCMS - version 1.46.0) [12] was used to automatically optimize XCMS [13] peak picking parameters. For each sample, a data matrix of metabolite features (m/z-retention time pairs) was constructed from raw LC-HRMS lipidomic data. Total (ESI +/ESI -) ion chromatograms showing different features obtained for lipidomics (C18+) (panel A) and metabolomics (HILIC-) (panel B) analysis at baseline and at follow-up were shown in supplementary Figure 2.

The preprocessed lipidomic data were further filtered by intensity and quality control (QC) criteria. The intensity threshold was set at 10,000 arbitrary units as the minimum suitable intensity for MS/MS experiments. The data for pooled QC samples were applied to perform QC filtering.

Metabolite features with lower RSD (relative standard deviation) in samples than in QC were deleted from the dataset. Features present in less than 80% of samples were also removed.

Before univariate statistical analysis, data were normalized by probabilistic quotient normalization (PQN) [14]. To assess lipidomic changes before and after the intensive treatment, paired Wilcoxon nonparametric test was used due to the low number of participants (less than 30), and a false discovery rate (FDR) correction was performed to correct false positive results ($q<0.05$). The preprocessed data obtained by XCMS in positive and negative ionization was further filtered by sample representativeness using the 80% rule and by intensity criteria and coefficient of variation (CV). The 80% rule consists of retaining those mzRT features found in 80% of the samples or at least in one experimental group. In the CV criteria filtration, a variation of 20% was the minimum accepted variation across samples to consider a biological variation. Additionally, the intensity threshold was set at 30,000. As this was an exploratory study, a formal sample size calculation was not done.

3. Results

3.1. Baseline characteristics of participants

Table 1 shows the baseline and follow-up clinical data of the participants. Most participants were male (58.3%), with a mean age of 25.2 years. As set out in the study protocol, none of the subjects had hypertension or dyslipidemia. The median time to achieve optimal glycemic control was 97.5 days (range: 79 to 179 days). The demographic, clinical and biochemical characteristics of subjects used in the current study did not differ from those who were excluded (Supplementary Table 1).

As expected, blood glucose levels and HbA1c significantly decreased after intensive treatment (p-value <0.001). There was also a significant decrease in total cholesterol and LDL cholesterol (LDLc) concentrations and a significant increase in mean BMI. No changes in other relevant clinical variables were seen after achieving optimal glycemic control.

3.2. Changes in serum lipid and metabolites after glycemic optimization (follow-up)

Compared with the baseline (HbA1c median = 11.5% (102 mmol/mol); min 9.2% (77 mmol/mol) to max 13.4% (123 mmol/mol)), achievement of optimal glycemic control (HbA1c median = 6.2% (44 mmol/mol); min 5.2% (33 mmol/mol) to max 6.7% (50 mmol/mol) resulted in changes in 123 lipids and 69 non-lipid metabolites from the two ionization modes. Up to 51 lipids and 5 non-lipid metabolites were identified with a mass accuracy lower than 8 ppm **(Table 2, Figure 1)**. After glycemic optimization, most glycerophosphocholine species, including phosphatidylcholine, lysophosphatidylcholine (PC(O-26:2/0:0)), and other phosphatidylcholine derivatives, significantly decreased compared to baseline. Other lipid species, including diacylglycerols, phosphatidylethanolamine, phosphatidylinositol, acylceramide, sterols, monoacylglycerol, and most of the triacylglycerols increased upon glycemic optimization. Some sphingomyelin (SM)

species were also higher at the onset of T1DM, whereas some dihydrosphingomyelin species were higher at follow-up. The relative abundance of cholesterol ester decreased from baseline to follow-up (**Table 2 and Figure 1).**

Beyond serum lipids, our analysis also identified changes in non-lipid metabolites in response to improved glycemic control. Serum glucose and gluconic acid were lower at follow-up, whereas levels of mannitol, mannitol acetate, and uric acid increased.

