Insulin resistance as a potential driving force of parental obesity-induced adverse metabolic programming mechanisms in children with obesity

BMC Medicine

RESEARCH

Open Access

Insulin resistance as a potential driving force of parental obesity-induced adverse metabolic programming mechanisms in children with obesity

Lucía Jurado-Sumariva[1], Álvaro González-Domínguez[1,2*], Otto Savolainen[3], Jesús Domínguez-Riscart[1,4], Rikard Landberg[3] and Raúl González-Domínguez[1*]

Abstract

Background Parental obesity has been identified as one of the most important early risk factors for childhood obesity, but molecular mechanisms driving this greater predisposition remain to be elucidated.

Methods In this study, we recruited a cohort comprising children with obesity (body mass index over two *z*-scores above the age/sex-adjusted mean of the Spanish reference population, age range: 6–12 years), born to parents with obesity ($N=18$) or without obesity ($N=41$), as well as matched healthy controls ($N=26$). Plasma and erythrocyte samples were collected for comprehensive biochemical and metabolomics analyses, this latter by applying high-throughput liquid chromatography–mass spectrometry. Then, a combination of multivariate and univariate statistical tools was applied to unravel the molecular pathogenic impairments that parental obesity may imprint in the offspring.

Results Interestingly, we found parental obesity to be associated with exacerbated unhealthy metabolic outcomes in the offspring with obesity, as mirrored in higher fasting insulin levels ($p=2.8\times10^{-8}$) and HOMA-IR scores ($p=1.3\times10^{-8}$). This was in turn accompanied by altered concentrations in 87 plasma and 51 erythroid metabolites ($p<0.05$) involved in a variety of obesity-related pathways that are known to be tightly regulated by insulin action, namely energy-related metabolism, branched-chain amino acids, nitrogen homeostasis, redox systems, and steroid synthesis (i.e., steroid hormones, bile acids). Additional analyses demonstrated that most metabolomics associations were largely attenuated after adjusting for the HOMA-IR scores.

Conclusions Therefore, we hypothesize that insulin resistance could be a major driving force in mediating deleterious programming mechanisms induced by parental obesity in the offspring.

Keywords Childhood obesity, Insulin resistance, Metabolomics, Parental obesity

*Correspondence:
Álvaro González-Domínguez
alvaro.gonzalez@inibica.es
Raúl González-Domínguez
raul.gonzalez@inibica.es
Full list of author information is available at the end of the article

Background

The etiology of obesity has a multifaceted nature involving complex interactions between lifestyle, environmental, genetic, endocrine, gut microbial, socioeconomic, psychological, and many other factors [1]. The prenatal and perinatal periods are especially sensitive to certain obesogenic determinants, such as parental diseases (e.g., obesity, gestational diabetes), malnutrition, and exposure to endocrine and microbiota-disrupting chemicals (e.g., smoking, antibiotics), which may elicit long-lasting deleterious consequences on the offspring health [2]. Among them, parental obesity (PO) stands out as one of the most important early risk factors for childhood obesity [3, 4]. In this respect, maternal obesity has repeatedly been reported to have a stronger impact on newborn health than paternal obesity since, besides the transgenerational inheritance transmitted by both progenitors during conception, the infant could be exposed to additional maternal stimuli along fetal (e.g., placental environment) and neonatal (e.g., breastfeeding) development [5, 6]. In contrast, other studies have not found differences between maternal and paternal obesity in their magnitude of association with childhood obesity at older ages [3, 7], as growing evidence suggests that paternal effects might appear later in life, possibly around 3–4 years of age [8, 9]. This greater predisposition to obesity and related cardiometabolic disorders has been allocated to the induction of altered developmental programming mechanisms affecting a variety of organs (e.g., adipose tissue, pancreas), ultimately leading to impairments in adipocyte remodeling, insulin secretion and function, appetite control via the hypothalamic–pituitary–adrenal axis, cytokine production, and epigenetic modifications [10, 11]. Nevertheless, the understanding of these PO-mediated metabolic adaptations mainly proceeds from animal models, which are not always translatable to humans [12], so there is an urgent need for further clinical research to clarify the mechanisms contributing to this increased metabolic risk. To this end, metabolomics has been established as a powerful tool to get deeper insights into the pathophysiology and susceptibility factors underlying the onset and progression of complex diseases, since metabolites accurately reflect the intertwined crosstalk between genetics background, endogenous metabolism, and exogenous inputs [13]. A number of authors have explored the influence of maternal adiposity in the offspring metabolome, as assessed both during the perinatal period [14–16] and, to a lesser extent, in mid-childhood [17, 18]. Conversely, scarce data are available on the impact of combined parental insults, being limited to a few studies performed in adult descendants using low-coverage metabolomics methods [19, 20]. Moreover, it should be stressed that most of these previous investigations were aimed at elucidating the involvement of family history of obesity in modulating adverse metabolic outcomes in general populations (i.e., non-obese offspring), which hinders establishing a direct pathophysiological link between parental and childhood obesity. Accordingly, further research in obesity cohorts is essential to better understand the role of PO in shaping obesity-related metabolic disturbances along childhood.

Herein, we recruited a population comprising children with obesity, born to parents with or without obesity, as well as matched healthy lean controls, to unravel the molecular pathogenic impairments that PO may imprint in the offspring (Fig. 1). To this end, paired plasma and erythrocyte samples were collected for high-throughput metabolomics analysis, thus enabling us to investigate obesity pathophysiology in a comprehensive manner, at both systemic and cellular levels. In this respect, it should be stressed that erythrocytes have been proven to be reliable and sensitive sensors of failures in energy homeostasis, oxidative stress, and inflammation [21], which stand out as the most relevant pathogenic events behind obesity and related comorbidities.

Methods

Study design

The study population consisted of 85 prepubertal children, aged between 6 and 12 years, who were recruited at "Hospital Universitario Puerta del Mar" (Cádiz, Spain). For the group of children with obesity, the eligibility criteria were presenting a body mass index (BMI) over two *z*-scores above the age/sex-adjusted mean of the Spanish reference population [22], being classified as stage 1 according to the Tanner scale for pubertal development, and having retrospectively registered data about PO (assessed by an endocrinologist at time of birth) in medical records. The information of parental weight at birth (i.e., BMI > or < 30 kg/m^2) was registered by endocrinologists in an internal database (Pediatric Endocrinology Unit, Hospital Universitario Puerta del Mar), which was surveyed at the time of enrollment to stratify our population (permission to access this database was granted by clinicians in charge of the recruitment, Dr. Alfonso Lechuga and Jesús Domínguez). On this basis, the participants were stratified as children with obesity and PO (PO+, $N=18$), when at least one of the parents had obesity (BMI > 30 kg/m^2); or as children with obesity without PO (PO−, $N=41$), when none of the parents had obesity (BMI < 30 kg/m^2). Within the PO+ group, five children were born to mothers with obesity, three children were born to fathers with obesity, and ten children were born to both parents with obesity. Furthermore, healthy controls (CNT, $N=26$) matched on age and sex were recruited among children without obesity nor PO who

