

Plasma Metabolomics Profile in Young Adults with a History of Extrauterine Growth Restriction: The BIORICA-10 Study

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Abstract

Background

Extrauterine growth restriction (EUGR) can exert long-term effects on cardiometabolic outcomes. However, whether metabolic alterations persist into adulthood remains poorly understood. Elucidating alterations in the plasma metabolome may facilitate the identification of early biomarkers and formulation of preventive strategies. Consequently, this study aimed to characterize the plasma metabolomic signatures of young adults with a history of EUGR.

Methods

This cross-sectional study included 36 young adults with a history of EUGR and 72 healthy participants as a reference group (control) born with adequate birth weight and gestational age. Plasma metabolomic profile was obtained using gas and liquid chromatography coupled with mass spectrometry-based techniques (GC-MS and LC-MS). Principal component analysis (PCA), sparse partial least squares discriminant analysis (sPLS-DA), receiver operating characteristic (ROC) curves, and integrative multiblock analysis using the DIABLO framework were applied to identify key discriminatory metabolic signatures and biomarkers.

Results

A total of 249 metabolites were identified. Unsupervised PCA revealed clear separation trends between study groups, driven by distinct profiles of steroid hormones and energy-related metabolites, such as glycerate and glycerol-1-phosphate. Consistently, individuals with EUGR exhibited elevated levels of proinflammatory mediators (oxylipins and lysophosphatidylcholines) alongside altered intermediates of glucose and lipid metabolism. Refinement using a sPLS-DA model explained 24% of the variance using only 12 key variables, while ROC curve analysis identified the glutamate/glutamine ratio as possessing the highest discriminative power. Finally, multiblock integrative analysis confirmed that inflammatory biomarkers, oxylipins, glutamate, and aspartate cooperatively discriminated between groups.

Conclusions

The present study reveals a distinct metabolic signature in young adults with a history of EUGR. Elevated markers of oxidative stress and increased products of lipolysis and fatty acid oxidation may indicate a predisposition to cardiovascular risk.

Introduction

The perinatal period represents a critical developmental window due to the substantial plasticity of infant organs, which enables phenotypic adaptation to nutritional and environmental factors (Moreno-Mendez et al. 2020). Low gestational age (GA) and low birth weight have been linked to long-term risk of hypertension, cardiovascular disease (CVD), and type 2 diabetes (T2D) (Crump 2020).

Extrauterine growth restriction (EUGR) is a common condition among premature infants, with an incidence of up to 70% reported in extremely premature infant (Chien et al. 2018; Devaram et al. 2026). Although the definition of EUGR remains controversial, a recent consensus has proposed more stringent criteria, specifically a weight below the 3rd percentile (p3) at 36 weeks of corrected GA and/or at hospital discharge (Fenton et al. 2020; Gür et al. 2026; Palomino-Fernández et al. 2024; Zozaya et al. 2018). This condition has been associated with neurodevelopmental, digestive and metabolic complications, among others (González-López et al. 2024; Ordóñez-Díaz et al. 2020a; Ordóñez-Díaz et al. 2020b; Ortiz Espejo et al. 2012; Ortiz-Espejo et al. 2014; Sun et al. 2024), including elevated blood pressure (BP), increased proinflammatory biomarkers, and alterations in the antioxidant enzyme system (Ordóñez-Díaz et al. 2021; Ordóñez-Díaz et al. 2020a; Ordóñez-Díaz et al. 2020b). Moreover, aggressive nutritional interventions during early development aimed at achieving rapid catch-up growth may also contribute to these neurodevelopmental and metabolic programming alterations (Chien et al. 2018; Parkinson et al. 2013).

Despite being a highly prevalent condition among preterm infants, the long-term health consequences of EUGR in adulthood remain largely unexplored. Metabolomics offers novel opportunities to identify metabolic signatures associated with disease risk, opening new frontiers for preventive and therapeutic strategies (Mussap et al. 2013). Recently, these approaches have been used to discover biomarkers and underlying biological pathways across various conditions, spanning from the perinatal period to adulthood (Rangel-Huerta et al. 2019; Wishart 2019). For instance, some studies have indicated that plasma metabolomic profiling of infants with growth failure reveals delayed maturation of the gut microbiota and metabolome (Dudzik et al. 2020; Fan et al. 2021). Furthermore, distinct differences in plasma, urine, and stool metabolite levels have been specifically reported between EUGR and well-grown infants (Duan et al. 2025; Meneghelli et al. 2025; Younge et al. 2019). Therefore, the objective of this study was to analyze the plasma metabolomic profile of young adults with a history of EUGR to identify potential biomarkers associated with the risk of developing metabolic diseases later in life.

Materials and Methods

Study design and participants

This study employed a descriptive cross-sectional design. Young adults with a history of EUGR were recruited from the BIORICA cohort (Ortiz Espejo et al. 2012; Ortiz-Espejo et al. 2014). For comparison, a healthy reference group (control) of individuals with an adequate birth weight and GA was recruited independently. All enrolled participants were at Tanner pubertal stage $\geq$ III (confirmed by sex hormones levels) and presented no metabolic disorders that could confound the study outcomes. Details regarding

sample size calculation, baseline participant characteristics, and physical evaluations at the prepubertal stage for the BIORICA cohort have been described previously (Ortiz Espejo et al. 2012; Ortiz-Espejo et al. 2014). Although 37 individuals with EUGRs and 76 individuals in the reference group were initially recruited, one individual with EUGR and four healthy individuals were excluded due to not meeting the inclusion criteria. Consequently, a total of 108 participants (36 EUGR and 72 controls) were included in the final analysis.

Clinical and biochemical data

Anthropometric, BP, and other clinical parameters were collected using standardized methods during this young adult stage, as previously described (Palomino-Fernández et al. 2024). Fasting blood samples were drawn to assess routine biochemical parameters, C-reactive protein (CRP), cytokines, and adipokines (Palomino-Fernández et al. 2024). Plasma aliquots were stored at -80°C until metabolomic analysis.

Dietary intake was evaluated using a validated food frequency questionnaire (FFQ) (Willett and Lenart 2012). Food items were categorized into 12 distinct groups according to the Spanish Community Nutrition Society (SENC) Guidelines (Sociedad Española de Nutrición Comunitaria 2004). Daily intake (g/day) for each food group was calculated based on consumption frequency and standardized portion sizes (Sociedad Española de Nutrición Comunitaria 2004). These food groups were subsequently incorporated into the integrative multiblock analysis.

Metabolomic Analyses

Targeted metabolomic analyses were performed across three complementary analytical platforms: amino acids (AAs), carboxylic acids and metabolic intermediates, which were determined by Gas Chromatography-Mass Spectrometry (GC-MS); bile acids, hydroxy fatty acids (OH-FAs), steroid hormones and oxylipins were quantified via Liquid Chromatography-Mass Spectrometry (LC-MS); acylcarnitines were determined via Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS); and free fatty acids (FFAs), lysophosphatidylcholines (LPC) and lysophosphatidylethanolamines (LPE) were profiled using liquid chromatography-quadrupole Time-of-Flight (LC-qTOF).

All GC- and LC-MS-based analyses were conducted at the Metabolomics Facility of the Center for Omic Sciences of the Joint Unit of Universitat Rovira i Virgili-Eurecat (Tarragona, Spain). To evaluate clinical-metabolomic relationships, several ratios and summations were computed (see **Supplementary Table S1**) to assess their clinical relevance and comparative correlations.