Figure 1 shows a bar plot containing each identified lipid or metabolite that significantly changed at follow-up and the fold-change (FC) from baseline to follow-up. The positive FCs (blue bars) correspond to those lipids significantly downregulated at T1DM onset that increased on glycemic optimization. The negative FCs (red bars) show the FC values for lipids significantly upregulated at baseline that decreased after glycemic optimization.

4. Discussion

In this study, we found changes in the serum lipidomic profile of subjects who achieved an HbA1c <7% after the onset of T1D. Besides glucose, other serum metabolites may be related to adverse derangements and protection against atherosclerosis in T1DM [15]. In line with our findings, the relative levels of different TG and phosphatidylcholine species were found to be altered in T1DM compared with subjects without diabetes [5], thereby demonstrating that individual lipid species are modified upon glucose control.

One of the most significant differences occurred in the long-chain fatty acid-containing triglycerides, which relatively increased after optimal glycemic control was achieved. This finding could be, at least in part, attributed to the favorable influence of insulin therapy on the gene expression of the mitochondrial isoform 1 of the glycerol-3-phosphate (G3P) acyltransferase-1 (GPAT1) [16]. GPAT isoforms catalyze a critical reaction in tissue TG synthesis, i.e., esterification of G3P to long-chain acyl-CoA [17]. In particular, GPAT1, which is highly expressed in the liver [18,19], might contribute to the relative elevations in circulating long-chain fatty acid-containing TGs found at follow-up. In addition to insulin-stimulated overexpression of GPAT1, this enzyme isoform is also controlled by the transcription factor-designated sterol regulatory element-binding protein (SREBP)-1c in the liver [20]. Interestingly, the expression of SREBP-1c is upregulated by insulin [21,22], which might further increase the hepatic expression of GPAT1 in T1DM subjects at follow-up. Conversely, our data also revealed significant relative reductions of two TG species, i.e., TG (45:0) and TG (47:1), after glycemic optimization. Although the content in specific fatty acyl groups was not further dissected in our study, the formula of these TG species suggests the presence of medium-chain fatty acids (less than C12) in their composition. Hydrolysis of TG by lipoprotein lipase (LPL) is more efficient in small and medium-chain fatty acids-containing TG mixtures than in long-chain fatty acid-containing TG mixtures [23]. In support of this, the LPL activity, which is strongly influenced by insulin, is reduced

in insulin-deficient states, as in T1DM, but increases upon insulin therapy [24]. Thus, our data could suggest enhanced LPL-mediated hydrolysis, especially of serum small and medium-chain fatty acid-containing TGs in T1DM subjects after introducing and optimizing insulin therapy.

The other leading altered lipid group was that of glycerophosphocholines. Of the 20 phosphatidylcholines that decreased at follow-up, 15 were ether-linked phosphatidylcholines. Ether-linked lipids contain an ether instead of an ester linkage between the glycerol backbone and one or both fatty acid chains. The role of this type of lipid in metabolic and cardiovascular diseases remains poorly defined. However, ether-linked phosphatidylcholines have been found to be elevated, acting as an antioxidant in response to oxidative stress in adverse metabolic conditions such as obesity [25]. This could be a plausible explanation for the increased levels of ether-linked phosphatidylcholines found in newly diagnosed T1DM subjects and their reduction after achieving glycemic control. On the other hand, phosphatidylcholines are the primary source of choline in the body. Choline is critical in epigenetic regulation [26] and modulates the secretion of TG-rich lipoproteins, influencing serum TG concentrations [27].

In the case of sphingolipids, we found that three SM species were significantly decreased at follow-up, while four dihydro-SM species were increased after treatment. The reason for changes in the concentration of these sphingolipids is unknown; however, SM may be a source of ceramides [28], a bioactive sphingolipid involved in diabetes-associated lipotoxicity in multiple target tissues, including the heart [29].