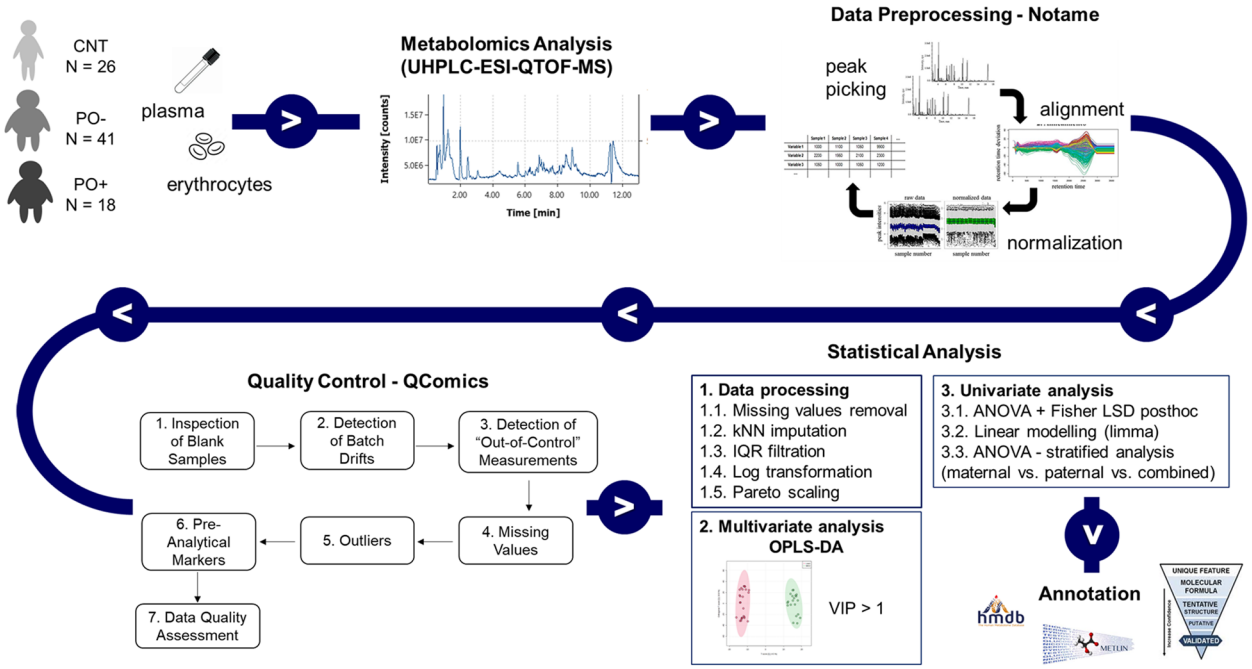

Fig. 1 Schematic workflow of the study design

were subjected to a routine blood test under medical prescription. Children suffering from other chronic diseases or acute infectious processes, or receiving any medical treatment, were not eligible for the study. The GRANMO 7.12 webtool was employed for statistical power calculations. For this purpose, means between groups under study (PO+, $N=18$; PO−, $N=41$; CNT, $N=26$) were compared using Cohen's d as effect size (assuming d 0.80 as large effect). Thus, using a sample size of 85 children and considering an alpha risk of 0.05, we found the statistical power of our comparisons to be above 80%. The Ethical Committee of "Hospital Universitario Puerta del Mar" (Cádiz, Spain) approved the study protocol (Ref. PI22/01899), and all participants and/or legal guardians provided written informed consent. The study was performed in accordance with the principles contained in the Declaration of Helsinki.

Sampling and biochemical data collection

After an overnight fasting of at least 12 h, blood samples were collected by venipuncture using BD Vacutainer EDTA tubes. The blood tubes were centrifuged at 1500 g for 10 min at 4 °C to separate the plasma, and cell pellets were washed three times with cold saline solution (9 g/L NaCl, 4 °C) to obtain erythrocytes after centrifuging at 1500 g for 5 min at 4 °C. A fresh aliquot of plasma was employed to determine the content of glucose, insulin, and lipids (i.e., total cholesterol; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; and triglycerides) using an Alinity automatic analyzer (Abbot, Madrid, Spain). The homeostatic model assessment for insulin resistance (HOMA-IR) score was calculated by applying the formula: HOMA-IR = (glucose × insulin) × 0.055/22.5, where glucose and insulin concentrations are expressed as mg/dL and μU/mL, respectively. The rest of the samples were stored at − 80 °C for metabolomics analysis.

Metabolomics analysis

Metabolomics analysis of plasma and erythrocyte samples was performed by applying the method described by González-Domínguez et al. [23]. Briefly, biological samples were subjected to protein precipitation with organic solvents and then analyzed by reversed-phase ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry, using the operational conditions described elsewhere [23]. Quality control (QC) samples were prepared by mixing equal aliquots of each individual sample under study and injected at intermittent points throughout the sequence run to monitor system stability and data quality, according to the QC*omics* guidelines [24]. Then, raw data were preprocessed using the "notame" workflow for peak picking, peak alignment, and analytical drift correction [25]. Finally, molecular features of interest were identified following the Metabolomics Standards Initiative (MSI) recommendations: (i) matching experimental data (i.e., accurate m/z and tandem spectra, maximum error mass: 10 ppm)

against databases (i.e., Human Metabolome Database, METLIN); (ii) analysis of pure standards [26]. The annotation of phospholipids and phase II metabolites was confirmed based on their characteristic fragmentation spectra [27, 28].

