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ORIGINAL ARTICLE

The metabolome of human placental tissue: investigation of first trimester tissue and changes related to preeclampsia in late pregnancy

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Abstract Unique biochemical and physical challenges to both mother and fetus are observed during human pregnancy, and the placenta plays an important role in protecting the fetus and supporting its development. Consequently, many pregnancy complications are associated with altered placental biochemistry and structure. Here we have further developed a combination of analytical tools for determining the tissue metabolome of placental tissue by applying a methanol/water/chloroform extraction method followed by analysis of the polar fraction (methanol/water) using GC–ToF–MS and of the non-polar fraction (chloroform) using UPLC–LTQ–Orbitrap–MS. This combination maximises the number of different metabolites detected and is the first holistic investigation of placental tissue applying UPLC–MS. Placental tissue differs between early and late first trimester pregnancies in that the developing placenta is exposed to significantly different oxygen tensions and undergoes a change from histiotrophic to haemotrophic nutrition. Application of these metabolomic methods detected 156 unique and chemically identified metabolites that showed statistically significant differences ($P < 0.05$). These included changes in di- and triglycerides, phospholipids, sphingolipids, fatty acids and fatty acid carnitines. This is the first metabolomics study to identify these changes that potentially show the initiation or switch to fatty acid beta-oxidation for mitochondrial ATP production. A separate study showed a small number of changes that were related to the position of sampling of the placental tissue and to the type of delivery from pregnancy. This result indicates that variations associated with sampling position and delivery type are small compared to between-subject variation. However, the authors recommend robust experimental design which may include sampling from the same position of the placenta and from the same delivery type. When comparing tissue from term-uncomplicated pregnancies with those exhibiting preeclampsia at term, 86 unique and chemically identified metabolites showed statistically significant differences ($P < 0.05$). Potential changes in metabolism operating in the mitochondria, in vitamin D metabolism and in oxidative and nitrative stress were observed. These proof-of-principle studies demonstrate the sensitivity of placental tissue metabolomics to define changes related to

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alterations in environment and perfusion and related to diseases of pregnancy including preeclampsia. Data are available on request.

Keywords Metabolomics · Mass spectrometry · Placenta · Tissue · Preeclampsia · First trimester · Systems biology

1 Introduction

In 2002 an estimated 210 million women became pregnant (Neilson et al. 2003). Human pregnancy presents unique biochemical and physical changes and challenges to both mother and fetus. Inadequate physiological responses to these challenges can result in complications and associated ill-effects including (among others) miscarriage, pre-eclampsia (PE), preterm birth, gestational diabetes and the birth of small for gestational age (SGA) infants. Approximately, 20 million mothers experienced pregnancy-related illness in 2002 (Neilson et al. 2003). In 2001, 510 000 mothers died during pregnancy and childbirth and 2.46 million babies died in the perinatal period (Guilbert 2003), with the largest proportion of these deaths being in the developing world. Pregnancy complications can also influence the long-term health of the mother and also increase the risk of disease of the child in adult life. For example, babies born from pregnancies complicated by PE or SGA have an increased risk of developing cardiovascular diseases later in life (Anderson 2007).

The placenta is a very important organ in pregnancy (Page 1993). The placenta is composed of fetal tissue, is attached to the maternal uterus, and is connected to the fetus by the umbilical cord. The placenta is a complex organ reflecting the many intricate interactions between mother, placenta and fetus. When considering pregnancy a systems-wide investigation is optimal to provide holistic views of these complex interactions.

The placenta fulfils a number of roles. Firstly, the placenta places the blood supplies of mother and fetus in close proximity, to maximise gas exchange and the supply of nutrients to and the removal of waste products from the fetus. The placenta (and amniotic fluid) acts as a barrier to prevent infection reaching the fetus, which is augmented by the transport of antibodies from mother to fetus. The placenta produces a range of hormones that maintain and promote pregnancy including progesterone, human chorionic gonadotropin (hCG), human placental growth hormone (hPGH) and human placental lactogen (hPL), all of which profoundly alter maternal physiology and metabolism (Whitridge Williams 2001).