Reagents and analytical standards

Milli-Q water (Millipore, USA), Formic Acid (Fisher Scientific, USA), LC-MS methanol, and 2-propanol were purchased from Merck (Darmstadt, Germany). Water and acetonitrile (MeCN) were obtained from Scharlab (Barcelona, Spain). Methoxyamine was purchased from Sigma-Aldrich, and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) + 1% Trimethylsilyl chloride (TMCS) was obtained from Thermo Fisher Scientific (USA).

A comprehensive panel of analytical standards –including lipids, bile acids, fatty acids, steroid hormones, oxylipins and acylcarnitines, alongside isotopically labeled internal standards, were utilized for LC-qTOF, LC-MS and LC-MS/MS analyses. Further analytical details are provided in **Supplementary Table S2**.

Extraction and sample preparation for metabolomics analysis

Extraction and sample preparation for metabolomics analysis

Figure 1 outlines the general workflow for sample preparation and metabolomic analyses. Detailed protocols and analytical parameters are provided in the **Supplementary Methods** (Sections S2 and S3) and **Supplementary Tables S3 and S4**.

Statistical analysis

Descriptive statistics were calculated for all continuous variables and are presented as median, minimum, and maximum values. Data normality was assessed using the Shapiro-Wilk test. Differences between groups were evaluated using the unpaired Student's t-test for normally distributed variables and the non-parametric Wilcoxon rank-sum test for non-normally distributed data. Statistical significance was initially set at $p < 0.05$. To control the false-discovery rate inherent to high-throughput metabolomic datasets, raw p-values were adjusted using the Benjamini-Hochberg False Discovery Rate (FDR) procedure, setting a strict threshold at q-value < 0.05 . Univariate analyses were performed in R packages *Dplyr* and *Hmisc* (R v.4.3.1).

Prior to multivariate analysis, metabolomic data were log-transformed and Pareto-scaled. Unsupervised Principal Component Analysis (PCA) was performed to evaluate data variance and clustering. Supervised partial least squares discriminant analysis (PLS-DA) and sparse PLS-DA (sPLS-DA) were applied to characterize plasma metabolic signatures and classify sample groups. The optimal number of components and variables in sPLS-DA models was determined via 10-fold cross-validation with 1000 repetitions, setting the threshold of maximum balanced error rate < 0.05 . In sPLS-DA models, a L1 (LASSO) penalty is applied to the loading vectors during each successive PLS dimension, shrinking the coefficients of uninformative variables to zero and thereby performing simultaneous variable selection and dimensionality reduction. Multivariate modeling was conducted in R using the mixOmics package (v.6.26) (Rohart et al. 2017). Only variables displaying significant differences between the EUGR and reference groups in univariate tests were included in the multivariate PCA, PLS-DA, and sPLS-DA models.

To evaluate predictive performance, Receiver Operating Characteristic (ROC) curves were constructed for all metabolites displaying a loading score ≥ 0.2 in the sPLS-DA model. ROC curves, area under the curve (AUC) values, 95% confidence intervals (CIs), sensitivity, and specificity were calculated using the R packages *pROC* and *ggplot2* (R v.4.3.1).

Integrative multiblock analysis was performed using the DIABLO framework (Data Integration Analysis for Biomarker discovery using Latent components), a supervised multiblock sPLS-DA approach designed to integrate multi-omics datasets based on a categorical outcome (Singh et al. 2019). DIABLO constructs latent variables by maximizing covariances across datasets while balancing discrimination and integration. For this analysis, metabolomic features were divided into four independent analytical blocks: LC-MS/MS acylcarnitines, GC-MS features (AAs, carboxylic acids and metabolic intermediates); LC-MS features (bile acids, OH-FAs, steroid hormones and oxylipins), and LC-qTOF features (FFAs, LPC and LPE) to evaluate relationships across distinct chemical classes. Biochemical parameters (including glucose, urea, creatinine, apolipoprotein A, apolipoprotein B, total cholesterol, HDL-C (High-Density Lipoprotein-Cholesterol), LDL-C (Low-Density Lipoprotein-Cholesterol), triglycerides, total protein, albumin, insulin, HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), TSH (Thyroid-Stimulating Hormone), thyroxine (T4), iron, ferritin, and hemoglobin) and inflammation-related biomarkers (including adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1), nerve growth factor (NGF), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL1 β) and leptin) were included as two separate independent blocks. Additionally, calculated metabolic ratios and summations (see **Supplementary Table S1**) were incorporated as a dedicated block.

An additional independent model was generated to incorporate dietary intake data as an extra block, restricted to participants with complete datasets across all blocks. Models were constructed using a full-weighted design matrix 10-fold cross-validation with 100 repetitions and a maximum balanced error rate threshold < 0.05 . Score and loading visualizations were generated using the mixOmics package (v.6.26) (Rohart et al. 2017), while inter-block associations and rankings were determined using the OmicsFold package (v.1.0.1).

Results

Overview of plasma metabolic alterations in young adults with a history of EUGR

A total of 249 plasma metabolites were detected across the EUGR and reference groups. Initial univariate analysis identified 76 metabolites that differed significantly between groups ($p < 0.05$). Following FDR correction for multiple testing, 51 metabolites remained significantly dysregulated ($q < 0.05$), confirming the presence of a robust metabolic signature associated with a history of EUGR.

To characterize the overall metabolic perturbation, significantly altered metabolites were classified according to their biochemical families (Fig. 2a), demonstrating that metabolic disturbances were distributed across several interconnected processes rather than being confined to a single metabolite class. Among all significantly altered metabolites, LPC and LPE represented the most affected family (35.29%), showing the largest changes in relative abundance. Metabolic intermediates and amino acids (15.68%) together with oxylipins (15.68%) followed in magnitude of variation. The combined magnitude and statistical significance of metabolite changes between groups are illustrated in the volcano plot (Fig. 2b). Metabolites exhibiting significant differences are mainly distributed toward positive fold-change values, reflecting increased levels in the EUGR group. Notable examples include glutamate, glycerate, aspartate, the oxylipins 5(S)-HETE, 15(S)-HETE and 12(S)-HETE, and the fatty acid 20:4n-6.

Analysis of the relative abundance of the top-ranked metabolites further revealed a remarkably consistent pattern, with all major discriminatory metabolites showing higher relative concentrations in the EUGR group than in the reference group (Fig. 2c). Collectively, these findings indicate that coordinated metabolic remodeling occurs rather than isolated metabolic alterations.

Biological characterization of the dysregulated metabolic pathways

The dysregulated metabolites were primarily amino acids, carboxylic acids, oxylipins, LPC, LPE, FFA, and bile acids (See **Supplementary Table S5**).

Among inflammatory lipid mediators, several oxylipins -including 5(S)-HETE, 12(S)-HETE, 15(S)-HETE, 5(S)-HEPE, 13-oxoODE, 9(S)-HODE, 13(S)-HODE and 14,15-DHET- were consistently increased in the EUGR group. Likewise, extensive remodeling of membrane lipids was evidenced by the upregulation of multiple LPCs (LPCs 14:0, 15:0, 16:0, 16:1, 18:0, 20:1, 20:2 and 22:4), LPE 18:0 and several FFAs, including arachidonic acid- and omega3- derived species.

Alterations in energy metabolism were also observed. Metabolites involved in glycolysis and the tricarboxylic acid cycle, including pyruvate, α -ketoglutarate, glycerate, and glycerol-1-phosphate, were significantly elevated. Similarly, AA metabolism was markedly altered, with increased levels of glutamate, aspartate, erythronate and threonate.