Statistical analysis

A combination of multivariate (orthogonal partial least squares discriminant analysis) and univariate (analysis of variance, linear modeling) approaches was applied to perform group comparisons, following the workflow described by Blaise et al. for metabolomics data analysis [29]. For handling missing values, we applied the strict 20% rule (i.e., removal of variables containing more than 20% missing values) to minimize as much as possible the presence of spurious signals in our datasets, thus strengthening the power of further statistical analyses (crucial if considering the relatively small size of the study population, as a consequence of our strict inclusion criteria and further stratification according to the PO status). The major drawback of this strategy is the possible loss of metabolites that are not expected to be widely detected (e.g., truly absent data), such as dietary or exposure compounds. However, we considered this risk assumable as the main aim of this work was investigating the molecular impairments that PO may imprint in the offspring, not assessing the impact of other lifestyle factors (as we do not have lifestyle data of this population). Anyway, it should be stressed that only 3.1% of all the molecular features were removed after applying the 20% rule, guaranteeing that the impact of this preprocessing was negligible. The remaining missing data were subjected to kNN imputation, as this method has been proven to provide better performance, robustness, and an overall marginal type 1 error rate [30, 31], especially when the proportion of missing data across samples for a molecular feature is relatively low (as in our datasets, with only 11.6% of imputable variables). Then, data was filtered based on the interquartile range to remove non-informative variables, log transformed to increase the symmetry of skewed distributions, and Pareto scaled to adjust for differences in concentration scales. As a first data exploration, orthogonal partial least squares discriminant analysis (OPLS-DA) was employed to inspect the discriminant capacity of plasma and erythroid metabolites in a multivariate manner. On the basis of these models, metabolites of interest were selected among those showing a "Variable Importance for the Projection" parameter greater than 1. Afterward, the significance of the associations observed in multivariate models was validated by analysis of variance (ANOVA) with Fisher LSD post hoc tests and false discovery rate (FDR) correction for multiple testing. Linear models with covariate adjustment were also computed using the limma package to study the influence of insulin resistance (IR), as assessed through the HOMA-IR score, as a confounding factor. Finally, secondary ANOVA analyses were performed to look for metabolomics differences within the PO + group depending on which parent presents obesity (i.e., maternal vs. paternal vs. combined). On the other hand, descriptive analysis of demographic, anthropometric, and biochemical variables was performed by means of the median and interquartile range for quantitative variables, or by percentages in case of qualitative ones. Data normality was checked by conducting the Shapiro–Wilk test. Then, differences between groups were assessed by applying the Kruskal–Wallis test. The significance of all comparisons was set at $p < 0.05$. All statistical analyses were conducted in the MetaboAnalyst 5.0 web tool (https://www.metaboanalyst.ca/).

Results

The characterization of the study population in terms of demographic, anthropometric, and biochemical data is summarized in Table 1. The participants were on average 9.0 years old and 54% were male, characteristics that were matched across the three groups under investigation. As defined by inclusion criteria, children with obesity presented higher weight ($p = 2.8 \times 10^{-11}$), BMI ($p = 7.7 \times 10^{-11}$), and waist circumference ($p = 2.1 \times 10^{-7}$) than healthy controls, with *Z*-scores above 2. However, these anthropometric measures were similar between PO + and PO − children, which enables us to hypothesize that differences found in other variables are not related to obesity degree, but driven by PO. Interestingly, gradual disturbances were observed in insulin homeostasis within the study population, with higher fasting insulin levels ($p = 2.8 \times 10^{-8}$) and HOMA-IR scores ($p = 1.3 \times 10^{-8}$) being detected among PO + and, to a lesser extent, PO − subjects when compared with the control group. In contrast, no differences were found in glucose levels, whereas obesity-related changes in the lipid profile (i.e., higher triglyceride levels, lower HDL-C levels) were comparable regardless of PO status.

Metabolomics analysis evidenced that childhood obesity may trigger numerous changes in the circulating metabolome (FDR-*p* values < 0.05), with differential levels being observed in 87 plasmatic and 51 erythroid metabolites (from a total of 680,708 and 547,904 molecular features that were detected in each study matrix), as depicted in Additional file 1: Tables S1–S2. Compared to controls, children with obesity presented higher concentrations in a variety of metabolites involved in energy homeostasis, branched-chain and aromatic amino acids, markers of oxidative stress, and nucleotide catabolites in both biological matrices, while other metabolic

Table 1 Demographic, anthropometric, and biochemical characteristics of the study population. Results are expressed as the median and interquartile range (except for sex, expressed as percentage). Superscript letters within each row indicate significant differences between groups that are marked with different letters, according to the Kruskal–Wallis test ($p < 0.05$). *NS*, non-significant

	CNT ($N = 26$)	PO− ($N = 41$)	PO+ ($N = 18$)	*p*-value
Demographic and anthropometric variables				
Age (years)	8.8 (7.5–10.0)	9.3 (8.2–10.1)	9.3 (8.5–10.5)	NS
Sex (% male)	57.7	48.8	61.1	NS
Weight (kg)	27.3 (23.3–30.2)[a]	55.6 (46.7–66.5)[b]	56.0 (50.7–68.8)[b]	2.8×10^{-11}
Weight (*Z*-score)	0.04 (− 0.5–0.3)[a]	4.9 (3.7–6.1)[b]	4.9 (3.6–5.8)[b]	1.1×10^{-10}
Body mass index (kg/m^2)	16.6 (15.7–17.5)[a]	28.0 (25.7–31.0)[b]	29.7 (25.3–33.4)[b]	7.7×10^{-11}
Body mass index (*Z*-score)	− 0.3 (− 0.8–0.3)[a]	4.4 (3.7–5.6)[b]	5.1 (3.1–6.1)[b]	3.3×10^{-11}
Waist circumference (cm)	58.4 (55.0–61.1)[a]	90.0 (83.5–97.0)[b]	94.5 (87.0–106.0)[b]	2.1×10^{-7}
Waist circumference (*Z*-score)	− 0.4 (− 0.8–0.1)[a]	4.6 (3.1–5.8)[b]	4.8 (3.7–6.3)[b]	1.1×10^{-10}
Biochemical variables				
Glucose (mg/dL)	83.0 (81.0–87.0)	85.5 (80.8–91.0)	84.5 (79.0–89.8)	NS
Insulin (μU/mL)	4.7 (4.0–5.9)[a]	14.0 (10.0–15.8)[b]	18.5 (15.8–25.5)[c]	2.8×10^{-8}
HOMA-IR	0.9 (0.8–1.3)[a]	2.8 (2.3–3.4)[b]	4.0 (3.2–5.5)[c]	1.3×10^{-8}
Total cholesterol (mg/dL)	165.0 (144.3–178.8)	168.0 (149.0–189.0)	149.0 (143.0–160.0)	NS
Low-density lipoprotein cholesterol (mg/dL)	90.5 (79.0–108.5)	99.0 (85.5–117.5)	97.0 (82.0–104.0)	NS
High-density lipoprotein cholesterol (mg/dL)	63.5 (57.3–69.3)[a]	48.0 (43.0–51.5)[b]	44.0 (41.0–45.0)[b]	7.5×10^{-7}
Triglycerides (mg/dL)	44.5 (37.3–55.8)[a]	78.0 (59.0–101.0)[b]	63.0 (50.0–91.0)[b]	2.0×10^{-4}

increments were exclusive for plasma (e.g., steroid hormones, bile acids) or erythrocytes (e.g., carnitine derivatives, methionine, N-acetylspermine, sphingolipids). Moreover, lower plasma levels were found for ketone bodies, hydroxylated fatty acids, xanthosine 5-triphosphate, and other amino acids (e.g., arginine, histidine, glutamine, N-acetylglycine). Considering the phospholipid profile, we observed a profound remodeling in fatty acid composition (i.e., raised content of saturated species, reduced content of polyunsaturated species), which was in turn accompanied by an overall decrease in lysophospholipids. Besides these differences in endogenous metabolism, we also found childhood obesity to be associated negatively with dietary metabolites (in both matrices) and positively with endocrine disrupting chemicals (in plasma). However, the most interesting results were obtained when studying the influence of PO in these obesity-related metabolic impairments. Children from the PO+ group showed more pronounced changes in many differential metabolites, affecting all the metabolic classes aforementioned (with the exception of phospholipids and sphingolipids). More interestingly, a few metabolites (e.g., plasma histidine, erythroid carnitine derivatives) were reported to exclusively differ in PO+ children, whereas similar levels were detected in control and PO− subjects, thus suggesting that PO might play an important role in modulating these metabolic programming mechanisms. In this respect, it should also be noted that the influence of PO was generally stronger in erythrocytes, with analog plasmatic metabolites showing similar levels regardless of PO status (e.g., carbohydrate-related metabolites, branched-chain amino acids, oxidative stress byproducts, nucleotide catabolites).

