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Dry blood spots as a sampling strategy to identify insulin resistance markers during a dietary challenge

Stephany Gonçalves Duarte[1], Carlos M. Donado-Pestana[1,2], Tushar H. More[3], Larissa Rodrigues[1], Karsten Hiller[3] and Jarlei Fiamoncini[1,2*]

Abstract

This study aimed to identify markers of postprandial dysglycemia in the blood of self-described healthy individuals using dry blood spots (DBS) as a sampling strategy. A total of 54 volunteers, including 31 women, participated in a dietary challenge. They consumed a high-fat, high-sugar mixed meal and underwent multiple blood sampling over the course of 150 min to track their postprandial responses. Blood glucose levels were monitored with a portable glucometer and individuals were classified into two groups based on the glucose area under the curve (AUC): High-AUC (H-AUC) and Low-AUC (L-AUC). DBS sampling was performed at the same time points as the assessment of glycemia using Whatman 903 Protein Saver filter paper. A gas chromatography-mass spectrometry-based metabolite profiling was conducted in the DBS samples to assess postprandial changes in blood metabolome. Higher concentrations of metabolites associated with insulin resistance were observed in individuals from the H-AUC group, including sugars and sugar-derived products such as fructose and threonic acid, as well as organic acids and fatty acids such as succinate and stearic acid. Several metabolites detected in the GC-MS analysis remained unidentified, indicating that other markers of hyperglycemia remain to be discovered in DBS. Based on these observations, we demonstrated that the use of DBS as a non-invasive and inexpensive sampling tool allows the identification of metabolites markers of dysglycemia in the postprandial period.

Keywords Microsampling, Postprandial, Metabolomics, Dietary challenge, Nutritional physiology

Introduction

In recent years, there has been increasing interest in understanding metabolic responses to food and nutrient intake due to their potential for detecting metabolic dysregulation. The physiological changes that take place after the ingestion of a meal (nutrient absorption, modulation of lipolysis, gluconeogenesis, glycogenesis, etc.) are largely regulated by insulin, and thus, the assessment of postprandial physiology can serve as a valuable tool to investigate the state of insulin sensitivity. Previous studies have reported metabolic differences in the postprandial period between groups of individuals that showed no differences in the fasted state [1–3].

*Correspondence:
Jarlei Fiamoncini
jarlei@usp.br
[1]Department of Food Science and Experimental Nutrition, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 14, São Paulo, SP CEP 05508-900, Brazil
[2]Food Research Center - FoRC, University of São Paulo, São Paulo, Brazil
[3]Braunschweig Integrated Centre of Systems Biology, Technische Universität Braunschweig, Braunschweig, Germany

Alterations in postprandial responses are often signs of metabolic dysregulation or are a consequence of dietary interventions. Changes in circulating levels of metabolites and signaling molecules in response to a dietary challenge such as the intake of a chemically-defined meal could be used as a diagnostic tool. These alterations are more informative about the metabolic status than the analysis of plasma samples collected in non-challenged conditions such as fasting [4]. An assay that includes the intake of a chemically-defined mixture of macronutrients (mixed meal) and blood sampling at successive time points for a few hours after food intake has proven to be of great value for the assessment of phenotypic flexibility [5]. The metabolic response to a mixed meal is a more suitable tool for the assessment of β-cell function reflecting daily life conditions when compared to the standard oral glucose tolerance test (oGTT), since the mixed meal contains fatty acids and amino acids, which are metabolites that can also stimulate insulin secretion [6].

Studies of postprandial physiology involve the sampling of blood through venipuncture by trained nursing staff, a process that is invasive and expensive [7, 8]. As an alternative, dry blood spots (DBS) offer a simple, non-invasive, and inexpensive tool that facilitates the collection of capillary blood samples by non-medically trained personnel, even in home-based settings [9]. Endogenous metabolites such as organic acids, hormones, and metabolites from dietary compounds, drugs or pollutants can be detected in DBS samples [10]. Several applications of metabolomics and lipidomics have been developed and validated for the analysis of biofluids collected in this way [11–13].

Plasma glucose levels serve as a reliable clinical biomarker for diabetes and pre-diabetes, being used as the diagnostic criteria for these conditions. Unfortunately, when plasma glucose levels are increased, the disease is already established, making its management more challenging. In the last couple of decades, several biomarkers have been reported with predictive capacity for diabetes and insulin resistance. Despite the need for further investigation in population-based studies, metabolites such as mannose [14] and fructose [15] have emerged as promising candidates for identifying insulin resistance and type 2 diabetes [16]. Altered plasma levels of branched-chain amino acids (BCAA) and phospholipids have also been associated with insulin resistance and metabolic disorders such as obesity and type 2 diabetes [17, 18].

In the present study, we have sampled blood in the form of DBS from self-reported healthy individuals undergoing a dietary challenge. Fasting and postprandial DBS samples were analyzed using gas chromatography coupled with mass spectrometry (GC-MS), leading to the identification of distinct plasma metabolite profiles associated with differential glycemic responses during the dietary challenge.

Materials and methods

Study population

The Ethics Committee of the School of Pharmaceutical Sciences of University of São Paulo approved this study (CAAE: 15438019.7.0000.0067). Individuals who agreed to participate in the research signed the informed consent form. The 54 eligible subjects included men and women, aged 18 to 54 years old, and body mass index (BMI) varying between 23.7 and 25.4 kg/m². Individuals meeting the following criteria were excluded: pregnant women, those diagnosed with diabetes, and individuals with casein allergy or lactose intolerance. The use of medications or dietary supplements was not considered an exclusion criterion. Measurements of anthropometric variables including abdominal diameter, waist and hip circumferences were performed by trained examiners.