The upregulation of sterols (i.e., cholesterol, C27H460; and pregna-5-20-dien-3beta-ol, C20H300) and downregulation of cholesterol esters (CE(18:2)) following glycemic optimization are also novel findings that deserve to be carefully analyzed. The liver is one of the main contributors of circulating cholesterol under fasting conditions [30], and therefore the relative rise of circulating sterols could be due to increased hepatic cholesterol production. Significantly, hepatic *de novo* synthesis of

cholesterol is selectively influenced by SREBP-2 [31]. Decreased SREBP-2 activity strongly depends on hepatic insulin signaling [32], which could be attenuated in the liver of T1DM subjects at baseline, being reestablished upon insulin therapy. On the other hand, decreased levels of cholesterol esters might be potentially related to the favorable influence of glycemic optimization on serum enzymes involved in lipoprotein remodeling and metabolism [33]. Circulating cholesterol is mainly transported by LDL, HDL and its esterified form in human serum. Thus, the decreased levels of serum CE18:2 in treated T1DM subjects could be linked to reduced serum LDLc concentrations at follow-up.

Metabolomic analyses further showed a significant decrease in glucose and gluconic acid and a significant increase in uric acid, mannitol, and mannitol-1-acetate after treatment. The significant decrease in serum glucose deserves no further comment. Likewise, gluconic acid, which is a product of glucose oxidation and is increased in diabetes conditions in humans [34], was decreased upon follow-up.

The association between uric acid and diabetes is controversial. However, a study of 14,144 subjects concluded that increased serum uric acid is negatively associated with diabetes mellitus [35], a finding that is in line with the results of the present study. Furthermore, another study demonstrated a negative association between serum uric acid and fasting blood glucose concentration, and significantly lower serum uric acid levels were found in a group of subjects with diabetes compared to healthy and prediabetic subjects [36]. A possible mechanism for the negative association between serum uric acid levels and diabetes mellitus may be linked to a decreased reabsorption of uric acid in the kidney's proximal tubule caused by high blood glucose levels [35,36]. This has been shown when comparing both plasma uric acid levels and fractional uric acid excretion between T1DM patients and healthy controls, as well as the relationship between both variables and hyperglycemia [37].

Finally, the mannitol and mannitol-1-acetate increase at follow-up may be related to diet changes. Mannitol is a sugar alcohol used as an alternative sweetener for people with diabetes [38].

The strength of this pilot study was its prospective longitudinal design. Limitations included the relatively small sample size, as only those T1DM subjects with HbA1c <7.0 % (53 mmol/mol), i.e., reaching an optimal glycemic control, were analyzed. Moreover, the contribution of different host- and environment-related variables, including dietary changes during the follow-up, could impact lipid profiles [39]. Taken together, we acknowledge that our results must be interpreted cautiously and confirmed in future studies. However, the observed fold changes were clearly significant and particularly large in a number of differentially regulated metabolites, most of them lipids, in serum from T1DM subjects at follow-up.

In conclusion, we have found potentially relevant beneficial changes in the lipidomic and metabolomic profile of subjects with T1DM after glycemic control. Further, our findings identified different circulating metabolites that may be involved in the development of chronic complications associated with diabetes. Additional studies are warranted to determine whether these novel biomarkers are associated with future risk of diabetic complications in T1DM subjects with poor glycemic regulation.

List of abbreviations

BMI: body mass index; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CVD: cardiovascular disease; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; FCs: fold-changes; HbA1c: glycosylated hemoglobin; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; PC: phosphatidylcholines; SBP: systolic blood pressure; SM: sphingomyelin; T1DM: type 1 diabetes mellitus; TG: triglycerides.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (Madrid, Spain) grant PI15/0625 (to DM and EC), PI17/01362 (to NA), and PI17/00232 (to JJ), FEDER “Una manera de hacer Europa”, and by Fundació La Marató de TV3 2016 (303/C/2016) (201602.30.31) (to NA and JJ). This research was supported by CIBER-Consorcio Centro de Investigación Biomédica en Red-CIBERDEM (CB15/00071), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación. Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau is accredited by the Generalitat de Catalunya as Centre de Recerca de Catalunya (CERCA).