The significance of most of these associations between childhood obesity, PO, and circulating metabolites was lost, or at least attenuated, after additionally adjusting for HOMA-IR as a covariate (Additional file 1: Tables S1–S2). Furthermore, secondary statistical analyses in the PO+ group demonstrated that none of the above-mentioned differential metabolites remained significant when considering maternal, paternal, or combined parental obesity as separate study groups (Additional file 1: Tables S1–S2), thus suggesting that the influence PO could be independent of which progenitor presents obesity.

Discussion

Family history of obesity is well-recognized as one of the most important risk factors for childhood obesity [3, 4], but mechanisms driving this greater predisposition remain to be elucidated. Herein, we found that children born to parents with obesity had increased fasting insulin levels and HOMA-IR scores, in agreement with previous clinical [32, 33] and experimental [10] studies describing that PO may disrupt β cell development in the offspring, thus impairing insulin production and function. Interestingly, this was in turn accompanied by altered levels

in a multitude of metabolites participating in a variety of pathways that are known to be tightly regulated by insulin action, as summarized in Fig. 2 and detailedly discussed in the following paragraphs. Additional statistical analyses showed that most of these associations between childhood obesity, PO, and blood metabolites were largely attenuated after adjusting for HOMA-IR scores (Additional file 1: Tables S1–S2). Altogether, our findings suggest that IR could represent a cornerstone in modulating PO-mediated exacerbations in the metabolic derangements typically underlying childhood obesity.

Supporting this hypothesis, we found that levels of numerous metabolites involved in carbohydrate metabolism and other energy-related pathways, which can be regarded as the central pathogenic hallmarks in the obesity-IR crosstalk, differed between PO+ and PO− children. Obesity has traditionally been associated with an excessive consumption and ineffective expenditure of

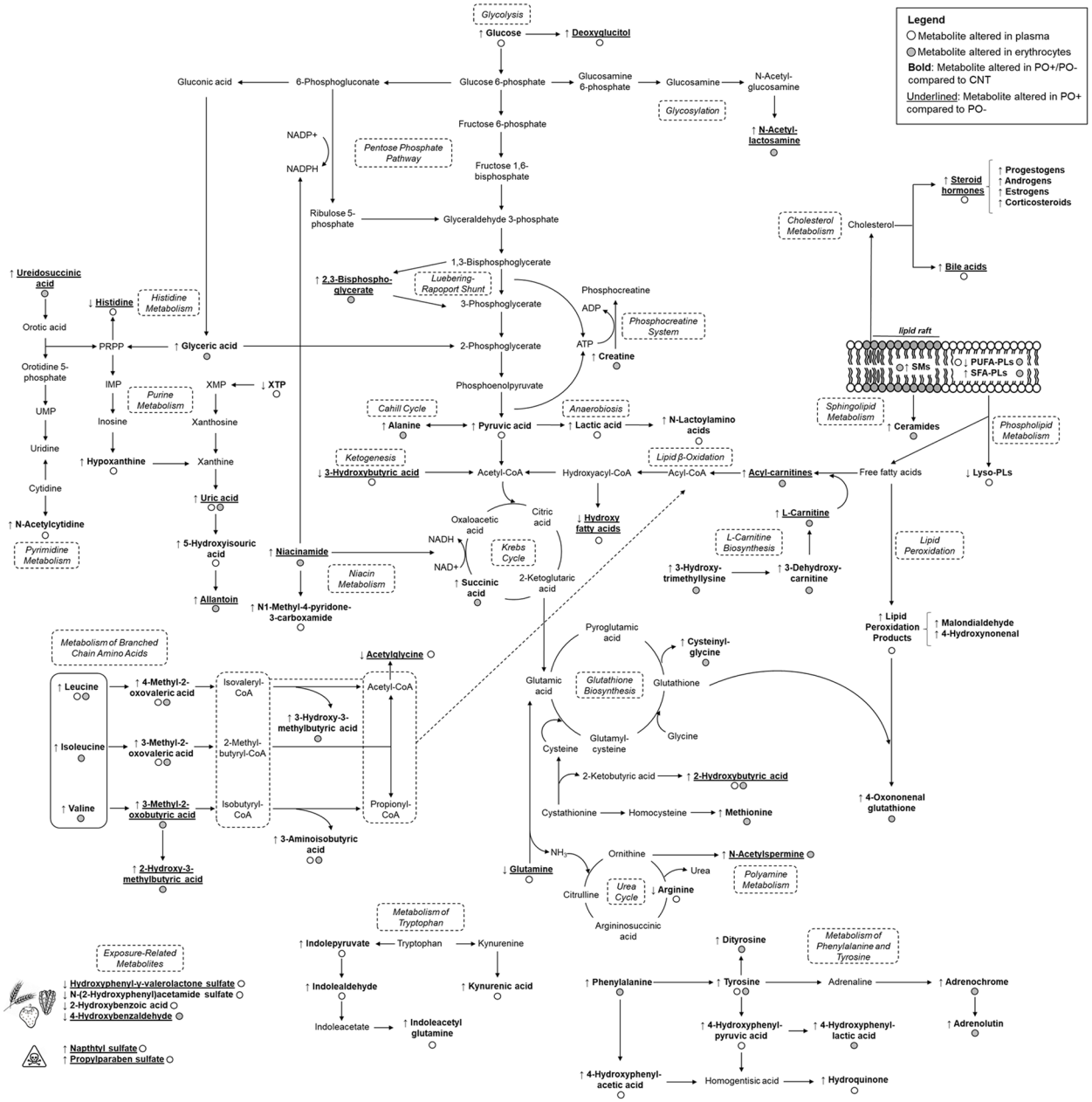

Fig. 2 Pathway analysis of differential plasma and erythroid metabolites found to be altered in children with obesity and/or influenced by parental obesity