Dietary challenge, measurement of fasting and postprandial glycemia and DBS sampling

The dietary challenge was conducted at the participants' homes and at the School of Pharmaceutical Sciences of the University of São Paulo. Participants were instructed to avoid intense physical activity, alcohol, and caffeine consumption for 24 h before the dietary challenge. Additionally, it was established that participants should not ingest any food or beverage (except water) starting at 10 p.m. the night before the test. After overnight fasting (10 h), volunteers were submitted to the dietary challenge that consisted of the ingestion of a liquid mixed meal containing 75 g of glucose, 60 g of canola oil and 20 g of micellar casein dissolved in filtered water up to 200 mL. The energy content of the mixed meal was 967 Kcal.

Capillary blood was obtained by pricking the fingertips with a sterile lancet, after sanitization with an isopropylic alcohol swab. Blood glucose was measured using a portable glucometer (ACCU-CHEK Guide®, Roche Diagnostic) at the fasting state (0 min), and 30, 60, 90, 120 and 150 min after meal intake. DBS sampling was carried out at the same time points as the measurements of glucose levels. After discarding the first drop of blood, the blood drop was directly placed on the DBS card (Whatman 903 Protein Saver filter paper), to obtain a blood stain (approximately 1 cm diameter). At the end of the procedure, DBS cards were dried for 2 h at room temperature, and stored at -80 °C in a foil bag (Whatman, GE Healthcare Life Sciences) containing a sachet of silica as desiccant material.

Glycemic response calculations

The study participants were grouped into two groups based on the glucose area under the curve (AUC). The

determination of AUC involved calculating the sum of the areas of triangles and trapezoids representing the response over a 150-min period [19]. The 54 participants were then ranked according to the AUC values and the 27 participants with the lowest AUC values were designated to the *low AUC* (L-AUC) group, while the 27 with the highest AUC values were assigned to the *high AUC* (H-AUC) group.

Gas chromatography-mass spectrometry-based DBS metabolite profiling

DBS samples were subjected to untargeted metabolomics analysis by gas chromatography-mass spectrometry (GC-MS). Two punches of 3 mm were extracted with 80 µl methanol: water (8:1) and shaken for 30 min at 500 rpm. The extracts were dried at 4 °C in a centrivap (Labconco) and underwent derivatization with methoxyamine hydrochloride in pyridine for 90 min at 40 °C, followed by N-methyl-N-trimethylsilyl-trifluoroacetamide for 30 min at 40 °C. Then, 1 µL was injected into an SSL injector at 270 °C for GC-MS analysis using an Agilent 7890 A GC with a DB-35MS+5 m Duraguard column. Helium was the carrier gas at 1.0 mL/min. The GC oven temperature ramped from 80 °C to 320 °C at 15 °C/min and held for 8 min, totaling a 25 min runtime per sample. The MSD operated at 70 eV, scanning m/z 70 to m/z 800 at 5.2 sca ns/s (https://doi.org/10.3390/metabo12111058).

MS data processing

The deconvolution of mass spectra, peak picking, integration, and retention index calibration were conducted using our proprietary software 'Metabolite detector' [20]. Compounds were identified through spectral and retention index similarity, utilizing an in-house mass spectral library. The deconvolution settings applied to the scan data were as follows: peak threshold: 5; minimum peak height: 5; bins per scan: 10; deconvolution width: 5; no baseline adjustment; minimum of 15 peaks per spectrum; no minimum required base peak intensity. Retention index calibration was performed based on a C10–C40 even n-alkane mixture (68281, Sigma-Aldrich, Munich, Germany). Relative quantification was achieved using the batch quantification function of metabolite detector software [20]. Data were normalized to the quality control pool measurement and the intensity of the internal standard (D6-Glutaric acid) and exported as .CSV file format.

Statistical analysis

The Shapiro-Wilk test was used to verify the normality of the data. Anthropometric variables showed a non-normal distribution and were thus analyzed with the non-parametric Mann-Whitney U test for non-normally distributed variables. A partial least squares discriminant analysis (PLS-DA) model was built considering anthropometric variables and data from the GC-MS analysis after unit variance scaling using SIMCA v.18 (Umetrics, Sweden). Linear mixed-models analysis was performed to test for differences in variables measured during the dietary challenge (multiple time points) between the two groups. To correct for multiple testing, a Geisser-Greenhouse correction was performed. When a p-value < 0.05 was reached, a Šídák's multiple comparison test was performed for each time point to determine when the difference between the two group was significant. Results were expressed as mean ± standard error. statistical analyses were carried out by GraphPad Prism version 8.02 for Windows (GraphPad Software, CA, USA).

Results

The focus of this study was to identify markers of distinct glycemic responses to a dietary challenge performed in healthy individuals. Blood was sampled as DBS, glycemia was measured with a portable glucometer and subjects were classified into two groups according to their glucose-AUC. Despite a self-described absence of disease, there was a significant difference in the glucose-AUC, suggesting that the group H-AUC had an impaired state of insulin sensitivity in comparison to the L-AUC. Following the segregation of the participants into two groups, a PLS-DA model was constructed to assess differences and identify the variables that best discriminate the two groups. The dataset consisted of 81 metabolites (identified or not) by the GC-MS analysis and 8 parameters of anthropometric data. Each metabolite measured multiple times in the postprandial period was considered as an individual variable in each time point, and thus 494 variables were used to build the PLS-DA model. The model has 4 components, $R^2 = 0.84$, $Q^2 = 0.47$, and p-value $= 0.0002$ for the ANOVA of the cross-validated residuals, indicating that the model is sound and there are differences between the groups. The metabolites detected by the GC-MS analysis with the top 20% VIP values in at least two different time points of the dietary challenge were subjected to mixed-effects analysis and only those that displayed significant differences between the two groups of participants were considered as putative markers of dysglycemia in DBS.

After selecting the variables with the top 20% VIP values, only 30% were associated to the L-AUC group and either did not receive a high VIP value in at least 2 time points or did not display a significant difference between the groups. As a result, only metabolites most abundant in the H-AUC group are considered and discussed in the study. Additionally, metabolites that did not receive high VIP values in the PLS-DA model, but displayed differences between the two groups after the mixed-effects analysis, were also included in the discussion.

