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Preliminary metabolomics analysis of placenta in maternal obesity

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ABSTRACT

Introduction: Metabolomics identifies phenotypical groups with specific metabolic profiles, being increasingly applied to several pregnancy conditions. This is the first preliminary study analyzing placental metabolomics in normal weight (NW) and obese (OB) pregnancies.
Methods: Twenty NW (18.5 $\leq$ BMI< 25 kg/m^2) and eighteen OB (BMI$\geq$ 30 kg/m^2) pregnancies were studied. Placental biopsies were collected at elective caesarean section. Metabolites extraction method was optimized for hydrophilic and lipophilic phases, then analyzed with GC-MS. Univariate and PLS-DA multivariate analysis were applied.
Results: Univariate analysis showed increased uracil levels while multivariate PLS-DA analysis revealed lower levels of LC-PUFA derivatives in the lipophilic phase and several metabolites with significantly different levels in the hydrophilic phase of OB vs NW.
Discussion: Placental metabolome analysis of obese pregnancies showed differences in metabolites involved in antioxidant defenses, nucleotide production, as well as lipid synthesis and energy production, supporting a shift towards higher placental metabolism. OB placentas also showed a specific fatty acids profile suggesting a disruption of LC-PUFA biomagnification. This study can lay the foundation to further metabolomic placental characterization in maternal obesity. Metabolic signatures in obese placentas may reflect changes occurring in the intrauterine metabolic environment, which may affect the development of adult diseases.

1. Introduction

Obesity is spreading worldwide with almost epidemic proportions, representing a risk factor for adverse pregnancy outcomes and offspring's complications [1,2]. Maternal obesity is characterized by calorie imbalance and incorrect dietary intake and has been associated with a lipotoxic placental environment, defined by decreased regulators of angiogenesis and increased markers of inflammation and oxidative stress [3]. This adverse intrauterine environment may directly affect placental function and metabolism [4,5]. Similarly to what occurs with maternal diabetes, increased maternal Body Mass Index (BMI), together with fetal sex, is associated with decreased placental efficiency and histopathologic findings typical of hypoxia and inflammation [3–6].

Metabolomics applies a holistic approach to study the whole metabolite content of cells, tissues or bio-fluids. Metabolomic analysis has recently found applications in several pregnancy-related conditions [7–13] allowing for the recognition of different phenotypical groups due to their characteristic metabolic profile. Most of these works reported metabolomic analysis of bio-fluids such as blood, urine or amniotic fluid [7–13].

To the best of our knowledge, there are only few metabolomic studies on placenta tissue extracts using Mass Spectrometry (MS) [14–20] or Nuclear Magnetic Resonance (NMR) spectroscopy [21–23]. Placental metabolome changes in relation to maternal

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Abbreviations

AMDIS	Automated Mass spectral Deconvolution and Identification System
ANOVA	ANalysis Of VAriance
BF3	Boron triFluoride
BMI	Body Mass Index
CV	Cross Validation
DHA	DocosaHexaenoic Acid
DNA	DeoxyriboNucleic Acid
FDR	False Discovery Rate
FIGO	International Federation of Obstetrics and Gynecology
GC-MS	Gas Chromatography-Mass Spectrometry
GDM	Gestational Diabetes Mellitus
GMD	Golm Metabolome Database
GWG	Gestational Weight Gain
HSD	Honest Significant Difference
IGF-1	Insuline-like Growth Factor-1
LC-MS	Liquid Chromatograph Mass Spectrometers
LC-PUFA	Long Chain-Polyunsaturated Fatty Acids
MS	Mass Spectrometry
MSTFA	N-Methyl-N-trimethylsilyltrifluoroacetamide
mTOR	mammalian Target Of Rapamycin
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NIST08	National Institute of Standards and Technology mass spectral database
NMR	Nuclear Magnetic Resonance
OGTT	Oral Glucose Tolerance Test
PBS	Phosphate Buffered Saline
PLS-DA	Partial Least Square-Discriminant Analysis
RT	Room Temperature

obesity were only investigated in rats following different diets [20].