calories, coupled to a shift toward glycolytic metabolism as the predominant energy source to compensate for mitochondrial impairments [34]. In this work, this was mirrored in increased plasma and/or erythroid contents of simple sugars (i.e., glucose, deoxyglucitol) and other intermediates participating in downstream metabolic processes, such as glycolysis (i.e., pyruvate), anaerobiosis (i.e., lactate, N-lactoylamino acids), Cahill cycle (i.e., alanine), Luebering-Rapoport shunt (i.e., glyceric acid 2,3-bisphosphate), pentose phosphate pathway (i.e., glycerate), glycosylation (i.e., N-acetyl-lactosamine), phosphocreatine system (i.e., creatine), and niacin metabolism (i.e., niacinamide, N1-methyl-4-pyridone-3-carboxamide), in line with recent metabolomics studies [21, 34]. Furthermore, this was accompanied by alterations in other metabolites suggesting mitochondrial dysfunctions affecting ketogenesis (i.e., lower plasma 3-hydroxybutyrate), lipid β-oxidation (i.e., lower plasma hydroxylated fatty acids and N-acetylglycine, higher erythroid acyl-carnitines), and Krebs cycle (i.e., higher erythroid succinate) [21, 34]. Noteworthy, this obesity-related metabolic fingerprint reflecting a widespread energy dyshomeostasis was found to be largely exacerbated in children born to parents with obesity when compared with the PO− group, but only when considering erythroid data (Additional file 1: Table S2), whereas most plasmatic energy intermediates showed similar concentrations regardless of PO status (Additional file 1: Table S1). More interestingly, the rise in short-chain acyl-carnitines was exclusively detected among PO+ children, whereas control and PO− subjects showed similar levels, thereby pinpointing to β-oxidation as a pivotal player in PO-mediated deleterious programming mechanisms, as reported in animal models [35, 36]. In close relationship with these findings, children with obesity also presented higher contents of branched-chain amino acids (BCAA) and derivatives when compared to controls. This increment in circulating levels of BCAAs has repeatedly been allocated to a complex interplay between higher dietary intake, abnormal expression of amino acid transporters, and blunted capacity to inhibit protein turnover [37]. In turn, this increased bioavailability may trigger skeletal muscle catabolism of BCAAs to produce α-ketoacids and, finally, short-chain acyl-coenzyme A species, which compete with lipid oxidation as a fuel source for entry into the Krebs cycle, thus causing the accumulation of incompletely oxidized intermediates (i.e., acyl-carnitines) [37]. When considering PO-based stratification, changes in BCAAs and catabolites were more pronounced among children within the PO+ group, but only when analyzing erythrocytes, as stated before for acyl-carnitines. This concurs with other studies describing that PO primarily influences mitochondrial β oxidation and skeletal muscle metabolism [17, 19]. In this respect, it should be noted that IR is closely involved in BCAAs and acyl-carnitine homeostasis and, at the same time, these metabolites may exacerbate insulin signaling disruption via different mechanisms [37], which altogether reinforce our rationale about the potential role of IR in mediating adverse programming mechanisms in the offspring.

Besides BCAAs, childhood obesity was also characterized by alterations in other amino acid classes. On the one hand, we observed an overall increase in aromatic amino acids (i.e., tyrosine, phenylalanine), probably because of their competition with BCAAs for the same cellular transporters [38]. Their catabolism may in turn release a myriad of uremic toxins (e.g., 4-hydroxyphenylacetic, -pyruvic, and -lactic acids) and pro-inflammatory compounds (e.g., kynurenic acid), which modulate oxidative stress and inflammation [39]. Children with obesity also had reduced plasma levels of arginine and glutamine, this latter exacerbated among PO+ subjects, indicative of failures in nitrogen metabolism. In this vein, it has been reported that rates of urea excretion and glutamine formation are generally decreased in obesity, being activated alternative mechanisms for excess nitrogen disposal, such as the synthesis of nitric oxide from arginine [40]. However, in our study, deficits in glutamine and arginine were accompanied by higher levels of N-acetylspermine, thereby suggesting that polyamines could serve as a complementary sink to manage nitrogen surplus. Although much less investigated, growing evidence also supports a pivotal role of histidine in the pathophysiology of obesity, with decreased concentrations of this essential amino acid being considered as a reliable marker of activated immune response [41] and impaired histaminergic regulation of food intake [42]. Notably, we found histidine plasma reductions to be exclusively detected in children with obesity and PO.

Oxidative stress is another of the pathogenic events most frequently linked to obesity, which is primarily triggered by sustained free radical generation as a consequence of mitochondrial electron transport chain overload due to excess calorie intake [43]. Not surprisingly, this stressful environment was ultimately mirrored in higher plasma and erythroid contents of a multitude of metabolites derived from the oxidative damage to proteins (i.e., dityrosine), lipids (i.e., malondialdehyde, 4-hydroxynonenal, 4-oxononenal glutathione), and catecholamines (i.e., adrenochrome, adrenolutin) among children with obesity. Furthermore, we observed an enhanced degradation of nucleotide bases, as reflected in lower levels of xanthosine 5-triphosphate and subsequent accumulation of purine (i.e., hypoxanthine, uric acid, 5-hydroxyisouric acid, allantoin) and pyrimidine (i.e., ureidosuccinic acid, N-acetylcytidine) catabolites.

Accompanying this rise in oxidative stress markers, childhood obesity was also associated with impairments in glutathione metabolism, one of the most important antioxidants in the human body. In particular, we detected higher levels of cysteinyl-glycine and 2-hydroxybutyrate, metabolites derived from the over-expression of the glutathione system under a pro-oxidative scenario [44]. As previously reported for energy-related intermediates, we found PO to predispose to exacerbated changes in some of these metabolites (i.e., 2-hydroxybutyric acid, uric acid, allantoin), but again only within erythrocytes. In this respect, it should be stressed that these cells contain powerful antioxidant systems to fight against the continuous exposure to free radicals generated during oxygen transport. Accordingly, it has been proposed that erythrocytes could serve as reliable systemic biomarkers to study redox physiology in health and disease [45], in line with our findings.