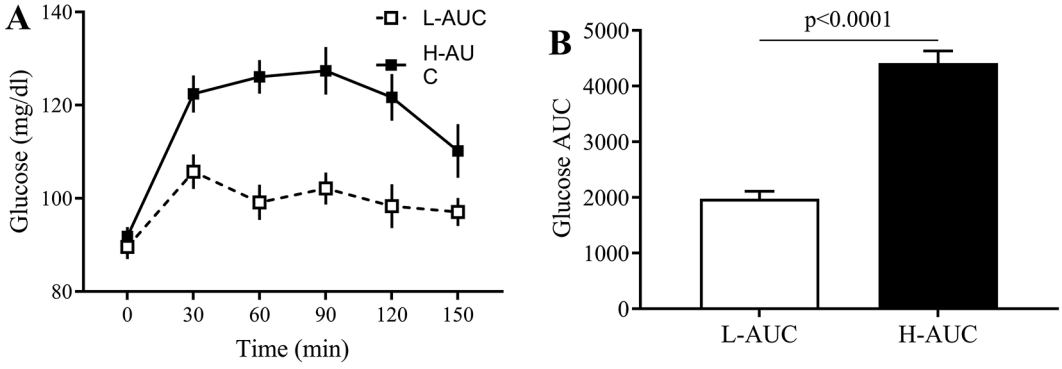

Fig. 1 **A**. Glycemic response during the dietary challenge. Participants were ranked and grouped into two groups (L-AUC and H-AUC) according to their glucose area under the curve (AUC); **B**. Glucose AUC during the dietary challenge. $n=27$ individuals/ group. The p-value of differences found in the Student's t-test is indicated. Data presented as the mean and standard error of the mean

Table 1 Anthropometric evaluation from participants. Values are means and standard error of the mean

	L-AUC $n=27$ (15 females)		H-AUC $n=27$ (16 females)		p-value*
	Mean	SEM	Mean	SEM	
Age (years)	28.1	1.9	34.6	2.5	0.0209
BW (kg)	66.3	2.8	72.6	3.4	0.1334
BMI (kg/m^2)	23.7	0.8	25.4	0.7	0.0464
Waist (cm)	81.5	4.7	82.0	2.8	0.2893
Abdomen (cm)	83.6	2.6	87.8	2.8	0.2418
Hip (cm)	95.1	2.0	99.5	2.1	0.0755
Waist: Height	0.47	0.03	0.49	0.01	0.2259
Waist: Hip	0.86	0.05	0.82	0.02	0.8388

* Mann-Whitney U test

During the postprandial period, blood glucose varied between 91.8 and 127.4 mg/dL for individuals classified in the H-AUC group and between 89.5 and 105.7 mg/dL in the L-AUC group (Fig. 1a), with AUC values of 1978±130 and 4416±215 (arbitrary units), for L-AUC and H-AUC respectively (Fig. 1b). The glucose-AUC was 123% higher ($p<0.0001$) in the H-AUC group compared to the L-AUC group. Although fasting glucose levels were similar between the groups (89.6±2.6 and 91.8±2.0 mg/dL for L-AUC and H-AUC, respectively), individuals in the H-AUC group exhibited a 31% higher increase in glucose levels in the first 30 min after meal ingestion in comparison to individuals from the L-AUC group and remained 20% higher in the first group compared to the latter until the end of the dietary challenge (150 min). Conversely, postprandial glucose levels in the L-AUC group increased only 17% in comparison to fasting levels, with no significant difference.

Individuals displayed no significant differences in anthropometric measurements between the groups (Table 1), except for age and BMI ($p=0.02$ and 0.05, respectively), with a mean value of hip circumference slightly higher in individuals from the H-AUC group, compared to those from the L-AUC group ($p=0.07$).

The GC-MS metabolite profiling of the DBS revealed that carbohydrates and carbohydrate-derived metabolites were detected at higher concentrations in individuals from the H-AUC group (Fig. 2). Fructose levels were higher in DBS from the H-AUC group compared to the L-AUC ($p=0.0199$) (Fig. 2a). When compared to the fasting levels, fructose levels showed a 72% increase in the H-AUC group ($p<0.0001$) within the first hour and remained higher until the end of the test, while the L-AUC group did not present postprandial variations in fructose levels. At fasting, mannose concentration was 31% higher in the DBS from H-AUC in comparison to the L-AUC group ($p<0.0001$). This difference tended to decrease towards the end of the challenge (150 min), when the concentration of mannose was 35% smaller in comparison to the fasted levels in the H-AUC ($p<0.0001$) (Fig. 2b). Threonic acid levels were in average 38% higher in DBS from the H-AUC compared to L-AUC group at fasting and throughout the dietary challenge (Fig. 2c). Although blood levels of myo-inositol and erythronic acid did not change due to the meal ingestion, participants from the H-AUC group displayed ~30% higher concentrations of these metabolites in blood, in comparison to L-AUC group ($p=0.0488$ and 0.0323, respectively) (Fig. 2d-e).

The DBS levels of some TCA cycle intermediates identified by the PLS-DA model as relevant for the discrimination between L-AUC and H-AUC participants did not show changes due to food intake. H-AUC group showed higher levels of citrate, fumarate and succinate in the blood after the overnight fasting and in the postprandial state, in comparison to the L-AUC group ($p=0.0067$, $p=0.0412$, $p=0.0093$ respectively) (Fig. 3). H-AUC participants showed a higher concentration of palmitate in blood when compared to the L-AUC group at fasting and throughout the test ($p=0.0147$) (Fig. 3d). Both