Metabolomics can play an important role in the study of complex mammalian systems (Dunn et al. 2011b; Atherton et al. 2009; Kaddurah-Daouk et al. 2008) and its functions in pregnancy research have been reviewed (Heazell et al. 2011; Horgan et al. 2009b). The biochemical changes associated with pregnancy have been studied for more than 50 years (Rauch et al. 1956). However, metabolomic studies have only recently been applied to investigate the complex molecular mechanisms associated with normal pregnancy and with pregnancies with complications. Early successes include the identification of symptomatic (Kenny et al. 2008) and pre-symptomatic (Kenny et al. 2010) putative metabolic biomarkers of preeclampsia. Similar work, though on a smaller scale, has been used to predict male infertility (Deepinder et al. 2007) and embryo viability and oocyte quality during in vitro fertilisation (IVF) (Brison et al. 2007; Seli et al. 2008). Metabolomic analysis of body fluids such as serum provides an essentially non-invasive strategy in these studies. Changes in the amniotic fluid related to fetal malformations, gestational diabetes and preterm delivery and early rupture of membranes have also been investigated using NMR spectrometry (Graca et al. 2009; Graca et al. 2010).

Holistic metabolic profiling studies of placental tissue have been limited. Tissot van Patot and colleagues have studied the metabolome of placental tissue biopsies, with biopsy collection immediately after delivery, with proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy from pregnancies occurring at sea-level and at 3100 m altitude (Tissot van Patot et al. 2010). Labour at sea level produced oxidative stress markers, most probably a result of the sudden ischemia/hypoxic insult, whereas markers of oxidative stress were absent in placentas collected for pregnancies which developed at 3100 m. The data suggest that development of a placenta at high altitudes provides hypoxic preconditioning before the sudden ischemic hypoxic insult of labour. This preconditioning mechanism appears to influence the placental metabolome for rapid and short ischemia/hypoxia insults but not for long periods of ischemia/hypoxia. This is shown by the fact that preeclampsia, which displays a hypoxic phenotype during large periods of pregnancy, is more prevalent at high altitude than at sea level (Palmer et al. 1999).

Other studies have concentrated on the culture of placental explants in serum-based media, including the effects of hypoxia (Heazell et al. 2008) and of pregnancies complicated by PE (Dunn et al. 2009) and SGA (Horgan et al. 2009a). The analysis of the culture medium, defined by the authors as the metabolic footprint (Allen et al. 2003; Kell et al. 2005), provided putative information on those aspects of intracellular metabolism that influence the extracellular culture medium. By filling the intervillous space, the culture medium mimics the maternal blood in these studies. 2-deoxyribose and threitol/erythritol were detected at increased concentrations in hypoxic culture conditions. In

the comparison of metabolic footprints of cultured placental tissue taken following uncomplicated pregnancies and pregnancies affected by preeclampsia, the results showed that preeclamptic placental tissue has an irreversible hypoxic phenotype, a finding that complements other data indicating the role of hypoxia in preeclampsia (Genbacev et al. 1996; Granger et al. 2002; Kell 2009; Rajakumar et al. 2001; Rampersad and Nelson 2007; Vaiman et al. 2005).

We here describe two separate studies of the metabolite composition of human placental tissue. First, we study tissue from early and late first trimester placental development, where large metabolic changes are expected due to significant changes in oxygenation and a change from histiotrophic to haemotrophic nutrition. Secondly, we evaluate term placental tissue to determine whether the metabolome following normal vaginal delivery, a proposed source of oxidative stress, differs from that following caesarean section, and to determine whether term placental tissue from preeclampsia and from uncomplicated term pregnancies differ in a manner that can be related to the disease phenotype.

2 Materials and methods

All chemicals and reagents applied were of Analytical Reagent or higher purity grade and were purchased from Sigma-Aldrich (Gillingham, UK) or Fisher Chemicals (Loughborough, UK).