The metabolism of cholesterol was also found to be altered in children with obesity and possibly influenced by PO. On the one hand, children with obesity showed higher plasma levels of numerous steroid hormones, including androgens, estrogens, progestogens, and corticosteroids. This enhanced steroidogenesis is known to be originated by various inter-related mechanisms mediated by IR and pro-inflammatory cytokines, such as the secretion of gonadotropin-releasing hormone, the over-expression of adrenocorticotropic hormone, and lowering of sex hormone-binding globulin levels [46]. Alternatively, obesity may also promote cholesterol metabolism toward the production of bile acids with the aim of facilitating the absorption of excess body fat [47], in line with our findings. The release of steroid compounds may in turn bidirectionally modulate insulin secretion and function by activating many signaling pathways, thus creating a vicious pathological cycle [48]. As a result of this tight interplay between insulin action and cholesterol homeostasis, other studies have evidenced that IR may imprint profound disturbances in the profile of steroid hormones and bile acids [21, 49]. Similarly, we have reported in the present work that children born to parents with obesity, who had higher HOMA-IR scores, manifested worsened impairments in steroid derivatives, further emphasizing the potential involvement of IR in driving these PO-related metabolic disruptions. Besides the aforementioned changes in cholesterol-related metabolites, children with obesity also presented lower levels of various lyso-phospholipids and phospholipids containing polyunsaturated fatty acids, as well as higher levels of saturated phospholipids and sphingolipids. In this vein, a recent study has reported similar results in children with obesity and IR, which were allocated to impaired lecithin cholesterol acyltransferase activity, changes in cell membrane phospholipid composition, and improved biosynthesis of ceramides and sphingomyelins [21]. However, none of these lipid species have been found to be influenced by PO.

Finally, childhood obesity was also closely associated with several metabolites derived from environmental and dietary sources, which tended to be exacerbated in children with PO. When compared to controls, children with obesity and PO, and to a lesser extent those born to lean progenitors, had higher plasma levels of propylparaben sulfate and naphthyl sulfate. These can be considered as markers of exposure to parabens and polycyclic aromatic hydrocarbons, respectively, which have extensively been documented to possess endocrine-disrupting and obesogenic properties [50]. Conversely, the opposite direction of association was observed for other metabolites coming from the consumption of polyphenol-rich foods and further microbial metabolization (i.e., hydroxyphenyl-γ-valerolactone sulfate, N-(2-hydroxyphenyl)acetamide sulfate, 2-hydroxybenzoic acid, 4-hydroxybenzaldehyde) [51], which could be indicative of lower adherence to healthy dietary habits. Concurring with our findings, it is well recognized that, besides genetics and metabolic programming, the greater predisposition to childhood obesity induced by PO might be related to lifestyle habits, since children grown by families with obesity often have lower preference for vegetables and higher preference for fatty/sugary foods [52]. However, the lack of dietary assessment data in our study population impedes us from corroborating these findings.

The major strength of this study has been the recruitment of a cohort of children with obesity, born to parents with or without obesity, to deepen into the role of PO in mediating deleterious metabolic programming mechanisms in the offspring. This differs from most previous metabolomics publications in this regard, which have often been conducted in general populations (i.e., non-obese offspring), thereby hindering elucidation of a direct pathophysiological link between parental and childhood obesity. In this respect, it should also be stressed that greater attention has traditionally been paid to investigating the impact of maternal obesity on offspring health, especially along the perinatal period, whereas the influence of paternal obesity has frequently been underestimated. However, growing evidence suggests that both maternal and paternal obesity are equally important determinants in predisposing individuals to obesity during mid-childhood. Accordingly, herein we decided to explore the influence of combined parental insults. Another strength was the use of a population exclusively comprising prepubertal children to minimize biological interferences (e.g., hormonal development, puberty-related physiological IR). Moreover, the combined

analysis of plasma and erythrocyte samples enabled us to comprehensively characterize the molecular mechanisms underlying such a complex and multifactorial disorder as obesity, and thus compare the utility of complementary biological matrices to identify markers associated with its pathophysiology and risk factors. Nonetheless, some limitations deserve to be mentioned as well. As aforementioned, unlike previous studies in this field, we exclusively recruited children with obesity. This, together with the strict inclusion criteria (i.e., Tanner stage 1, matching for BMI) and further stratification according to PO status, considerably limited our sample size. Furthermore, although our secondary analyses supported that the influence of PO is independent of which progenitor presents obesity, in line with growing scientific evidence [3, 7], we cannot discard that, at least in part, this lack of observed differences could be due to limited statistical power (i.e., reduced sample size, unequal number of subjects within groups). Accordingly, future studies separately addressing maternal, paternal, and combined parental obesity in larger populations would be of interest. In this vein, to investigate potential differences in parental influences over children with and without obesity, further investigations also considering the PO status in healthy control children are needed (not feasible here, as none of the control children were born to parents with obesity in our study population). To conclude, it should be highlighted that, given its multifactorial origin, obesity pathophysiology may be mediated by a variety of risk factors, many of which are closely associated with PO. In this respect, it is noteworthy that PO primarily predisposes to childhood obesity through complex biological mechanisms (e.g., genetic inheritance, induction of adverse metabolic programming), but also by influencing deleterious lifestyle habits (e.g., children grown by families with obesity frequently have sedentary behaviors and overeating-type eating styles). Thus, an in-depth characterization of risk factors behind childhood obesity would be necessary to better understand the overall impact of PO, and its relationship with other obesogenic determinants, on offspring health.

Conclusions

This study evidences that PO may induce a multitude of deleterious programming mechanisms in the offspring, affecting energy-related, amino acid, redox, and steroid metabolisms, thereby worsening metabolic health and predisposing them to childhood obesity. Furthermore, our findings interestingly suggest that IR, which was exacerbated among children born to parents with obesity, could be a pivotal driving force triggering these metabolic disturbances. Noteworthy, we found erythrocytes to be a more valuable study matrix rather than plasma, the most common biological material in biomedical research, to investigate the influence of PO on offspring health. Altogether, this study highlights the great impact of PO as an important early risk factor in shaping obesity-related metabolic impairments during childhood.

Abbreviations

BCAA	Branched-chain amino acid
BMI	Body mass index
CNT	Healthy control
HOMA-IR	Homeostatic model assessment for insulin resistance
PO	Parental obesity
PO +	Children with obesity born to parents with obesity
PO −	Children with obesity born to parents without obesity

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04282-w.

Additional file 1: Tables S1-S2. Table S1 - Summary of differential plasma metabolites between children with obesity and parental obesity (PO+), children with obesity without parental obesity (PO-), and healthy control children (CNT). Table S2 - Summary of differential erythroid metabolites between children with obesity and parental obesity (PO+), children with obesity without parental obesity (PO-), and healthy control children (CNT).

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization, R.G.-D.; methodology, Á.G.-D., O.S., and R.G.-D.; formal analysis, L.J.-S., Á.G.-D., O.S., and R.G.-D.; investigation, L.J.-S., Á.G.-D., O.S., R.L., and R.G.-D.; resources, J.D.-R., R.L., and R.G.-D.; data curation, R.G.-D.; writing—original draft preparation, L.J.-S. and R.G.-D.; writing—review and editing, L.J.-S., Á.G.-D., R.L., and R.G.-D.; supervision, R.G.-D.; project administration, R.G.-D.; funding acquisition, R.G.-D. All authors read and approved the final manuscript.