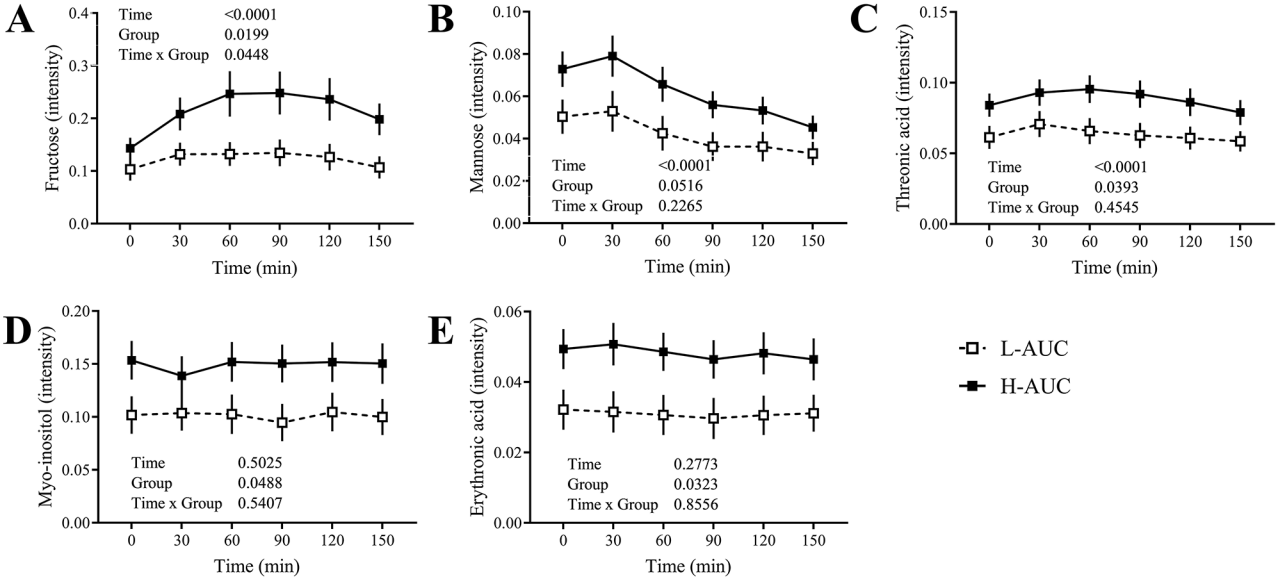

Fig. 2 Sugars and sugar-derived metabolites markers of postprandial hyperglycemia. **A**. Fructose; **B**. Mannose; **C**. Threonic acid; **D**. Myo-inositol; **E**. Erythronic acid. $n=27$ individuals/group. $n=27$ individuals/ group. The *p*-values of differences found in a mixed-effects analysis are indicated. Data is presented as the mean and standard error of the mean

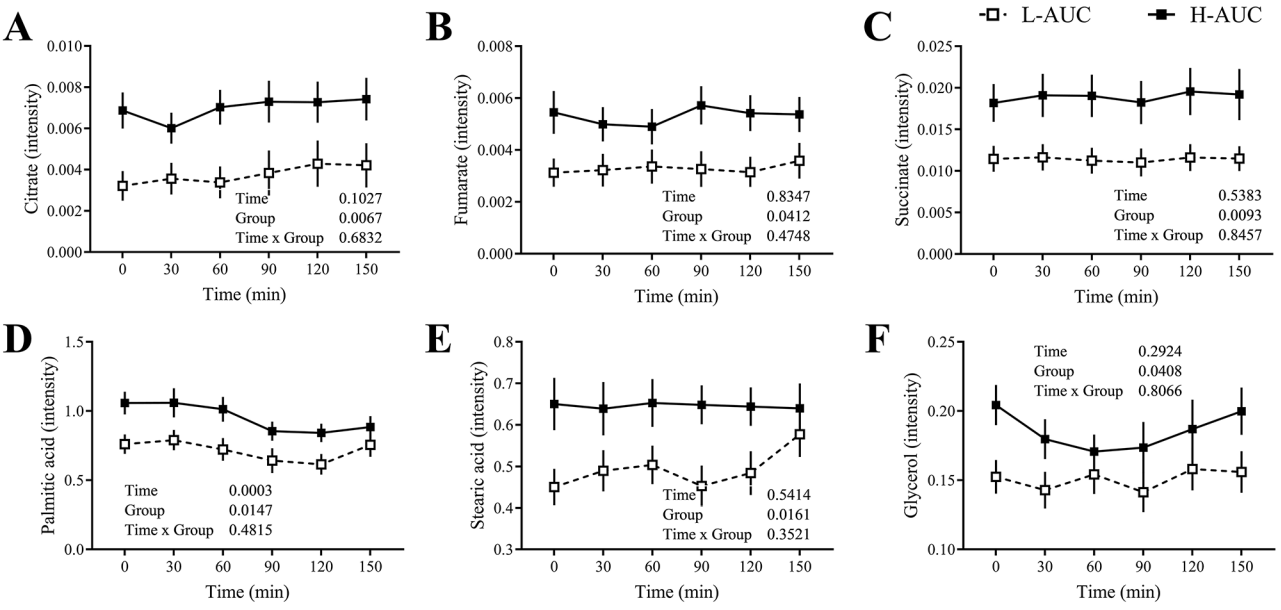

Fig. 3 TCA cycle intermediates and markers of lipid metabolism associated to postprandial hyperglycemia. **A**. Citrate; **B**. Fumarate; **C**. Succinate; **D**. Palmitic acid; **E**. Stearic acid; **F**. Glycerol. $n=27$ individuals/ group. The *p*-values of differences found in a mixed-effects analysis are indicated. Data is presented as the mean and standard error of the mean

groups displayed a decrease in the concentration of palmitic acid 90 min after food intake ($p=0.0003$). Similarly, stearic acid levels were higher in H-AUC than in L-AUC ($p=0.0161$), and there were no differences between fasting and postprandial levels (Fig. 3e). Individuals from the H-AUC group displayed 25% higher levels of glycerol in the DBS in comparison to the L-AUC group ($p=0.048$). The levels of this metabolite in the DBS described a u-shaped kinetic profile. In the first group, glycerol levels exhibited a slight decrease at 60 min compared to the fasted state (13.5%), followed by a rebound at the end of the challenge. Conversely, the L-AUC group presented no variations in glycerol levels during the postprandial period (Fig. 3f).