Authors' contributions

EC and DM designed and conceptualized the study. EC, MH, MG-C, NA and JJ contributed to researched data; IG, PQ, JJ, OY, MB, MH, AJ, JC, MG-C, JR, NA, EC and DM analyzed and interpreted data; JJ, MG-C, EC and DM contributed to the discussion and reviewed the manuscript; IG, MB, PQ and JJ wrote the original draft of the manuscript; IG, JJ, MG-C, NA, EC and DM reviewed/edited the manuscript; DM supervised the study; funding acquisition was provided by NA, JJ, EC and DM. All authors read and approved the manuscript.

Data availability statement

Proposals relating to the data access should be directed to the corresponding author. To gain access, data requestors will need to sign a data access agreement.

Acknowledgements

We want to particularly acknowledge the participants, and the IGTP-HUGTP (B.0000643) and IRBLleida Biobank (B.0000682) integrated in the PLATAFORMA BIOBANCOS (PT20/00050 and PT20/00021, respectively). The authors are also thankful to the COST Action EpiLipidNET, CA19105 - Pan-European Network in Lipidomics and EpiLipidomics.

References:

[1] Kerner W, Brückel J. Definition, Classification and Diagnosis of Diabetes Mellitus. Experimental and Clinical Endocrinology & Diabetes 2014;122:384–6. https://doi.org/10.1055/s-0034-1366278.

[2] Perkins BA, Sherr JL, Mathieu C. Type 1 diabetes glycemic management: Insulin therapy, glucose monitoring, and automation. Science (1979) 2021;373:522–7. https://doi.org/10.1126/science.abg4502.

[3] Watkins RA, Evans-Molina C, Blum JS, DiMeglio LA. Established and emerging biomarkers for the prediction of type 1 diabetes: a systematic review. Transl Res 2014;164:110–21. https://doi.org/10.1016/j.trsl.2014.02.004.

[4] Wang M, Wang C, Han RH, Han X. Novel advances in shotgun lipidomics for biology and medicine. Prog Lipid Res 2016;61:83–108. https://doi.org/10.1016/j.plipres.2015.12.002.

[5] Suvitaival T. Lipidomic Abnormalities During the Pathogenesis of Type 1 Diabetes: a Quantitative Review. Curr Diab Rep 2020;20:46. https://doi.org/10.1007/s11892-020-01326-8.

[6] Ritchie RH, Zerenturk EJ, Prakoso D, Calkin AC. Lipid metabolism and its implications for type 1 diabetes-associated cardiomyopathy. J Mol Endocrinol 2017;58:R225–40. https://doi.org/10.1530/JME-16-0249.

[7] Eid S, Sas KM, Abcouwer SF, Feldman EL, Gardner TW, Pennathur S, et al. New insights into the mechanisms of diabetic complications: role of lipids and lipid metabolism. Diabetologia 2019;62:1539–49. https://doi.org/10.1007/s00125-019-4959-1.

[8] Dashti M, Kulik W, Hoek F, Veerman EC, Peppelenbosch MP, Rezaee F. A Phospholipidomic Analysis of All Defined Human Plasma Lipoproteins. Sci Rep 2011;1:139. https://doi.org/10.1038/srep00139.

[9] Castelblanco E, Hernández M, Ortega E, Amigó N, Real J, Granado-Casas M, et al. Outstanding improvement of the advanced lipoprotein profile in subjects with new-onset type 1 diabetes mellitus after achieving optimal glycemic control. Diabetes Res Clin Pract 2021;182. https://doi.org/10.1016/j.diabres.2021.109145.

[10] Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12. https://doi.org/10.7326/0003-4819-150-9-200905050-00006.