2.1 Tissue collection

Ethical approval was provided by the North-West research ethics committees (Reference: 08/H1008/28 and 08/H1008/55). Two separate studies were performed with collection of placentas performed at different times. In study one, placentas were collected from women undergoing termination of pregnancy for non-medical reasons; these were divided into those with a gestation confirmed by ultrasound of less than 8 completed weeks ($n = 6$) and those with greater than 10 completed weeks gestation ($n = 6$). In study two, placentas were obtained from uncomplicated term pregnancies ($n = 11$; $n = 6$ for vaginal delivery and $n = 5$ for caesarean section) and preeclamptic term pregnancies ($n = 6$; $n = 5$ for vaginal delivery and $n = 1$ for caesarean section). The demographic characteristics of all subjects in study two are shown in Table 1. Pre-eclampsia was defined as a blood pressure of >140/90 mmHg on two or more occasions after the 20th week of pregnancy in a previously normotensive woman in the presence of significant proteinuria (either >300 mg/l in a 24 h period or >2+ on a voided urine sample in the absence of urinary tract infection (Davey and Macgillivray 1988)). No significant differences were observed between the three classes studied. The term placentas were collected within 20 min of delivery, tissue biopsies were sampled and frozen within 30 min of delivery. For studies of term placenta, tissue was taken from the centre of the placenta, the edge of the placenta and a midpoint in the radius between the centre and edge. All tissue samples were stored at −80°C until sample preparation.

2.2 Sample preparation

All tissues for each study were extracted on a single day in a randomised order, applying the following procedure for each tissue being extracted. Samples from each study were extracted at different times.

The tissue weight was determined by placing in a cooled and pre-weighed Eppendorf tube and rapidly weighing. 100 mg of frozen tissue was allowed to defrost on ice, followed by five rapid washes with saline (0.85% sodium chloride in water, 4°C) to remove residual blood and centrifugation at 4°C (5 min, 4000×g). Tissue was then rapidly placed in 0.6 ml of a 4:1 methanol:water extraction solvent at 4°C and homogenised in an ice-cooled mortar and pestle followed by transfer to an Eppendorf tube. Following homogenisation and transfer, 0.4 ml of

Table 1 Demographic characteristics of study participants

Characteristic	Normal-vaginal deliveries	Normal-caesarean section	Pre-eclampsia	*P*-value
Maternal age (years)	31 (27–34)	31 (27–35)	34 (30–39)	NS
Maternal BMI (kg/m^2)	25.5 (23.5–28.5)	26.0 (23.5–30.0)	29.0 (21.5–31,5)	NS
Blood pressure (mmHg)	116/70 (110–128/63–78)		165/106 (155–178/93–110)	$P < 0.001$
Gestation at delivery (weeks$^{+\text{days}}$)	39^{+6} (38^{+2}–40^{+3})	39^{+6} (37^{+6}–41^{+0})	38^{+0} (35^{+2}–39^{+3})	NS
Ethnicity	Caucasian (4)	Caucasian (4)	Caucasian (5)	NS
	Asian (2)	Asian (1)	Asian (1)	
Customised birthweight centile	47 (26–73)	68 (39–86)	24 (5–57)	NS

Figures are defined as median with interquartile range in parentheses. Customised birthweight centiles are adjusted for maternal height, weight, ethnicity, parity, gestation and gender (Gardosi et al. 1992). *NS* not significantly different

chloroform was added to the tissue homogenate and the sealed Eppendorf tube was placed in liquid nitrogen for one minute after which it was removed, allowed to thaw on ice and vortex mixed for 30 s. The freeze–thaw process was repeated a further four times. Following the freeze–thaw process 0.4 ml of water was added to the tissue homogenate followed by centrifugation at 4°C (15 min, 3000×*g*). A biphasic solution was present after centrifugation. The top layer (water/methanol, approximate volume of 1.0 ml) and bottom layer (chloroform, approximate volume of 0.4 ml) were transferred to separate Eppendorf tubes and were lyophilised (HETO VR MAXI vacuum centrifuge attached to a HETO CT/DW 60E cooling trap; Thermo Life Sciences, Basingstoke, UK). The volume of extraction solution was normalised to 100 mg of tissue weight before lyophilisation with 800 µl being dried down for 100.0 mg of tissue. For example, 889 µl of extract was lyophilised for a tissue biopsy of 90 mg (100/90 * 800).

A pooled quality control (QC) sample was prepared for each study by combining 100 µl aliquots of each extraction solution and the remaining sample volumes after normalisation for tissue weight. The pooled QC sample was vortex mixed. 800 µl (methanol/water extract) and 400 µl (chloroform extract) aliquots were then transferred to Eppendorf tubes and lyophilised.