Finally, conjugated bile acids, including taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA) and glycocholic acid (GCA), were significantly increased in participants with previous EUGR.

Overall, these findings reveal a coordinated metabolic phenotype characterized by persistent activation of inflammatory lipid pathways, along with alterations in energy metabolism, AA homeostasis and lipid remodeling.

Phenotypic Clustering and Supervised Metabolic Discriminators

To determine whether these metabolic alterations defined a global metabolic phenotype, unsupervised PCA was performed. The PCA score plot demonstrated a clear separation between EUGR and reference participants based on the first two principal components (PC) (Fig. 3), which together explained 31% of the total variance (PC1: 22%; PC2: 9%). These findings indicate that the overall metabolomic profile differs substantially between the two groups.

To further characterize the metabolic architecture within each group, a three-dimensional PCA (3D-PCA) was performed separately for the EUGR and reference groups. Only metabolites with loading ≥ 0.4 were included (see **Supplementary Tables S6 and S7**).

In the EUGR group, the first three PCs explained 35.1% of the total variance (PC1:13.9%; PC2:12.0%; PC3:9.2%). PC1 was mainly driven by steroid hormones, oxylipins and pyruvate, whereas PC2 was characterized by LPCs, LPEs and oxylipins, and PC3 by FFAs, oxylipins and metabolic intermediates.

Conversely, the reference group exhibited a different metabolic organization, with PC1 (12.7% of variance) enriched in LPCs, AAs and metabolic intermediates, whereas PC2 (11.7% of variance) was dominated by FFAs, LPCs and LPEs, and PC3 (9.7% of variance) was characterized by FFAs, steroid hormones, oxylipins and pyruvate.

To maximize discrimination between groups, PLS-DA was subsequently performed. The optimized two-component model achieved a clear separation between EUGR and reference individuals (Fig. 4). The PLS-DA model (Fig. 4a) contained PCs that explained 30% of the variance. However, the sPLS-DA (Fig. 4b) explained 24% of the variance, identifying a reduced panel of highly informative metabolites (see **Supplementary Table S8**).

Among these, oxylipins and AA-related metabolites emerged as the strongest contributors to the EUGR phenotype. Specifically, 5(S)-HETE, 15 (S)-HETE, glutamate, and the glutamate/glutamine (Glu/Gln) ratio exhibited the highest loading values together with perfect stability scores (1.00), indicating excellent robustness across model calibration.

Collectively, these multivariate analyses demonstrate that the metabolic alterations associated with EUGR constitute a coherent metabolic signature rather than isolated biochemical changes.

Evaluation of the discriminatory performance of candidate metabolites

Given the marked discrimination achieved by the multivariate analysis, the diagnostic performance of the principal metabolites was evaluated using ROC curve analysis.

Among all candidates, the Glu/Gln ratio showed outstanding discriminative performance, with an AUC of 0.984 (Fig. 5a). Similarly, oxylipins demonstrated excellent diagnostic accuracy, with 5(S)-HETE achieving perfect discrimination (AUC = 1) and 15(S)-HETE showing an AUC of 0.985 (Fig. 5b-c).

Metabolites involved in central energy metabolism also displayed high predictive performance, including glycerate (AUC = 0.985) and aspartate (AUC = 0.973) (Fig. 5d-e).

These findings identify several metabolites with an excellent discriminatory capacity and support their potential utility as candidate biomarkers of the long-term metabolic consequences of EUGR.

Integration of metabolic, inflammatory and dietary datasets

To investigate interactions across biological datasets, multiblock analysis (DIABLO) was performed including only participants with complete datasets (n = 88).

The initial multiblock model yielded an optimal two-component solution with a predicted balanced error rate of 0.05. Correlation analysis between latent components showed that acylcarnitines exhibited weak relationships with the remaining datasets (correlation values ≤ 0.25), suggesting limited contribution to group discrimination. In contrast, metabolomic platforms (GC-MS, LC-MS and LCqTOF), the biochemical parameters, inflammatory biomarkers, and calculated metabolic ratios showed substantially stronger correlations (≥ 0.5), indicating coordinated biological alterations (more details are provided in **Supplementary Fig. S1**). Selected variables together with their loading and stability values are presented in **Supplementary Table S9**. Variables with a stability value > 0.8 were considered relevant and had a high degree of confidence.

A second model incorporating dietary intake data produced a similarly robust two-component solution, with an overall classification error rate of $3.27e^{-02}$. Although the dietary block exhibited relatively weak correlations with the remaining datasets (≤ 0.36), the overall correlation structure remained highly consistent with that observed in the initial model (**Supplementary Fig. S2**), supporting the robustness of the integrated network.

Score plots for each data block (Fig. 6) showing excellent discrimination between groups for metabolomic datasets, inflammatory biomarkers and metabolic ratios, whereas acylcarnitines, biochemical parameters and dietary intake showed greater overlap. **Supplementary Table S10** summarizes the variables in the optimal model, including the loadings and stability of each component.

Network analysis based on block associations (**Supplementary Fig. S3**) identified two dietary components with potential biological relevance. Nut consumption was inversely associated with glutamate, aspartate, glycerate, HOMA-IR, the Glu/Gln ratio and several oxylipins, whereas vegetable intake was positively associated with FFAs, carnitines and iron levels, and inversely associated with IL8 concentrations.

Taken together, these integrative analyses demonstrate that the metabolic signature associated with EUGR is closely interconnected with inflammatory mechanisms, supporting the existence of coordinated biological networks that may contribute to the long-term cardiometabolic phenotype.

Discussion

This study offers a comprehensive metabolomic characterization of young adults with a history of EUGR. We identified a coordinated metabolic signature encompassing inflammatory lipid mediators, AA metabolism, intermediary metabolism, and lipid remodeling. Instead of standalone metabolic anomalies, our results show a unified surge across pathways, suggesting that early growth restriction triggers persistent metabolic remodeling into adulthood. Importantly, several of these molecules showed excellent discriminatory capacity, highlighting their potential utility as biomarkers of the long-term metabolic consequences of EUGR.

These findings are consistent with the concept that EUGR induces long-lasting metabolic programming, predisposing individuals to an adverse cardiometabolic phenotype later in life. Previous studies from our group have shown that children with a history of EUGR exhibit increased oxidative stress (OS), higher blood pressure and insulin resistance (IR) (Ordóñez-Díaz et al. 2020a; Ordóñez-Díaz et al. 2020b; Ortiz-Espejo et al. 2014) and similar changes in young adults (Palomino-Fernández et al. 2024). The present metabolomic analysis extends these observations by identifying specific metabolic processes that may underline these previously described clinical alterations.

This interpretation is supported by previous studies in preterm infants, which have shown that persistent postnatal undernutrition during critical developmental windows may disrupt normal metabolic programming, leading to long-term alterations in energy homeostasis mitochondrial function and substrate utilization (Dudzik et al. 2020; Younge et al. 2019). Notably, preterm infants who develop EUGR often exhibit limited metabolic recovery despite adequate nutritional support. This observation suggests that nutritional rehabilitation alone may be insufficient to fully restore metabolic adaptations established during early postnatal life. A better understanding of the metabolic pathways underlying impaired nutrient utilization and long-term metabolic programming is therefore needed (Younge et al. 2019). Collectively, these observations provide a biological framework for interpreting the persistent metabolic alterations identified in our young adult cohort.