The aim of this preliminary study is to examine key placental metabolites associated with maternal obesity. Obese patients were also evaluated according to gestational diabetes. Hydrophilic and lipophilic metabolites were studied through GC-MS (Gas Chromatography-Mass Spectrometry) platform, followed by multivariate statistic protocols.

2. Methods

2.1. Population

The protocol of the study was approved by the Ethical Committee of the Sacco Hospital (Milan) and all women signed a written informed consent. Only singleton spontaneous pregnancies, with maternal age between 18 and 40 years and of Caucasian ethnicity were included in the study. Exclusion criteria were maternal preexisting diseases, fetal and maternal infections, alcohol or drugs abuse, fetal malformations or chromosomal disorders, BMI< 18.5 or BMI between 25 and 30. Pregnant women were allocated into two different groups based on their pre-gestational BMI according to the Institute of Medicine (IOM) guidelines [24]:

- **Normal weight** (NW) ($18.5 \leq$ BMI< 25 Kg/m^2), n = 20
- **Obese** (OB) (BMI≥ 30 Kg/m^2), n = 18

Obese patients were given specific nutritional advice and recommendations on weight gain in pregnancy. Eight obese women had a diagnosis of Gestational Diabetes Mellitus (GDM) [OB/GDM(+)] based on an Oral Glucose Tolerance Test (OGTT, 75 g), according to FIGO guidelines [25]. OB/GDM(+) were constantly checked for glycaemia and were given lifestyle and dietary indications for glycemic control. None of the studied women needed insulin therapy.

Maternal medical history, demographic, anthropometric, and obstetric data, as well as neonatal outcome data were recorded at recruitment and after delivery. Maternal gestational weight gain (GWG) was recorded.

2.2. Sample collection

Placentas from elective caesarean section were measured recording placental weight, area and thickness as previously described [5]. Placental tissue was collected from a not-impaired part of the placental disc, after discarding the maternal decidua layer, washed in PBS, then cut into small pieces and immediately frozen in liquid nitrogen. The tissue was then transferred into a −80 °C freezer. Samples were sent to the University of Cagliari to be analyzed by the GC-MS platform.

2.3. Sample preparation

The extraction method was optimized from literature methods [17,18,26,27]. A piece of placental tissue of about 100 mg was rapidly weighed, put in a glass mortar on ice with 1.9 mL of chloroform/methanol/water (1.4/1.4/1, 700/700/500 μl) and homogenized with a Potter-Elvehjem homogenizer for 2 min. The mixture was kept at 4 °C for 15 min, then centrifuged at 14000 rpm for 10 min at 4 °C. The upper (hydrophilic) and lower (lipophilic) phases were separated: the hydrophilic was dried in a vacuum concentrator (Eppendorf Concentrator Plus) overnight; the lipophilic in a glass vacuum desiccator under fume-hood for 2 h. The volume of extraction solution was normalized to 100 mg of tissue, with 1000 μL of the upper phase and 600 μL of the lower being dried down for 100 mg of tissue [17]. The dried fractions were stored at −80 °C until analysis.

2.3.1. Hydrophilic phase

30 μL of a solution of methoxylamine hydrochloride in pyridine (0.24 M) were added to each sample, then vortex mixed and left for 17 h at room temperature (RT). 30 μL of MSTFA were added and left for 1 h at RT. Just before GC-MS analysis, samples were diluted with a hexane solution (600 μL) of tetracosane (0.006 mg/mL) as internal standard, then analyzed using a Agilent 5977B interfaced to the GC 7890B with a DB-5ms column (J & W) [injector temperature at 230 °C, detector temperature at 280 °C, helium carrier gas flow rate of 1 mL/min]. The GC oven temperature program was 90 °C for 1 min, ramped by 10 °C/min to 270 °C with 7 min hold time. The sample (1 μL) was injected in split (1:10) mode. After a solvent delay of 3 min, mass spectra were acquired in full scan mode using 2.28 scans/s with a mass range of 50–700 Amu.

2.3.2. Lipophilic phase

150 μL of chloroform:methanol (1:1) and 100 μL of 14% BF_3 in methanol were added to each vial, samples were vortex mixed and left for 90 min at 80 °C into an heating block. Once cooled, 600 μL hexane and 300 μL water were added, samples were vortex mixed