Participants from the H-AUC group presented higher levels of BCAAs in the DBS, compared to the L-AUC group. The concentrations of the branched-chain amino acids (BCAAs) in the DBS declined after the meal, with

this decrease being steeper in the H-AUC group ($p<0.02$) (Fig. 4a- c). A postprandial decline was also observed for the levels of other amino acids, including tyrosine ($p=0.0008$) and glycine ($p=0.0011$). It was observed that the H-AUC group showed an increased concentration of both amino acids compared to the L-AUC group ($p=0.0415$ vs. $p=0.0346$, respectively) (Fig. 4d-e).

At least 30 different features, detected but not identified by the GC-MS analysis, received high VIP values in the PLS-DA model This indicates that other metabolites, which discriminate L-AUC and H-AUC, remain to be discovered in the DBS (Suppl. Fig. S1).

Discussion

Dry blood spots are a blood sampling technique with numerous recognized advantages that include a patient-friendly sample collection as well as minimal sample processing for metabolite screening. In this report, we describe findings from a study where DBS were collected mostly at the participants' homes during a dietary challenge, allowing the characterization of participants otherwise considered healthy into two groups based on their postprandial glycemic response. We report the feasibility of using DBS samples to identify recognized biomarkers of insulin resistance in individuals with an exacerbated postprandial glycemic response. Markers of lipolysis, such as fatty acids and glycerol, organic acids, sugars and sugar-derived products, BCAAs, and other amino acids, previously described as indicators of insulin resistance, were identified as having elevated circulating levels during the postprandial period in individuals with high postprandial glycemia. The analysis of DBS extracts by GC-MS, recognized as a robust though less sensitive technique compared to LC-MS, allowed for the detection of these markers, highlighting the feasibility of using DBS as a sampling strategy in clinical studies of this nature.

Monosaccharides or derived metabolites, such as fructose, mannose, myo-inositol, and threonic acid, were reproducibly detected in DBS samples. Circulating levels of mannose were increased in individuals of the H-AUC group. Mannose can be synthesized from glucose via fructose-6-phosphate and elevated levels have been previously associated with an increased risk of developing type 2 diabetes and cardiovascular disease [21]. Lee et al.(2016) identified and validated circulating mannose as being highly associated with insulin resistance, independent of BMI. These authors concluded that mannose levels could serve as a marker of insulin resistance in clinical practice, potentially enhancing the accuracy of assessing insulin resistance [14]. Fructose is metabolized in various tissues of the body, including the liver, muscle, and adipose tissue. In the liver, fructose undergoes a series of enzymatic reactions, being converted into fructose-1-phosphate by the enzyme fructokinase. This process generates products that can be directed to the glycolytic pathway or converted into glucose or triglycerides [15, 22]. Excessive fructose levels can contribute to fat accumulation in the liver and the development of insulin resistance, which can lead to long-term metabolic complications such as non-alcoholic fatty liver disease

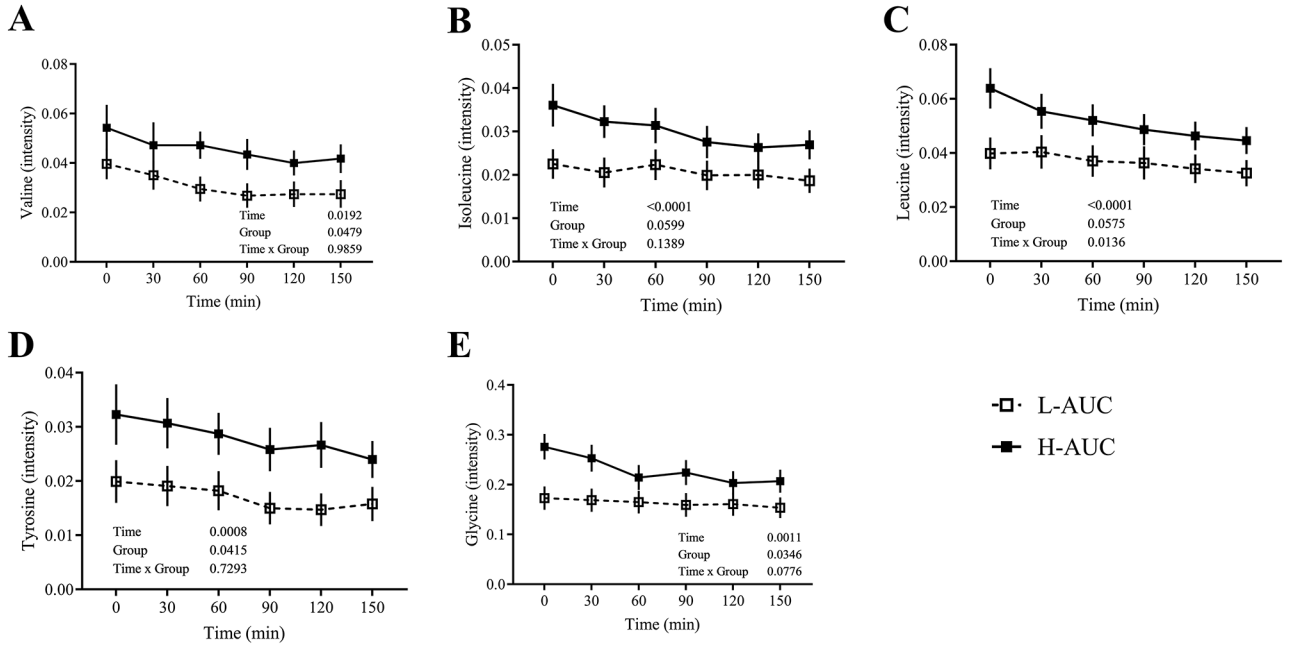

Fig. 4 Amino acid markers of postprandial hyperglycemia. **A**. Valine; **B**. Isoleucine; **C**. Leucine; **D**. Tyrosine; **E**. Glycine. $n=27$ individuals/ group. The *p*-values of differences found in a mixed-effects analysis are indicated. Data is presented as the mean and standard error of the mean

and type 2 diabetes [23]. Postprandial fructose levels were increased in individuals with type 2 diabetes after the consumption of a standardized mixed meal [24]. In accordance with our observations, a recent study demonstrated that over 40 different sugar and sugar-related products, including threonic acid, erythronic acid, and fructose, among others, exhibited circulating profiles that mimic those of glucose. These profiles showed slow clearance during an oGTT in insulin-resistant or diabetic individuals, but not in healthy subjects [25].