Persistent lipid remodeling and inflammatory signaling

One of the most prominent findings of this study was the marked alteration of lipid-derived metabolites, particularly oxylipins, LPCs, LPEs, and FFAs. Together, these metabolites suggest persistent remodeling of systemic lipid metabolism paired with heightened proinflammatory signaling.

Oxylipins represented one of the dominant metabolic families in the EUGR metabolome. 3D-PCA further supported this observation, as oxylipins contributed predominantly to the variance explained by the first principal component in the EUGR group, whereas in the reference controls, their contribution was restricted to the third principal component. This distinct distribution indicates that oxylipins constitute a major determinant of the metabolic phenotype associated with previous EUGR.

Oxylipins are bioactive lipid mediators generated through the enzymatic oxidation of polyunsaturated fatty acids, particularly arachidonic and linoleic acids (Misheva et al. 2022). In the literature, elevated circulating oxylipins are associated with chronic low-grade inflammation, OS, endothelial dysfunction, development of hypertension, CVD and T2D (Ağagündüz et al. 2024; Caligiuri et al. 2017). Accordingly, increased concentrations of arachidonic acid metabolites (including 5(S)-HETE, 12(S)-HETE, and 15(S)-HETE) together with linoleic acid derivatives such as 9(S)-HODE, 13(S)-HODE and 13-oxoODE, suggest persistent activation of proinflammatory lipid cascades in individuals with a history of EUGR.

While our study design was not intended to establish direct causality, these metabolic alterations are consistent with a state of chronic low-grade inflammation and redox imbalance that may contribute to the increased cardiometabolic susceptibility previously reported in this population (Ordóñez-Díaz et al. 2021; Palomino-Fernández et al. 2024). This interpretation is further supported by the excellent discriminatory performance of several oxylipins, particularly 5(S)-HETE and 15(S)-HETE, which emerged among the strongest candidate biomarkers identified by both sPLS-DA and ROC analyses.

In addition to oxylipins, plasma concentrations of multiple LPCs and LPE 18:0 were consistently increased in EUGR participants. LPCs are generated during phospholipid hydrolysis and lipid peroxidation and are increasingly recognized as active mediators of vascular inflammation rather than passive products of membrane degradation (Law et al. 2019). Clinical studies have demonstrated that LPCs promote endothelial activation, platelet aggregation and atherosclerotic plaque instability, contributing to CVD progression (Ren et al. 2022; Tan et al. 2022). Elevated circulating LPC concentrations have also been associated with T2D, obesity, cancer, cardiovascular and neurodegenerative diseases, and several chronic inflammatory conditions (Ren et al. 2022).

Therefore, the simultaneous increase in oxylipins, LPCs and FFAs supports the existence of coordinated lipid remodeling rather than isolated disturbances in individual lipid species. Taken together, these insights reinforce the hypothesis that persistent alterations in inflammatory lipid metabolism may represent one of the central biological mechanisms linking EUGR with long-term cardiometabolic vulnerability.

Altered AA and intermediary metabolism

Our metabolomic analysis revealed substantial alterations in AA and intermediary metabolism, suggesting persistent metabolic reprogramming that extends beyond inflammatory lipid processes. Among these alterations, the Glu/Gln ratio emerged as one of the most relevant metabolic features associated with previous EUGR.

Participants with a history of EUGR exhibited higher circulating glutamate and aspartate concentrations together with lower glutamine and ornithine levels than the reference group. Moreover, glutamate, aspartate and the Glu/Gln ratio were among the variables contributing most strongly to group discrimination in the sPLS-DA model, highlighting their relevance within the metabolic phenotype identified in this study.

Accumulating evidence indicates that disturbance in glutamate metabolism is closely linked to metabolic dysfunction. Elevated circulating glutamate has consistently been associated with IR, subclinical atherosclerosis, heart failure, metabolic syndrome and obesity (Chen et al. 2023; Diniz et al. 2024; Papandreou et al. 2020). In contrast, glutamine has been widely recognized for its anti-inflammatory, antioxidant and cytoprotective properties, with higher circulating concentrations being associated with lower risks of hypertension, dyslipidemia, obesity and T2D (Chen et al. 2023; Diniz et al. 2024). Consequently, the balance between these two metabolites, rather than their individual concentrations, has emerged as a robust indicator of metabolic health.

Notably, the Glu/Gln ratio was significantly increased in the EUGR group and was positively associated with fasting insulin concentrations and HOMA-IR. Previous metabolomic studies have demonstrated that an increased conversion of glutamine into glutamate has been associated with IR and predicts future risk of T2D and microvascular complications (Diniz et al. 2024; Guasch-Ferré et al. 2016). Our findings therefore suggest that alterations in glutamine metabolism may represent one of the metabolic mechanisms linking EUGR with impaired glucose homeostasis later in life.

Furthermore, the Glu/Gln ratio also exhibited excellent discriminatory performance in ROC analysis, together with high stability within the multivariate models. Although external validation in independent cohorts is still required, these findings support this ratio as a promising candidate biomarker for identifying young adults who may be at increased cardiometabolic risk following EUGR.

Alterations in intermediary metabolism further reinforced this metabolic phenotype. Aspartate, glycerate, glycerol-1-phosphate and α -ketoglutarate were all significantly increased in EUGR participants. These metabolites occupy central positions within glycolysis, gluconeogenesis and the tricarboxylic acid cycle, suggesting persistent adaptations in energy metabolism (Gaudet et al. 2000; Holeček 2023; Skowrońska and Albrecht 2013; Sullivan et al. 2015).

Among these, glycerate displayed one of the highest discriminatory performances observed in the present study. Although relatively little is known about its role in cardiometabolic diseases, increased glycerate concentrations have previously been associated with impaired glucose metabolism and altered carbohydrate utilization (Gaudet et al. 2000). Likewise, elevated aspartate levels may reflect disturbances in mitochondrial metabolism and nitrogen handling, processes that have been linked to OS and chronic inflammation (Holeček 2023).

Taken together, these findings suggest that the metabolic alterations observed in EUGR extend beyond inflammatory lipid mediators and involve central mechanisms regulating AA homeostasis, mitochondrial function and energy metabolism. These changes appear to constitute an integrated metabolic phenotype consistent with impaired metabolic flexibility.

The consistency of the identified metabolites across complementary analytical approaches further supports the robustness of the metabolic phenotype associated with EUGR. Importantly, these candidate biomarkers not only exhibited excellent discriminatory performance but also mapped to key biological

processes involved in inflammatory lipid signaling, AA homeostasis and energy metabolism, reinforcing their biological plausibility and translational relevance. Although external validation is warranted, our findings suggest that a multidimensional metabolic signature may represent a more reliable strategy for identifying young adults at increased cardiometabolic risk following EUGR.

Multi-omics integration of metabolic and inflammatory pathways

Beyond the identification of individual metabolites, one of the major strengths of the present study is in the integration of metabolomic, biochemical, inflammatory and dietary data through multiblock analysis. This systems biology approach demonstrated that the metabolic alterations associated with EUGR do not occur independently but rather form coordinated biological networks.