2.3 GC–ToF–MS analysis

Lyophilised tissue extracts and QC samples derived from the polar methanol:water extract were chemically derivatised in a two-stage process and analysed applying gas chromatography-time of flight-mass spectrometry (GC–ToF–MS). *O*-methylhydroxylamine solution (50 µl, 20 mg ml^{-1} in pyridine) was added to the lyophilised sample and heated at 60°C for 30 min followed by addition of 50 µl MSTFA (*N*-acetyl-*N*-(trimethylsilyl)-trifluoroacetamide) and heating at 60°C for 30 min. To the derivatised solution was added 20 µl of a retention index (RI) solution (0.6 mg ml^{-1} C_{10}, C_{12}, C_{15}, C_{19} and C_{22} *n*-alkanes) to provide normalisation of retention times. Particulate matter was removed by centrifugation (15 min, 13 363×*g*) followed by transfer of the supernatant to 300 µl glass inserts placed in 2 ml chromatography vials which were sealed with a PTFE/rubber septum containing screw cap.

Samples were analysed in a random order on a Pegasus III electron impact mass spectrometer (LECO UK, Stockport, UK) coupled to a MPS2L autosampler (Gerstel, Baltimore, MD) and 6890 N gas chromatograph (Agilent Technologies, Stockport, UK). The GC and MS operating parameters were as previously described (Begley et al. 2009). All samples were analysed within 36 h of derivatisation completion. The first five injections were a QC sample, followed by a QC sample every 7th injection and two QC samples at the end of the analytical batch. Samples from the first trimester study were analysed in October 2010 and the samples from the preeclampsia study were analysed in July 2010.

2.4 UPLC–MS analysis

Lyophilised tissue extracts and QC samples derived from the nonpolar chloroform extract were reconstituted in 100 µl 50:50 methanol:water, vortex mixed and particulates were removed by centrifugation (15 min, 13 363×*g*). The supernatant was transferred to a 2 ml low-volume chromatography vial, sealed with a septum containing screw cap and stored in an autosampler at 4°C. All samples were analysed in a random order on a Acquity UPLC system (Waters, Corporation, Elstree, UK) coupled to an electrospray LTQ–Orbitrap XL hybrid mass spectrometer (ThermoFisher, Bremen, Germany). Chromatographic separations were performed employing an Acquity UPLC BEH 1.7 µm-C_{18} column at a flow rate of 0.40 ml min^{-1} in positive (UPLC–MS (+)) and negative (UPLC–MS (−)) ion modes, separately. The column was eluted with 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). The column was held at 100% A for 1 min and subsequently ramped to 100% B (curve 5) over 6 min, followed by a 2.5 min period at 100% B before a rapid return to 100% A and an equilibration period of 2.5 min. The column and samples were maintained at temperatures of 50 and 4°C, respectively. A 10 µl sample volume was introduced onto the column and 50% of the column effluent was transferred to the mass spectrometer. Centroided accurate mass spectra were acquired in the *m/z* range of 50–1000 using the Orbitrap mass analyser operating with a target mass resolution of 30,000 (Full Width Half Maximum as defined at *m/z* 400) and a scan time of 0.4 s. All samples were analysed within 48 h of reconstitution. Mass calibration was performed according to the manufacturer's guidelines using a manufacturer defined mixture of sodium dodecyl sulphate, sodium taurocholate, the tetrapeptide MRFA and Ultramark 1621. The first ten injections were a QC sample, followed by a QC sample every 7th injection and two QC samples at the end of the analytical batch. Samples from the first trimester study were analysed in October 2010 and the samples from the preeclampsia study were analysed in July 2010.

2.5 Data pre-processing

Raw data files (.peg format) acquired from the GC–ToF–MS platform were directly processed applying the ChromaTof software (Leco Corp. v3.25) as previously described (Begley et al. 2009).

Raw data files (.raw format) acquired from the UPLC–MS platform were converted to the NetCDF format using the File converter program in the XCalibur software package (ThermoFisher Scientific, Bremen, Germany). Deconvolution of data was performed using XCMS, running on R version 2.6.0, an open-source deconvolution program available for LC–MS data (Smith et al. 2006) using identical settings to those reported previously (Dunn et al. 2008).

Pre-processed data were exported for univariate and multivariate data analysis as a data matrix of metabolite feature versus sample and with chromatographic peak areas included for each feature detected in each sample. Associated information was included for each metabolite feature for identification purposes. For GC–MS retention index and EI-fragmentation mass spectrum were included and for UPLC–MS retention time and accurate *m/z* were included.