Funding

This research was funded by the Spanish Government through "Instituto de Salud Carlos III" (PI22/01899). Á.G.-D. was supported by an intramural grant from the Biomedical Research and Innovation Institute of Cádiz (LII19/16IN-CO24) and a Scientific Exchange grant from EMBO (ref. 9400). J.D.-R. thanks the "Río Hortega" program from "Instituto de Salud Carlos III" (CM23/00026). R.G.-D. was the recipient of a "Miguel Servet" fellowship (CP21/00120) funded by "Instituto de Salud Carlos III" and a "José Castillejo" mobility grant (CAS22/00080) funded by Spanish "Ministerio de Educación".

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The Ethical Committee of "Hospital Universitario Puerta del Mar" (Cádiz, Spain) approved the study protocol (Ref. PI22/01899), and all participants and/or legal guardians provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details
[1]Instituto de Investigación e Innovación Biomédica de Cádiz (INiBICA), Hospital Universitario Puerta del Mar, Universidad de Cádiz, Cádiz 11009, Spain. [2]Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York City, NY 10029, USA. [3]Division of Food and Nutrition Science, Department of Life Sciences, Chalmers University of Technology, Gothenburg SE-412 96, Sweden. [4]Unidad de Endocrinología Pediátrica y Diabetes, Servicio de Pediatría, Hospital Universitario Puerta del Mar, Cádiz 11009, Spain.

Received: 20 January 2025 Accepted: 15 July 2025
Published online: 05 August 2025

References

1. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol. 2021;12: 706978.
2. Larqué E, Labayen I, Flodmark CE, Lissau I, Czernin S, Moreno LA, et al. From conception to infancy - early risk factors for childhood obesity. Nat Rev Endocrinol. 2019;15:456–78.
3. Lee JS, Jin MH, Lee HJ. Global relationship between parent and child obesity: a systematic review and meta-analysis. Clin Exp Pediatr. 2022;65:35–46.
4. Hieronimus B, Ensenauer R. Influence of maternal and paternal preconception overweight/obesity on offspring outcomes and strategies for prevention. Eur J Clin Nutr. 2021;75:1735–44.
5. Linabery AM, Nahhas RW, Johnson W, Choh AC, Towne B, Odegaard AO, et al. Stronger influence of maternal than paternal obesity on infant and early childhood body mass index: the Fels Longitudinal Study. Pediatr Obes. 2013;8:159–69.
6. Whitaker KL, Jarvis MJ, Beeken RJ, Boniface D, Wardle J. Comparing maternal and paternal intergenerational transmission of obesity risk in a large population-based sample. Am J Clin Nutr. 2010;91:1560–7.
7. Wang Y, Min J, Khuri J, Li M. A Systematic Examination of the Association between Parental and Child Obesity across Countries. Adv Nutr. 2017;8:436–48.
8. Botton J, Heude B, Maccario J, Borys JM, Lommez A, Ducimetière P, et al. Parental body size and early weight and height growth velocities in their offspring. Early Hum Dev. 2010;86:445–50.
9. Knight B, Shields BM, Hill A, Powell RJ, Wright D, Hattersley AT. The impact of maternal glycemia and obesity on early postnatal growth in a nondiabetic Caucasian population. Diabetes Care. 2007;30:777–83.
10. Cechinel LR, Batabyal RA, Freishtat RJ, Zohn IE. Parental obesity-induced changes in developmental programming. Front Cell Dev Biol. 2022;10: 918080.
11. Batra V, Norman E, Morgan HL, Watkins AJ. Parental Programming of Offspring Health: The Intricate Interplay between Diet, Environment. Reproduction and Development Biomolecules. 2022;12:1289.
12. Rabadán-Diehl C, Nathanielsz P. From Mice to Men: research models of developmental programming. J Dev Orig Health Dis. 2013;4:3–9.
13. González-Domínguez Á, González-Domínguez R. How far are we from reliable metabolomics-based biomarkers? The often-overlooked importance of addressing inter-individual variability factors. Biochim Biophys Acta Mol Basis Dis. 2024;1870: 166910.
14. Yuan X, Ma Y, Wang J, Zhao Y, Zheng W, Yang R, et al. The influence of maternal prepregnancy weight and gestational weight gain on the umbilical cord blood metabolome: a case-control study. BMC Pregnancy Childbirth. 2024;24:297.
15. Guixeres-Esteve T, Ponce-Zanón F, Morales JM, Lurbe E, Alvarez-Pitti J, Monleón D. Impact of Maternal Weight Gain on the Newborn Metabolome. Metabolites. 2023;13:561.
16. Schlueter RJ, Al-Akwaa FM, Benny PA, Gurary A, Xie G, Jia W, et al. Prepregnant Obesity of Mothers in a Multiethnic Cohort Is Associated with Cord Blood Metabolomic Changes in Offspring. J Proteome Res. 2020;19:1361–74.
17. Francis EC, Kechris K, Cohen CC, Michelotti G, Dabelea D, Perng W. Metabolomic Profiles in Childhood and Adolescence Are Associated with Fetal Overnutrition. Metabolites. 2022;12:265.
18. Rahman ML, Doyon M, Arguin M, Perron P, Bouchard L, Hivert MF. A prospective study of maternal adiposity and glycemic traits across pregnancy and mid-childhood metabolomic profiles. Int J Obes. 2021;45:860–9.
19. Romero-Ibarguengoitia ME, Vadillo-Ortega F, Caballero AE, Ibarra-González I, Herrera-Rosas A, Serratos-Canales MF, et al. Family history and obesity in youth, their effect on acylcarnitine/aminoacids metabolomics and non-alcoholic fatty liver disease (NAFLD). Structural equation modeling approach PLoS One. 2018;13: e0193138.
20. Santos Ferreira DL, Williams DM, Kangas AJ, Soininen P, Ala-Korpela M, Smith GD, et al. Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. PLoS Med. 2017;14: e1002376.
21. González-Domínguez Á, Savolainen O, Domínguez-Riscart J, Landberg R, Lechuga-Sancho A, González-Domínguez R. Probing erythrocytes as sensitive and reliable sensors of metabolic disturbances in the cross-talk between childhood obesity and insulin resistance: findings from an observational study, in vivo challenge tests, and ex vivo incubation assays. Cardiovasc Diabetol. 2024;23:336.
22. Hernández M, Castellet J, Narvaiza JL, Rincón JM, Ruiz I, Sánchez E, et al. Curvas y Tablas de Crecimiento. Instituto de Investigación sobre Crecimiento y Desarrollo, Fundación Faustino Orbegozo. Madrid: Editorial Garsi; 1988.
23. González-Domínguez Á, Armeni M, Savolainen O, Lechuga-Sancho AM, Landberg R, González-Domínguez R. Untargeted Metabolomics Based on Liquid Chromatography-Mass Spectrometry for the Analysis of Plasma and Erythrocyte Samples in Childhood Obesity. In: González-Domínguez R, editor. Mass Spectrometry for Metabolomics (Methods in Molecular Biology, vol 2571). Humana; New York, NY, USA: 2023. pp. 115–122.
24. González-Domínguez Á, Estanyol-Torres N, Brunius C, Landberg R, González-Domínguez R. QComics: Recommendations and Guidelines for Robust, Easily Implementable and Reportable Quality Control of Metabolomics Data. Anal Chem. 2024;96:1064–72.
25. Klåvus A, Kokla M, Noerman S, Koistinen VM, Tuomainen M, Zarei I, et al. "notame": Workflow for Non-Targeted LC-MS Metabolic Profiling. Metabolites. 2020;10:135.
26. Blaženović I, Kind T, Ji J, Fiehn O. Software Tools and approaches for compound identification of LC-MS/MS Data in Metabolomics. Metabolites. 2018;8:31.
27. González-Domínguez Á, Santos-Martín M, Sayago A, Lechuga-Sancho AM, Fernández-Recamales Á, González-Domínguez R. Analysis and annotation of phospholipids by Mass Spectrometry-based metabolomics. In: Bhattacharya SK, editor. Lipidomics (Methods in Molecular Biology. Volume 2625). New York: Humana; 2023. pp. 79–88.
28. González-Domínguez R, Urpi-Sarda M, Jáuregui O, Needs PW, Kroon PA, Andrés-Lacueva C. Quantitative dietary fingerprinting (QDF)-A Novel Tool for Comprehensive Dietary Assessment based on urinary nutrimetabolomics. J Agric Food Chem. 2020;68:1851–61.
29. Blaise BJ, Correia GDS, Haggart GA, Surowiec I, Sands C, Lewis MR, et al. Statistical analysis in metabolic phenotyping. Nat Protoc. 2021;16:4299–326.
30. Krutkin DD, Thomas S, Zuffa S, Rajkumar P, Knight R, Dorrestein PC, et al. To Impute or Not To Impute in Untargeted Metabolomics—That is the Compositional Question. J Am Soc Mass Spectrom. 2025;36:742–59.
31. Do KT, Wahl S, Raffler J, Molnos S, Laimighofer M, Adamski J, et al. Characterization of missing values in untargeted MS-based metabolomics data and evaluation of missing data handling strategies. Metabolomics. 2018;14:128.
32. Martínez-Villanueva J, González-Leal R, Argente J, Martos-Moreno GÁ. La obesidad parental se asocia con la gravedad de la obesidad infantil y de sus comorbilidades [Parental obesity is associated with the severity of childhood obesity and its comorbidities]. An Pediatr. 2019;90:224–31.
33. González-Domínguez Á, Jurado-Sumariva L, Domínguez-Riscart J, Saez-Benito A, González-Domínguez R. Parental obesity predisposes to exacerbated metabolic and inflammatory disturbances in childhood obesity within the framework of an altered profile of trace elements. Nutr Diabetes. 2024;14:2.
34. De Spiegeleer M, De Paepe E, Van Meulebroek L, Gies I, De Schepper J, Vanhaecke L. Paediatric obesity: a systematic review and pathway mapping of metabolic alterations underlying early disease processes. Mol Med. 2021;27:145.