Levels of palmitic acid, stearic acid, and glycerol were higher in DBS from the H-AUC group compared to the L-AUC group. Saturated long-chain fatty acids such as stearic acid and palmitic acid are key contributors to the development of insulin resistance and glucose intolerance [26]. A large population-based study observed that levels of glycerol and saturated fatty acids were increased in categories of fasting and 2 h hyperglycemia, predicting the worsening of hyperglycemia and development of incident type 2 diabetes [27]. The underlying mechanisms involved in the fatty acid-induced loss of insulin sensitivity are still unclear but they may be related to different effects in specific sites, including muscle tissue and the pancreas. Previously, it was demonstrated that palmitic acid induces insulin resistance in myocytes by activating C-Jun N-terminal kinase (JNK), which acts as an effector in phosphorylating serine (Ser307) of the insulin receptor substrate 1 (IRS-1) [28]. Furthermore, fatty acids induce pancreatic β-cell dysfunction and death, eliciting endoplasmic reticulum stress, oxidative stress, mitochondrial dysfunction, impaired autophagy, and inflammation [29].

Our study also identified the organic acids citrate, fumarate and succinate in the DBS as metabolites that discriminate participants from H-AUC and L-AUC. TCA cycle metabolites might be a link between mitochondrial dysfunction and insulin resistance. Mitochondrial dysfunction, including alterations in mitochondrial content or activity, has been partially linked to the emergence of insulin resistance, impacting various tissues such as skeletal muscle, liver, adipose tissue, heart, blood vessels, and pancreas. Mitochondria play a central role in ATP production through oxidative phosphorylation, a process involving the oxidation of NADH and FADH2 to efficiently generate ATP in aerobic organisms [30]. The decline in oxidative capacity of mitochondria and the ATP synthesis rate with advancing age and elevation of fatty acids over longer periods, contributes to insulin resistance. These alterations result in intracellular lipid accumulation inside the liver, muscle or β-cells, leading to a deterioration in insulin action [31]. Impaired mitochondrial fatty acid oxidation and energy production may lead to an abnormal accumulation of mixed carbon substrates such as the organic acids citrate, fumarate, and succinate. The high flux of these substrates leads to the mitochondrial congestion and indecisiveness of metabolic switches, resulting in increased VLDL production, and elevated postprandial glucose levels [32]. These organic acids serve as intermediates in metabolic pathways, and their buildup may indicate disruptions in mitochondrial function. Succinate, for instance, plays a crucial role in mitochondrial metabolism, and its accumulation has been associated with the induction of mitochondrial reactive oxygen species (ROS) production, which can lead to oxidative stress and mitochondrial dysfunction [33]. Studies have suggested that citrate metabolism is involved in various biological processes, including inflammation, cancer, insulin secretion, and non-alcoholic fatty acid liver [34]. Additionally, previous reports have shown that both fumarate and succinate levels are elevated in metabolic diseases, playing an important role in the development and progression of obesity-induced inflammation, insulin resistance, and diabetes [35, 36].

Elevated circulating levels of BCAAs and related metabolites have been identified among the strongest biomarkers of insulin resistance, obesity, and type 2 diabetes [37]. A previous study showed that levels of 3-hydroxyisobutyrate (3-HIB), a valine catabolite, are elevated in muscle tissues from *db*/*db* mice with diabetes and in diabetic human subjects when compared to those without diabetes. When secreted by muscle cells, 3-HIB can act as a paracrine regulator of trans-endothelial fatty acid transport, stimulate muscle fatty acid uptake, and promote lipid accumulation in muscle, leading to insulin resistance [38]. Interestingly, similar abnormalities are also observed with isoleucine, which increases both muscle and fat mass, causes insulin resistance, and upregulates levels of key adipogenic and myogenic proteins under obesogenic conditions [39]. Furthermore, circulating levels of amino acid such as leucine and tyrosine have been observed to be elevated in the postabsorptive state of *ob*/*ob* mice compared to lean mice [40]. Both amino acids were positively correlated with HOMA-IR in non-diabetic subjects [41]. In a longitudinal study with obese children, tyrosine levels were closely associated with HOMA-IR index, suggesting that tyrosine alterations precede changes in BCAA metabolism [42].

Unexpectedly, this study identified higher levels of glycine in the DBS from participants of the H-AUC group. Previous studies found that elevated concentrations of glycine are associated with improved insulin sensitivity, while lower plasma glycine concentrations have been related to obesity, diabetes, and non-alcoholic fatty liver disease [43, 44]. Indeed, dietary glycine supplementation may improve insulin responses and glucose tolerance and reduce systemic inflammation [45]. Nevertheless, one must keep in mind that our results derive from whole blood (in the form of DBS). Thus the metabolite contents of red blood cells, leucocytes and platelets can lead to

different observations than those derived from the analysis of isolated plasma or serum. Indeed, some discrepancies have been found in previous reports comparing plasma and DBS samples in metabolomics applications.