2.6 Data analysis

Interspersed quality control (QC) samples (pooled aliquots from all samples) as standards for subsequent signal correction and quality assurance (QA) were used in both GC–ToF–MS and UPLC–MS analysis (Dunn et al. 2011). The GC–ToF–MS data in Study 1 was normalised to an internal standard (succinic d_4 acid). An interfering species did not allow accurate peak area determination of the internal standard in study 2 and therefore data was normalised to the total peak area. These normalisation methods showed tight clustering of the interspersed QC samples using Principal Components Analysis (PCA), in contrast to the variability (or greater dispersion) of the biological samples. The tight clustering of the QC samples (multiple injections of the same sample), which illustrate appropriate method reproducibility, indicated that drift in signal response during the analytical run was low and therefore no signal correction was required for GC–ToF–MS data, and none was performed. In the UPLC–MS data for Study 1 good tight clustering of the QC samples applying PCA was observed and no further signal correction was required or performed. In Study 2 with a larger number of sample injections there was more evidence of machine drift as shown by a greater dispersion of QC samples in relation to biological samples after PCA. For this reason each detected metabolite feature was normalised to the QC sample using the quality control-robust loess signal correction (QC–RLSC) where a low-order nonlinear locally weighted spline (LOESS) is fitted to the QC data with respect to the order of injection (Dunn et al. 2011). A correction curve for the whole analytical run is then interpolated, to which the total data set for that peak is normalized. Using this procedure any attenuation of peak response over an analytical run, due to injection order, was minimised. Following this process, Quality Assurance (QA) was performed. Tolerance limits were set such that the measured response detected in 60% of QC samples for each metabolite feature was within 20% (UPLC–MS) or 30% (GC–ToF–MS) of the QC mean and metabolite features not present in at least 60% of the samples or that did not pass the QA criteria were removed from the dataset and ignored in subsequent data analysis. A measure of method reproducibility (for study 2) is shown in Table 3 for each of the three analytical platforms. In univariate analysis all missing values were annotated as 'NaN' and in multivariate analysis were entered as 0.

Within Matlab (http://mathworks.com), exploratory multivariate analysis was performed using principal components analysis (PCA), an unsupervised approach. PCA was performed on data normalised to zero mean and unit variance. The first 3 PCs were investigated visually.

Univariate analysis was performed using the non-parametric Mann-Witney test which makes no assumptions about the normal distribution or otherwise of the variance for any metabolite. For all tests in this discovery phase, features were considered significant where the P-value <0.05. As the experimental objective was to perform discovery studies to define changes related to molecular pathophysiological changes (e.g. metabolism) rather than to define metabolite biomarkers (which require a more robust statistical analysis (Broadhurst and Kell 2006), a non-conservative P-value was applied. Further screening of statistically significant metabolic features was performed by grouping of metabolites from the same metabolite class, same metabolic pathway or similar chemical structure (e.g. triglycerides). Although changes in biochemical parameters can influence the concentrations of individual metabolites (Kell and Westerhoff 1986), we here discuss only groups of metabolites, rather than single metabolites, from a biological point of view. The a priori probability of multiple metabolite features from the same metabolite class being statistically significant arguably decreases as the number of metabolite features in the class increases and therefore provides increased confidence in their biological relevance.

2.7 Metabolite identification

The putative or definitive identification of metabolite features detected on the GC–ToF–MS platform was performed by the comparison of retention index and electron-impact mass spectrum to those recorded for authentic chemical standards and present in freely available mass spectral libraries (e.g., Golm metabolome database (Kopka et al. 2005) or present in the MMD in-house library (Brown et al. 2009)) or in commercially available mass spectral libraries (e.g. NIST/EPA/NIH05 libraries). A definitive identification was assigned if the retention index (± 10) and mass

spectrum (match >70%) were within the stated ranges. A putative identification was assigned if a match to a mass spectrum only was observed, but where the retention index data for authentic standards were not available.