[11] Chambers MC, Maclean B, Burke R, Amodei D, Ruderman DL, Neumann S, et al. A cross-platform toolkit for mass spectrometry and proteomics. Nat Biotechnol 2012;30:918–20. https://doi.org/10.1038/nbt.2377.

[12] Libiseller G, Dvorzak M, Kleb U, Gander E, Eisenberg T, Madeo F, et al. IPO: a tool for automated optimization of XCMS parameters. BMC Bioinformatics 2015;16:118. https://doi.org/10.1186/s12859-015-0562-8.

[13] Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching, and Identification. Anal Chem 2006;78:779–87. https://doi.org/10.1021/ac051437y.

[14] Dieterle F, Ross A, Schlotterbeck G, Senn H. Probabilistic Quotient Normalization as Robust Method to Account for Dilution of Complex Biological Mixtures. Application in ^{1}H NMR Metabonomics. Anal Chem 2006;78:4281–90. https://doi.org/10.1021/ac051632c.

[15] Nahmias A, Stahel P, Xiao C, Lewis GF. Glycemia and Atherosclerotic Cardiovascular Disease: Exploring the Gap Between Risk Marker and Risk Factor. Front Cardiovasc Med 2020;7. https://doi.org/10.3389/fcvm.2020.00100.

[16] Shin DH, Paulauskis JD, Moustaïd N, Sul HS. Transcriptional regulation of p90 with sequence homology to Escherichia coli glycerol-3-phosphate acyltransferase. J Biol Chem 1991;266:23834–9.

[17] Coleman RA, Lewin TM, Muoio DM. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. Annu Rev Nutr 2000;20:77–103. https://doi.org/10.1146/annurev.nutr.20.1.77.

[18] Lewin TM, Granger DA, Kim JH, Coleman RA. Regulation of mitochondrial sn-glycerol-3-phosphate acyltransferase activity: response to feeding status is unique in various rat tissues and is discordant with protein expression. Arch Biochem Biophys 2001;396:119–27. https://doi.org/10.1006/abbi.2001.2604.

[19] Hammond LE, Gallagher PA, Wang S, Hiller S, Kluckman KD, Posey-Marcos EL, et al. Mitochondrial glycerol-3-phosphate acyltransferase-deficient mice have reduced weight and liver triacylglycerol content and altered glycerolipid fatty acid composition. Mol Cell Biol 2002;22:8204–14. https://doi.org/10.1128/MCB.22.23.8204-8214.2002.

[20] Horton JD, Shimomura I. Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. Curr Opin Lipidol 1999;10:143–50. https://doi.org/10.1097/00041433-199904000-00008.

[21] Azzout-Marniche D, Bécard D, Guichard C, Foretz M, Ferré P, Foufelle F. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. Biochem J 2000;350 Pt 2:389–93.

[22] Dif N, Euthine V, Gonnet E, Laville M, Vidal H, Lefai E. Insulin activates human sterol-regulatory-element-binding protein-1c (SREBP-1c) promoter through SRE motifs. Biochem J 2006;400:179–88. https://doi.org/10.1042/BJ20060499.

[23] Bach AC, Babayan VK. Medium-chain triglycerides: an update. Am J Clin Nutr 1982;36:950–62. https://doi.org/10.1093/ajcn/36.5.950.

[24] Taskinen M-R. Lipoprotein lipase in diabetes. Diabetes / Metabolism Reviews 1987;3:551–70. https://doi.org/10.1002/dmr.5610030208.

[25] Donovan EL, Pettine SM, Hickey MS, Hamilton KL, Miller BF. Lipidomic analysis of human plasma reveals ether-linked lipids that are elevated in morbidly obese humans compared to lean. Diabetol Metab Syndr 2013;5:24. https://doi.org/10.1186/1758-5996-5-24.

[26] Zeisel SH. Dietary choline deficiency causes DNA strand breaks and alters epigenetic marks on DNA and histones. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 2012;733:34–8. https://doi.org/10.1016/j.mrfmmm.2011.10.008.