A recent study applied plasma- and DBS-based metabolomics to explore dysregulated metabolites in patients with diabetes mellitus, demonstrating a strong correlation between plasma and DBS for only half of the detected metabolites [46]. Gut-derived metabolites, including trimethylamine N-oxide (TMAO) and its precursors and derivatives such as choline, carnitine, and acetylcarnitine, displayed moderate correlation (for TMAO and carnitine) and poor correlation (for choline and acetylcarnitine) when analyzed in plasma and DBS samples [47]. Meanwhile, in plasma and DBS samples obtained from pregnant women with HIV, more than half of the metabolites (>60%) were detected in both matrices. However, plasma and DBS samples yielded distinct metabolite profiles that were highly predictive of individual subjects [48]. These discrepancies are likely due to the additional metabolites present in DBS and derived from the hematocrit, which could "mask" metabolites that would normally be detected in plasma and/or serum samples. This bias is not necessarily a problem, as it could help address the underrepresentation of several intracellular metabolic pathways [46, 49]. Despite the promising results, this study has some limitations that need to be considered for an adequate interpretation of the findings. One of the main limitations is the lack of comparison between the conventional plasma matrix and the DBS matrix, to obtain a reliable and validated analytical method for our study. Second, in the GC-MS metabolomic profiling, most of the peaks remain unidentified even after extensive data processing (See Supplementary data). Once these challenges are overcome, specific DBS metabolites with potential clinical usefulness may be identified as biomarkers associated with insulin resistance. Furthermore, the sample size is another limitation of our study, that should be replicated in a larger and more homogeneous population.

Conclusion

In conclusion, our findings provide a basis for utilizing DBS sampling as a valuable tool in clinical studies aimed at investigating postprandial metabolic responses. We report the association between exacerbated postprandial glycemic response with markers measured in DBS, including sugars and sugar-derived products, fatty acids, organic acids, BCAAs and other amino acids. This clinical study, employing DBS as a sampling strategy, successfully describes a signature of metabolic dysregulation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12263-024-00752-7.

Supplementary Material 1

Supplementary Material 2

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Author contributions

SGD Formal analysis, Methodology, Investigation, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing. CMDP Formal analysis, Funding acquisition, Investigation, Visualization, Writing - Original Draft, Writing - Review & Editing. THM Investigation, Software. LR Investigation. Visualization, Writing - Original Draft, Writing - Review & Editing. KH Investigation, Software, Resources. JF Conceptualization, Data curation, Methodology, Project administration, Funding acquisition, Formal analysis, Supervision, Visualization, Writing - Original Draft, Writing - Review & Editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The Ethics Committee of the School of Pharmaceutical Sciences approved this study (CAAE: 15438019.7.0000.0067).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Fiamoncini J, Donado-pestana CM, Biude G, et al. Plasma metabolic signatures of healthy overweight subjects challenged with an oral glucose. Tolerance Test. 2022;9:1–17.
2. Morris C, O'Grada CM, Ryan MF, Gibney MJ, Roche HM, Gibney ER, Brennan L. Modulation of the lipidomic profile due to a lipid challenge and fitness level: a postprandial study. Lipids Health Dis. 2015;14:1–11.
3. Pimentel G, Burton KJ, Pralong FP, Vionnet N, Portmann R, Vergères G. The postprandial metabolome — a source of nutritional biomarkers of health. Curr Opin Food Sci. 2017;16:67–73.
4. Pellis L, van Erk MJ, van Ommen B, et al. Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. Metabolomics. 2012;8:347–59.
5. van Ommen B, van der Greef J, Ordovas JM, Daniel H. Phenotypic flexibility as key factor in the human nutrition and health relationship. Genes Nutr. 2014. https://doi.org/10.1007/s12263-014-0423-5.
6. Lages M, Barros R, Moreira P, Guarino MP. Metabolic effects of an oral glucose tolerance test compared to the mixed meal tolerance tests: a narrative review. Nutrients. 2022. https://doi.org/10.3390/nu14102032.