The first multiblock model clearly discriminated individuals with previous EUGR from the reference group, and confirmed that AAs, oxylipins, LPCs, LPEs, and FFAs represented the principal contributors to the metabolic phenotype identified throughout the study. In contrast, acylcarnitines showed relatively weak correlations with the remaining datasets. These findings are consistent with previously published studies focusing on the metabolome of EUGR populations in the first year of life that reported an initial increase in acylcarnitines that gradually declined, with long-chain acylcarnitines being more elevated in the general population (Dudzik et al. 2020; Younge et al. 2019). Taken together, these data suggest that alterations in fatty acid transport play a less prominent role during young adulthood than inflammatory lipid metabolism or intermediary metabolic pathways (Brejchova et al. 2023).

Importantly, the integration of inflammatory biomarkers into the multiblock model further strengthened the biological interpretation of our findings. Reduced circulating adiponectin emerged as one of the variables associated with the EUGR metabolic phenotype. Adiponectin is widely recognized for its antiatherosclerotic, antithrombotic, antioxidant, and anti-inflammatory properties and has consistently been associated with improved insulin sensitivity and reduced cardiovascular risk (Cândido et al. 2021; Palomino-Fernández et al. 2024). Therefore, the coexistence of reduced adiponectin together with increased oxylipins, LPCs, LPEs and glutamate supports the presence of a coordinated metabolic environment favoring chronic low-grade inflammation and CVD rather than isolated biochemical abnormalities (Ağagündüz et al. 2024; Knuplez and Marsche 2020). This integrated perspective is particularly relevant because cardiometabolic disease arises from complex biological interactions.

Dietary contribution to the metabolic phenotype

To explore whether current dietary habits could explain the observed metabolic alterations, dietary intake data were incorporated into the multiblock model. Although dietary variables modestly improved the integrated network, they did not substantially modify the overall metabolic signature identified in EUGR participants.

Interestingly, the nuts and vegetables group emerged as the dietary components most closely associated with the metabolic network. However, the metabolic signature remained unchanged,

indicating that diet was not a determining factor in this profile with respect to the condition studied. Among these, nut consumption showed an inverse relationship with glutamate, aspartate, glycerate, the Glu/Gln ratio, oxylipins and HOMA-IR. These observations are biologically plausible given the well-established anti-inflammatory and insulin-sensitizing effects of nuts within Mediterranean dietary patterns (Alves et al. 2019; Estruch et al. 2018; Salas-Salvadó et al. 2011; Taghizadeh et al. 2023).

Likewise, vegetable intake was positively associated with components most closely related to alterations in lipid and energy metabolism (particularly the β -oxidation of fatty acids) FFA, acylcarnitines and iron concentrations, while showing an inverse relationship with IL-8, supporting previous evidence that plant-based dietary patterns contribute to attenuation of systemic inflammation (Alves et al. 2019; Menzel et al. 2020; Taghizadeh et al. 2023).

However, despite these associations, the overall metabolic phenotype remained remarkably stable after the inclusion of dietary information. This finding suggests that the metabolic alterations observed in young adults with previous EUGR are unlikely to be explained by current dietary habits and instead may reflect the persistent metabolic programming established during early life.

Strengths and limitations

The main strength of this study lies in the comprehensive multi-omics characterization of the long-term metabolic consequences of EUGR, integrating multiplatform metabolomics with biochemical, inflammatory and dietary data. The consistency of the findings across complementary analytical approaches strengthens the robustness of the identified metabolic signature. Nevertheless, several limitations should be acknowledged. The cross-sectional design precludes causal inference, and the relatively modest sample size requires external validation. Future longitudinal studies integrating metabolomics with other omics technologies will be essential to validate these candidate biomarkers and further elucidate the mechanisms linking EUGR to adult cardiometabolic disease.

Conclusion

To our knowledge, this is the first study that reveals a distinctive metabolic signature in young adults with a history of EUGR. This group is characterized by the presence of elevated levels of OS markers, along with increased products of lipolysis and fatty acid oxidation. These metabolic disturbances converge into interconnected biological networks associated with long-term cardiovascular risk. However, further research is needed to establish specific biomarkers to plan strategies and targeted interventions to prevent the development of metabolic diseases in the future.

Abbreviations

A separate list of abbreviations is provided in **Supplementary file 1** (Section Supplementary Abbreviations).

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments and was approved by the Institutional Hospital Ethical Committee (ref 3729). The participants were informed of the study, and written consent was obtained. Those under 18 years of age consented verbally, and their legal guardians provided written consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to data regulations and for ethical reasons, considering that this information might compromise research participants' acceptance because our participants only gave their consent for the use of their data by the original team of investigators. However, collaborations for data analyses can be requested by sending an email to the corresponding author.

Competing interests

The authors declare that they have no affiliations or involvement with any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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Authors' Contribution

ODRH and MGC contributed to the study conception and design. Material preparation, data collection, and data analysis were performed by LPF, IV, BPV, ODRH and MGC. The first draft of the manuscript was written by LPF, IV, BPV, ODRH, AG and MGC, and all authors reviewed and provided feedback on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