The putative identification of metabolite features detected on the UPLC–MS platform was performed by applying the PUTMEDID–LCMS set of workflows as recently described (Brown et al. 2011). As different metabolites can be detected with the same accurate *m/z* (for example, isomers with the same molecular formula), multiple 'identifications' can be observed for a single metabolic feature. Also, a single metabolite can be detected as multiple features, particularly as a different type of ion (for example, protonated and sodiated ions). In the results section the number of *metabolic features* showing a statistically significant difference ($P < 0.05$) is defined and is followed by reporting the number of unique and putatively identified metabolites.

3 Results and discussion

3.1 Placental tissue acquired from early and later first trimester pregnancies

The physical and biochemical development of placental tissue is fast in the first trimester of the pregnancy with a rapid increase in placental size and mass. Remodelling of maternal spiral arteries in the uterus during the first trimester, to increase the arterial diameter, provides an increased maternal blood flow to the placenta. Changes in the nature of nutritional supply are observed during the change to an increased maternal blood flow with histiotrophic nutrition provided by the endometrial glands in early first trimester tissue changing to haemotrophic nutrition provided by the increase in maternal blood perfusion in late first trimester tissue (Burton et al. 2010). The increased perfusion of maternal blood in late first trimester also provides a significant increase in the oxygenation of tissue from <20 mmHg before 8 weeks gestation to >50 mmHg by 12 weeks of pregnancy (Jauniaux et al. 2000). These changes were hypothesised to result in large metabolic differences in the placenta. The present study was designed to investigate whether metabolic profiling of placental tissue can provide valid and useful information on metabolic changes during first trimester development of placental tissue.

3017, 1552 and 111 metabolic features were detected on the UPLC–MS (+), UPLC–MS (−) and GC–ToF–MS analytical platforms, respectively, after signal correction and/or quality assurance processes. Multivariate PCA was performed. Data acquired on the GC–ToF–MS platform showed no separation of placental tissue collected in early and late first trimester pregnancies (data not shown) in PC1-3. Scores plots for PC1 versus PC2 UPLC–MS (+) and for PC1 versus PC3 for UPLC–MS (−) are shown in Fig. 1. The QC samples cluster together in a less dispersed manner than do the tissue extract samples, showing appropriate technical reproducibility. Biological variation is considerably greater than technical variation. Some separation of tissue from early and late first trimester pregnancies is observed for data acquired on the UPLC–MS platforms. This shows that changes in lipid metabolism during development are observed, but not major changes in