35. Zhang L, Wang Z, Wu H, Gao Y, Zheng J, Zhang J. Maternal High-Fat Diet Impairs Placental Fatty Acid β-Oxidation and Metabolic Homeostasis in the Offspring. Front Nutr. 2022;9: 849684.
36. Ornellas F, Carapeto PV, Mandarim-de-Lacerda CA, Aguila MB. Obese fathers lead to an altered metabolism and obesity in their children in adulthood: review of experimental and human studies. J Pediatr. 2017;93:551–9.
37. Vanweert F, Schrauwen P, Phielix E. Role of branched-chain amino acid metabolism in the pathogenesis of obesity and type 2 diabetes-related metabolic disturbances BCAA metabolism in type 2 diabetes. Nutr Diabetes. 2022;12:35.
38. Polidori N, Grasso EA, Chiarelli F, Giannini C. Amino acid-related metabolic signature in obese children and adolescents. Nutrients. 2022;14:1454.
39. García-Carrasco A, Izquierdo-Lahuerta A, Medina-Gómez G. The kidney-heart connection in obesity. Nephron. 2021;145:604–8.
40. Alemany M. The problem of nitrogen disposal in the obese. Nutr Res Rev. 2012;25:18–28.
41. Thalacker-Mercer AE, Gheller ME. Benefits and Adverse Effects of Histidine Supplementation. J Nutr. 2020;150:2588S-2592S.
42. Khouma A, Moeini MM, Plamondon J, Richard D, Caron A, Michael NJ. Histaminergic regulation of food intake. Front Endocrinol. 2023;14:1202089.
43. Panic A, Stanimirovic J, Sudar-Milovanovic E, Isenovic ER. Oxidative stress in obesity and insulin resistance. Explor Med. 2022;3:58–70.
44. Sousa AP, Cunha DM, Franco C, Teixeira C, Gojon F, Baylina P, et al. Which role plays 2-Hydroxybutyric acid on insulin Resistance? Metabolites. 2021;11:835.
45. Cortese-Krott MM, Shiva S. The redox physiology of red blood cells and platelets: implications for their interactions and potential use as systemic biomarkers. Curr Opin Physiol. 2019;9:56–66.
46. Burt Solorzano CM, McCartney CR. Obesity and the pubertal transition in girls and boys. Reproduction. 2010;140:399–410.
47. Giannini C, Mastromauro C, Scapaticci S, Gentile C, Chiarelli F. Role of bile acids in overweight and obese children and adolescents. Front Endocrinol. 2022;13:1011994.
48. Curley S, Gall J, Byrne R, Yvan-Charvet L, McGillicuddy FC. Metabolic inflammation in Obesity-At the crossroads between fatty acid and cholesterol metabolism. Mol Nutr Food Res. 2021;65: e1900482.
49. González-Domínguez Á, Domínguez-Riscart J, Savolainen O, Lechuga-Sancho A, Landberg R, González-Domínguez R. Identifying metabotypes of insulin resistance severity in children with metabolic syndrome. Cardiovasc Diabetol. 2024;23:315.
50. Catalán V, Avilés-Olmos I, Rodríguez A, Becerril S, Fernández-Formoso JA, Kiortsis D, et al. Time to consider the Exposome hypothesis in the development of the obesity pandemic. Nutrients. 2022;14:1597.
51. Castellano-Escuder P, González-Domínguez R, Wishart DS, Andrés-Lacueva C, Sánchez-Pla A. FOBI: an ontology to represent food intake data and associate it with metabolomic data. Database. 2020;2020:baaa033.
52. Wardle J, Guthrie C, Sanderson S, Birch L, Plomin R. Food and activity preferences in children of lean and obese parents. Int J Obes. 2001;25:971–7.

Publisher's Note