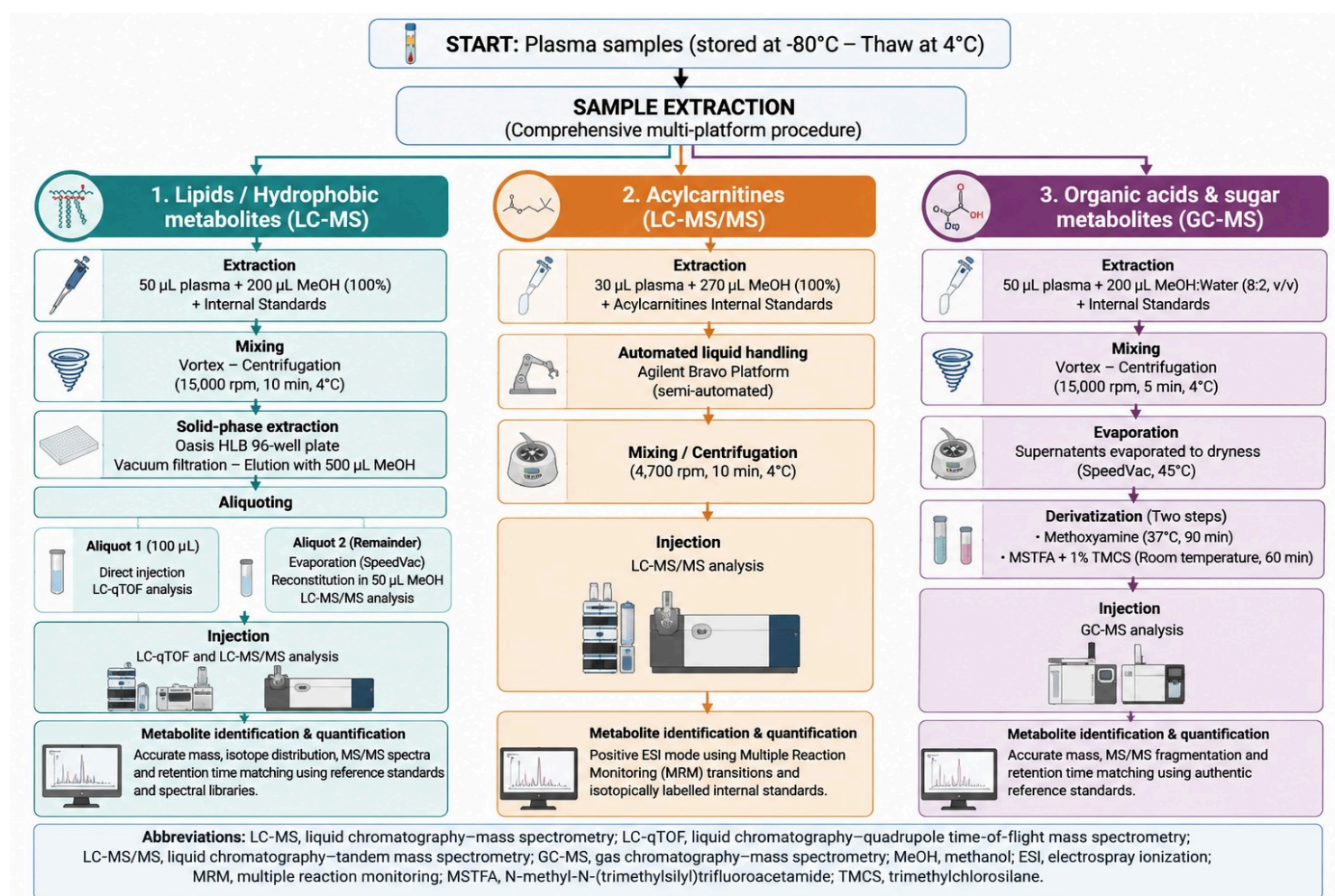


Figure 1

Analytical Workflow for Plasma Sample Preparation and Metabolomics/Lipidomics Profiling

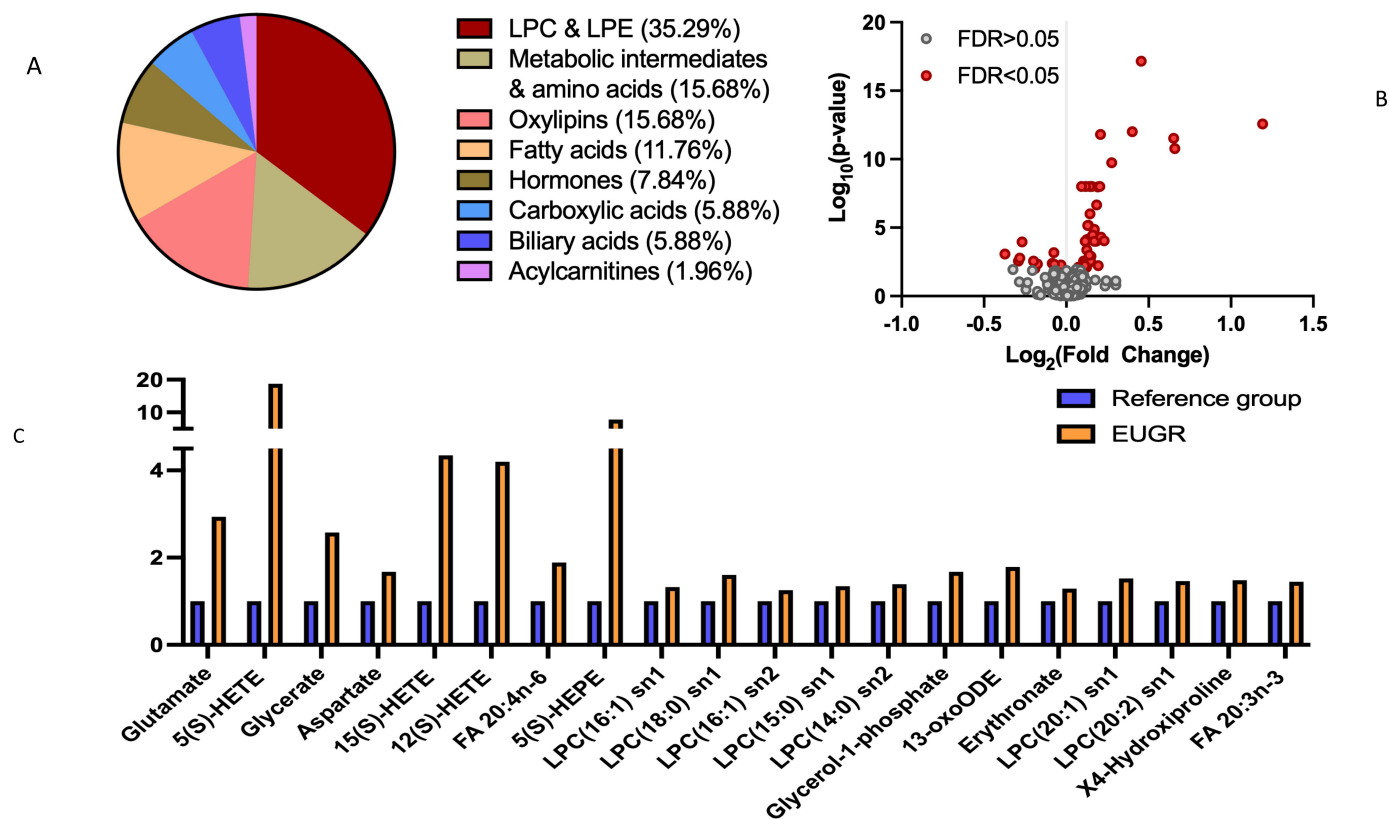


Figure 2

FDR-adjusted univariate analysis and screening of differentially expressed metabolites between the EUGR and reference groups. **A)** Pie chart of percentage distribution of the 51 metabolites that were significantly different between groups ($q < 0.05$) by biochemical class. **B)** Volcano plot of differential genes expression distribution. A total of 51 FDR-corrected ($q < 0.05$) significant metabolites (highlighted in red) were found to be significantly dysregulated in EUGR group vs reference group. **C)** Relative abundance of the top 20 most significant differential metabolites. Values were expressed as a ratio to the healthy reference group used as a baseline reference (value=1). Benjamini-Hochberg adjusted P value < 0.001 .