7. Berry SE, Valdes AM, Drew DA, et al. Human postprandial responses to food and potential for precision nutrition. Nat Med. 2020;26:964–73.
8. Ou SJL, Yang D, Pranata HP, Tai ES, Liu MH. Postprandial glycemic and lipidemic effects of black rice anthocyanin extract fortification in foods of varying macronutrient compositions and matrices. NPJ Sci Food. 2023. https://doi.org/10.1038/s41538-023-00233-y.
9. Milosheska D, Grabnar I, Vovk T. Dried blood spots for monitoring and individualization of antiepileptic drug treatment. Eur J Pharm Sci. 2015;75:25–39.
10. Demirev PA. Dried blood spots: analysis and applications. Anal Chem. 2013;85:779–89.
11. Joye T, Sidibé J, Déglon J, Karmime A, Sporkert F, Widmer C, Favrat B, Lescuyer P, Augsburger M, Thomas A. Liquid chromatography-high resolution mass spectrometry for broad-spectrum drug screening of dried blood spot as microsampling procedure. Anal Chim Acta. 2019;1063:110–6.
12. Thevis M, Walpurgis K, Thomas A. DropWise: current role and future perspectives of dried blood spots (DBS), blood microsampling, and their analysis in sports drug testing. Crit Rev Clin Lab Sci. 2023;60:41–62.
13. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. Mass Spectrom Rev. 2014;35:361–8.
14. Lee S, Zhang C, Kilicarslan M, et al. Integrated Network Analysis Reveals an Association between plasma mannose levels and insulin resistance. Cell Metab. 2016;24:172–84.
15. Fiehn O, Timothy Garvey W, Newman JW, Lok KH, Hoppel CL, Adams SH. Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. PLoS ONE. 2010;5:1–10.
16. Suhre K, Meisinger C, Döring A, et al. Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PLoS ONE. 2010. https://doi.org/10.1371/journal.pone.0013953.
17. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009;9:311–26.
18. Chang W, Hatch GM, Wang Y, Yu F, Wang M. The relationship between phospholipids and insulin resistance: from clinical to experimental studies. J Cell Mol Med. 2019;23:702–10.
19. Vrolix R, Mensink RP. Variability of the glycemic response to single food products in healthy subjects. Contemp Clin Trials. 2010;31:5–11.
20. Hiller K, Hangebrauk J, Jäger C, Spura J, Schreiber K, Schomburg D. MetaboliteDetector: Comprehensive analysis tool for targeted and nontargeted GC/MS based metabolome analysis. Anal Chem. 2009;81:3429–39.
21. Ferrannini E, Bokarewa M, Brembeck P, et al. Mannose is an insulin-regulated metabolite reflecting whole-body insulin sensitivity in man. Metabolism. 2020;102:153974.
22. Herman MA, Birnbaum MJ. Molecular aspects of fructose metabolism and metabolic disease. Cell Metab. 2021;33:2329–54.
23. Ter Horst KW, Serlie MJ. Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. Nutrients. 2017;9:1–20.
24. Wopereis S, Stroeve JHM, Stafleu A, Bakker GCM, Burggraaf J, van Erk MJ, Pellis L, Boessen R, Kardinaal AAF, van Ommen B. Multi-parameter comparison of a standardized mixed meal tolerance test in healthy and type 2 diabetic subjects: the PhenFlex challenge. Genes Nutr. 2017;12:1–14.
25. Mack CI, Ferrario PG, Weinert CH, Egert B, Hoefle AS, Lee YM, Skurk T, Kulling SE, Daniel H. Exploring the diversity of sugar compounds in healthy, prediabetic, and diabetic volunteers. Mol Nutr Food Res. 2020;64:1901190.
26. Nunes EA, Rafacho A. Implications of palmitoleic acid (palmitoleate) on glucose homeostasis, insulin resistance and diabetes. Curr Drug Targets. 2017;18:619–28.
27. Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, Uusitupa M, Ala-Korpela M, Laakso M. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. Diabetes Care. 2013;36:3732–8.
28. Han MS, Lim YM, Quan W, et al. Lysophosphatidylcholine as an effector of fatty acid-induced insulin resistance. J Lipid Res. 2011;52:1234–46.
29. Lytrivi M, Castell AL, Poitout V, Cnop M. Recent insights into mechanisms of β-Cell lipo- and glucolipotoxicity in type 2 diabetes. J Mol Biol. 2020;432:1514–34.
30. Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. Circ Res. 2008;102:401–14.
31. Wiederkehr A, Wollheim CB. Minireview: implication of mitochondria in insulin secretion and action. Endocrinology. 2006;147:2643–9.
32. Tippairote T, Bjørklund G, Yaovapak A. The continuum of disrupted metabolic tempo, mitochondrial substrate congestion, and metabolic gridlock toward the development of non-communicable diseases. Crit Rev Food Sci Nutr. 2022;62:6837–53.
33. Zhang Y, Zhang M, Zhu W, Yu J, Wang Q, Zhang J, Cui Y, Pan X, Gao X, Sun H. Succinate accumulation induces mitochondrial reactive oxygen species generation and promotes status epilepticus in the kainic acid rat model. Redox Biol. 2020;28:101365.
34. Iacobazzi V, Infantino V. Citrate - new functions for an old metabolite. Biol Chem. 2014;395:387–99.
35. Chen M, Zheng H, Xu M, et al. Changes in hepatic metabolic profile during the evolution of STZ-induced diabetic rats via an 1H NMR-based metabonomic investigation. Biosci Rep. 2019;39:1–12.
36. van Diepen JA, Robben JH, Hooiveld GJ, et al. SUCNR1-mediated chemotaxis of macrophages aggravates obesity-induced inflammation and diabetes. Diabetologia. 2017;60:1304–13.
37. White PJ, McGarrah RW, Herman MA, Bain JR, Shah SH, Newgard CB. Insulin action, type 2 diabetes, and branched-chain amino acids: a two-way street. Mol Metab. 2021;52:101261.
38. Jang C, Oh SF, Wada S, et al. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. Nat Med. 2016;22:421–6.
39. Liu S, Sun Y, Zhao R, Wang Y, Zhang W, Pang W. Isoleucine increases muscle mass through promoting myogenesis and intramyocellular fat deposition. Food Funct. 2021;12:144–53.
40. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am J Physiol Endocrinol Metab. 2007;293:E1552–63.
41. Yamada C, Kondo M, Kishimoto N, Shibata T, Nagai Y, Imanishi T, Oroguchi T, Ishii N, Nishizaki Y. Association between insulin resistance and plasma amino acid profile in non-diabetic Japanese subjects. J Diabetes Investig. 2015;6:408–15.
42. Hellmuth C, Kirchberg FF, Lass N, Harder U, Peissner W, Koletzko B, Reinehr T. Tyrosine is Associated with insulin resistance in Longitudinal Metabolomic profiling of obese children. J Diabetes Res. 2016. https://doi.org/10.1155/2016/2108909.
43. Gaggini M, Carli F, Rosso C, et al. Altered amino acid concentrations in NAFLD: impact of obesity and insulin resistance. Hepatology. 2018;67:145–58.
44. Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J, Hu FB. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care. 2016;39:833–46.
45. Yan-Do R, MacDonald PE. Impaired glycine-mia in type 2 diabetes and potential mechanisms contributing to glucose homeostasis. Endocrinology. 2017;158:1064–73.
46. Chiu HH, Lin SY, Zhang CG, Tsai CC, Tang SC, Kuo CH. A comparative study of plasma and dried blood spot metabolomics and its application to diabetes mellitus. Clin Chim Acta. 2024;552:117655.
47. Lu WH, Chiu HH, Kuo HC, Chen GY, Chepyala D, Kuo CH. Using matrix-induced ion suppression combined with LC-MS/MS for quantification of trimethylamine-N-oxide, choline, carnitine and acetylcarnitine in dried blood spot samples. Anal Chim Acta. 2021;1149:338214.
48. Tobin NH, Murphy A, Li F, et al. Comparison of dried blood spot and plasma sampling for untargeted metabolomics. Metabolomics. 2021;17:62.
49. Stringer KA, Younger JG, McHugh C, Yeomans L, Finkel MA, Puskarich MA, Jones AE, Trexel J, Karnovsky A. Whole blood reveals more metabolic detail of the human metabolome than serum as measured by 1H-NMR spectroscopy: implications for sepsis metabolomics. Shock. 2015;44:200–8.

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