[27] Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 2007;45:1366–74. https://doi.org/10.1002/hep.21655.

[28] Gault CR, Obeid LM, Hannun YA. An Overview of Sphingolipid Metabolism: From Synthesis to Breakdown, 2010, p. 1–23. https://doi.org/10.1007/978-1-4419-6741-1_1.

[29] Kovilakath A, Jamil M, Cowart LA. Sphingolipids in the Heart: From Cradle to Grave. Front Endocrinol (Lausanne) 2020;11. https://doi.org/10.3389/fendo.2020.00652.

[30] Persson L, Cao G, Ståhle L, Sjöberg BG, Troutt JS, Konrad RJ, et al. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. Arterioscler Thromb Vasc Biol 2010;30:2666–72. https://doi.org/10.1161/ATVBAHA.110.214130.

[31] Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 2004;86:839–48. https://doi.org/10.1016/j.biochi.2004.09.018.

[32] Miao J, Haas JT, Manthena P, Wang Y, Zhao E, Vaitheesvaran B, et al. Hepatic insulin receptor deficiency impairs the SREBP-2 response to feeding and statins. J Lipid Res 2014;55:659–67. https://doi.org/10.1194/jlr.M043711.

[33] Vergès B. Dyslipidemia in Type 1 Diabetes: A Masked Danger. Trends in Endocrinology & Metabolism 2020;31:422–34. https://doi.org/10.1016/j.tem.2020.01.015.

[34] Chen L, Cheng C-Y, Choi H, Ikram MK, Sabanayagam C, Tan GSW, et al. Plasma Metabonomic Profiling of Diabetic Retinopathy. Diabetes 2016;65:1099–108. https://doi.org/10.2337/db15-0661.

[35] Bandaru P, Shankar A. Association between Serum Uric Acid Levels and Diabetes Mellitus. Int J Endocrinol 2011;2011:1–6. https://doi.org/10.1155/2011/604715.

[36] Haque T, Rahman S, Islam S, Molla NH, Ali N. Assessment of the relationship between serum uric acid and glucose levels in healthy, prediabetic and diabetic individuals. Diabetol Metab Syndr 2019;11:49. https://doi.org/10.1186/s13098-019-0446-6.

[37] Lytvyn Y, Škrtić M, Yang GK, Yip PM, Perkins BA, Cherney DZI. Glycosuria-mediated urinary uric acid excretion in patients with uncomplicated type 1 diabetes mellitus. American Journal of Physiology-Renal Physiology 2015;308:F77–83. https://doi.org/10.1152/ajprenal.00555.2014.

[38] MSWolever T, Piekarz A, Hollands M, Cde R, Younker K, Cde MR. Sugar Alcohols and Diabetes: A Review. vol. 26. 2002.

[39] Hyötyläinen T, Orešič M. Optimizing the lipidomics workflow for clinical studies—practical considerations. Anal Bioanal Chem 2015;407:4973–93. https://doi.org/10.1007/s00216-015-8633-2.

FIGURE LEGENDS

Figure 1. Fold-change from baseline to follow-up in the levels of lipid and non-lipid metabolites that significantly changed (FDR q-value < 0.05) after achievement of optimal glycemic control.

Figure foot 1

The positive FCs (blue bars) correspond to those lipids significantly increased at follow-up. The negative FCs (red bars) show the FC values for lipids significantly decreased at follow-up. CE, Cholesterol ester; DG, Diacylglycerol; MG, Monoacylglycerols; PE, Phosphatidylethanolamine; PC, Glycerophosphocholines; PI, Phosphatidylinositol; SM, Sphingomyelin; TG, Triacylglycerols.

Supplementary Figure 2. Total Ion Chromatograms showing of different features obtained for lipidomics (C18+) (panel A) and metabolomics (HILIC-) (panel B) analysis. All datasets are represented. Red: follow-up; soft blue: baseline.