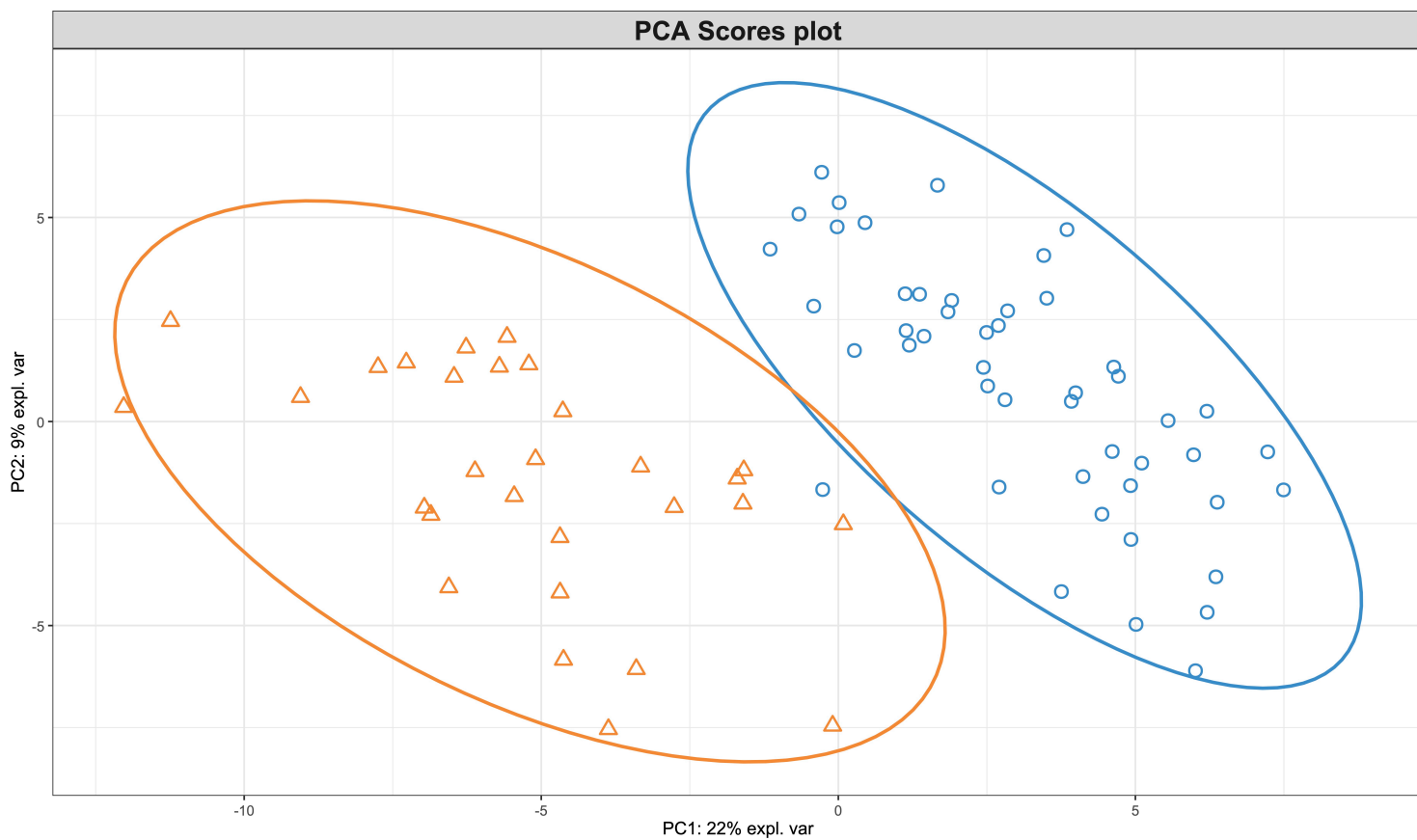


Figure 3

Scores plot corresponding to principal component analysis (PCA), including samples from young subjects with a history of EUGR and a reference group.

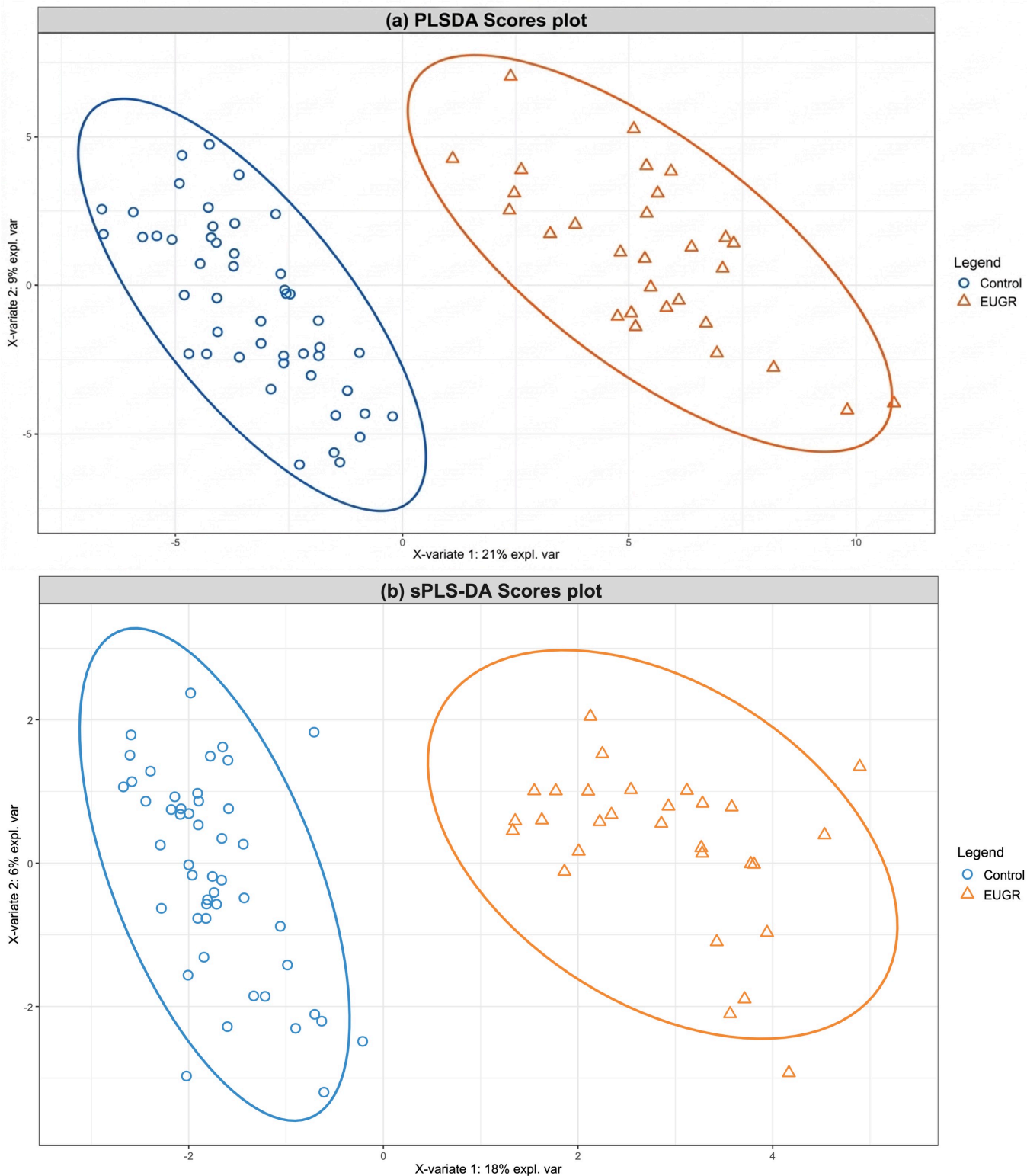


Figure 4

Comparison between young adults with a history of EUGR and a reference group. **(a)** Partial least squares discriminant analysis (PLS-DA) scores plot from the model; **(b)** sparse variant of PLS-DA (sPLS-DA) scores plot from the model.

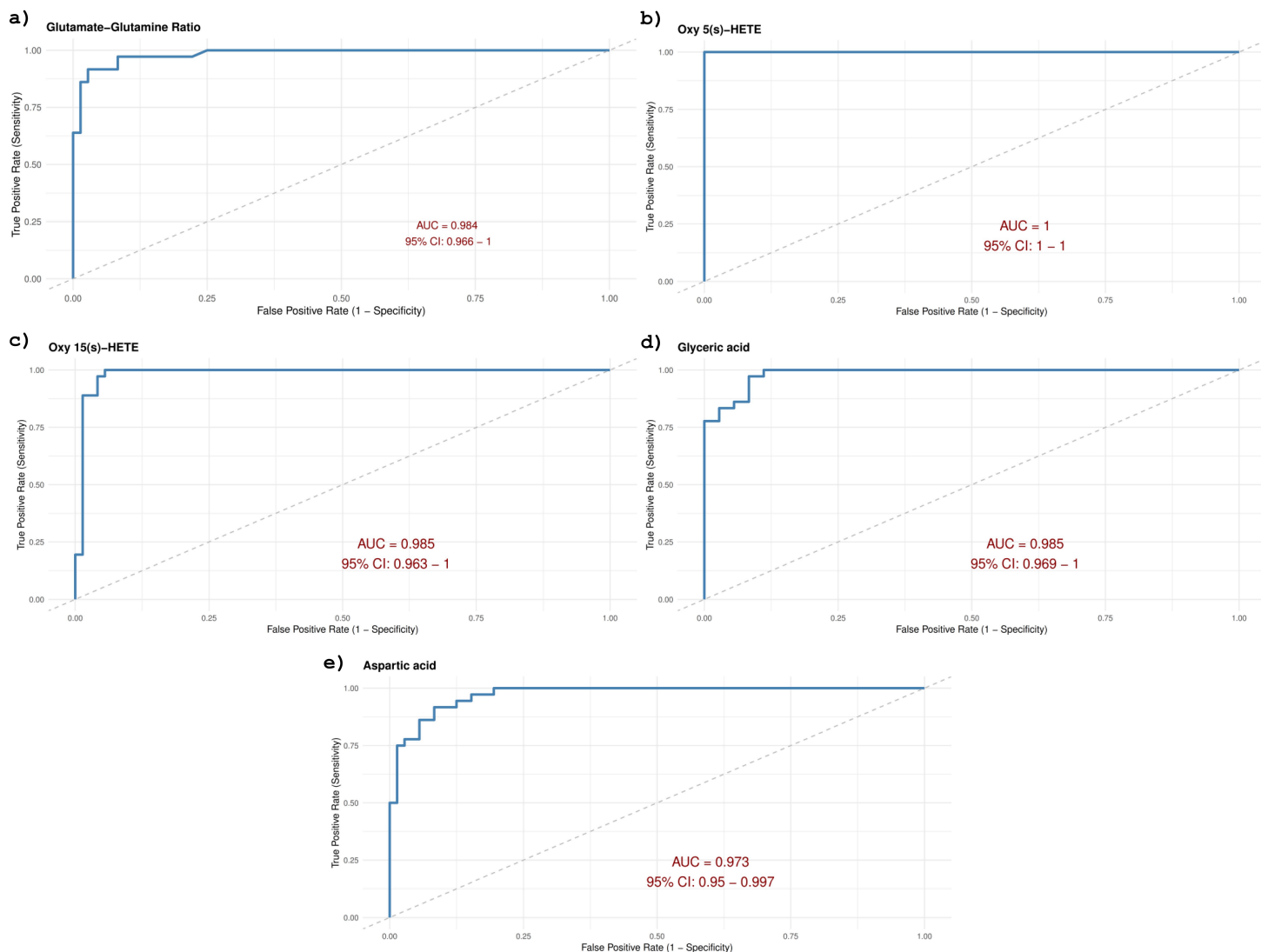


Figure 5

ROC curve showing the sensitivity and specificity of **(a)** Glu/Gln ratio, **(b)** oxy 5(S)-HETE, **(c)** oxy 15(S)-HETE, **(d)** glyceric acid and **(e)** aspartic acid in the EUGR group vs the reference group. The area under the curve (AUC) and 95% confidence interval (CI) are shown to indicate diagnostic performance.

DIABLO – Model with diet

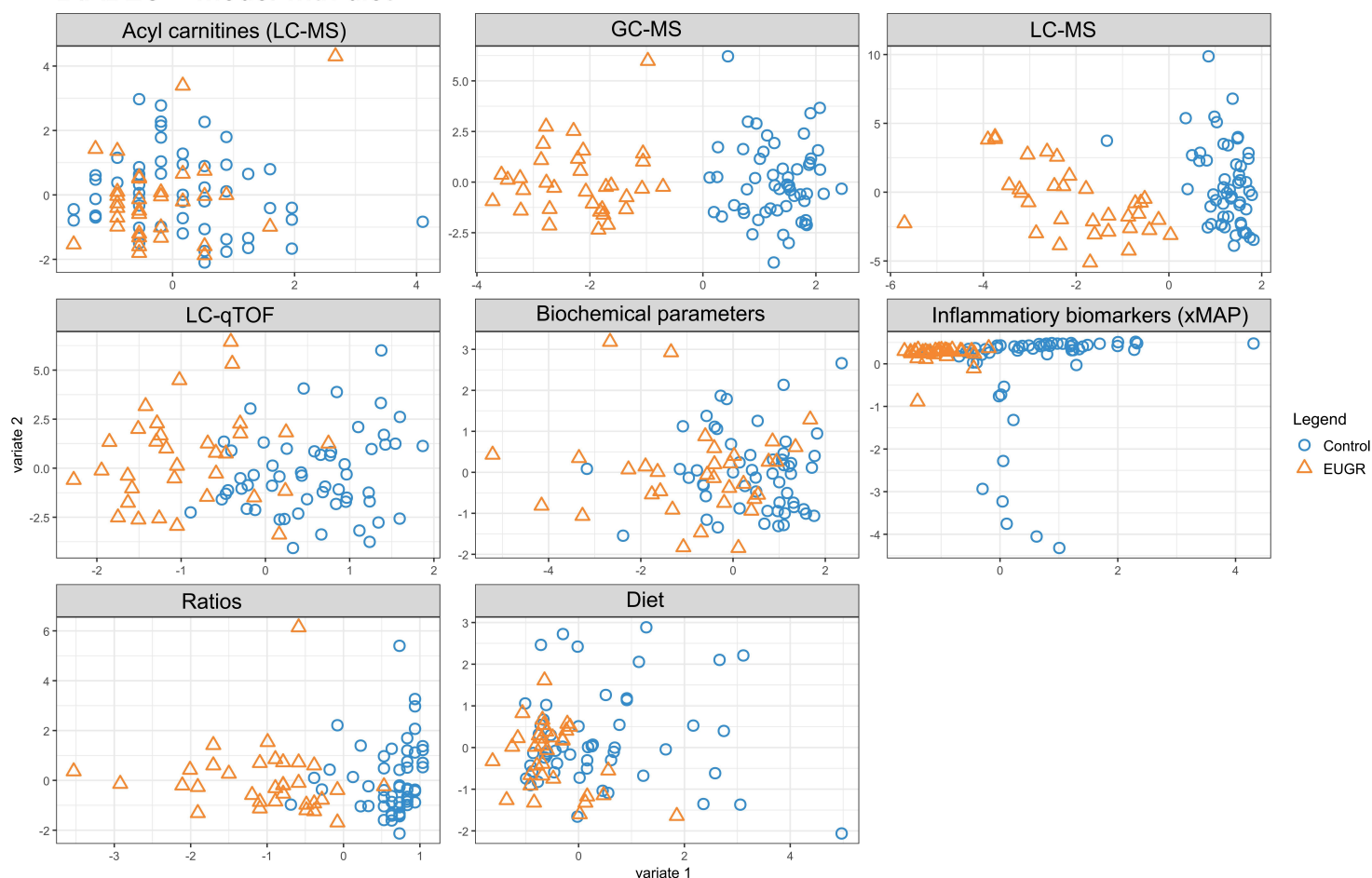


Figure 6

DIABLO (Data Integration Analysis for Biomarker Discovery using Latent Variable Approaches for Omics Studies) score plot for each data block included in the multiblock model comparing young participants with a history of EUGR and the healthy reference group.

Supplementary Files

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- [SupplementarymaterialPalominoFernandezetal.docx](#)