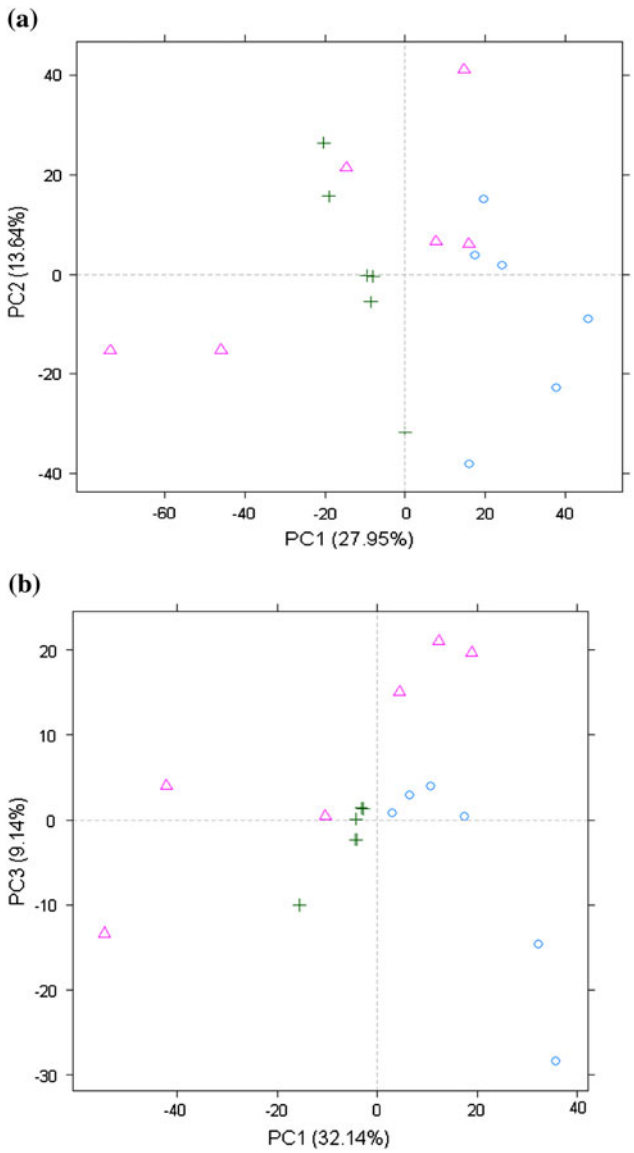

Fig. 1 PCA scores plots for data acquired on (**a**) UPLC–MS (+) and (**b**) UPLC–MS (−) platforms for non-polar chloroform extracts of early (*blue circle*) and late (*pink triangle*) first trimester placental tissue samples. QC samples from injection 9 are shown (*green cross*) (Color figure online)

the overall metabolism of polar metabolites as judged from the chemical discrimination provided by the extraction method (polar methanol/water versus non-polar chloroform) and analytical platforms applied.

Univariate analysis was then performed to define specific metabolic differences between placental tissue metabolomes collected from early and late first trimester pregnancies. UPLC–MS (+), UPLC–MS (−) and GC–MS defined 564 (18.7% of all detected features), 177 (11.4% of all detected features) and 3 (2.7% of all detected features) metabolic features which were statistically significant at a *P*-value of $P < 0.05$. 110, 45 and 1 unique metabolic features, respectively, were putatively chemically identified and are shown in Table 2. For a single metabolite identified multiple times (for example in UPLC–MS (+) and UPLC–MS (−)), the metabolite is reported only once with the lowest *P*-value being reported. These results emphasise that the metabolites detected in the polar extract by GC–MS do not change in their relative concentration between samples collected at different times of the first trimester. Organic acids, amino acids and two glycolytic intermediates were detected. Although, no changes were observed in central metabolism between the different tissues this does not imply that central metabolism is not important in placental development at different periods of the pregnancy.

The relative concentrations of 156 unique and putatively identified metabolite features were observed to change between early and late first trimester tissue samples. These changes are related to the onset of effective perfusion with oxygenated blood: the early samples will not be effectively perfused before remodelling of arteries in the uterus whereas the later samples will be perfused with oxygenated blood (Jauniaux et al. 2000). Other changes include the change from histiotrophic nutrition in early first trimester to haemotrophic nutrition in late first trimester tissue (Burton et al. 2010) which may lead to changes in nutrient supply involving lipids being more readily available to tissue once effective perfusion of blood supply commenced. A large number of metabolites in a small number of metabolite classes were observed to be statistically significantly different; these classes include triglycerides (14) and diglycerides (11), phospholipids (53, including lysophosphatidylethanolamines, phosphatidylcholines and phosphotidylethanolamines), sphingolipids (21), fatty acids and related metabolites (14), nucleotides (4), fatty acyl carnitines (3) and vitamin D related metabolites (3). 38 of 53 phospholipids, 17 of 21 sphingolipids, 9 of 11 diglycerides, 4 of 4 vitamin D-related metabolites, 3 of 4 nucleotides and 2 of 3 acyl carnitines all showed a relative increase in concentration in the late trimester tissue. 14 of 14 triglycerides showed a relative decrease in concentration in the late trimester tissue. No clear discrimination was observed for fatty acids as a group, in that 7 showed a decrease and 7 showed an increase in their relative concentration in late trimester tissue. The split between fatty acids is not related to chain length nor degree of saturation. Only one metabolite associated with central metabolism of polar metabolites (4-aminobutanoate) showed a statistically significant difference between early and late first trimester tissue showing that lipid metabolism is more variable in placental development than is central metabolism (including the glycolytic, tricarboxylic acid and pentose phosphate pathways).

These data show that large biochemical changes are occurring during early development of the placenta either side of the onset of effective perfusion with oxygenated blood. The decrease in the relative concentrations of many triglycerides and increase in the concentration of diglycerides and fatty acyl carnitines in late first trimester tissue may indicate an increase in the utilisation of specific fatty acids by release of the fatty acids from triglycerides and subsequent conversion to fatty acyl carnitines for transfer from cytoplasm to mitochondria for beta-oxidation and ATP production for energy. These changes may be expected when the oxygen concentration in tissue significantly increases to allow beta-oxidation and oxidative phosphorylation in the mitochondria. The changes in nucleotides potentially indicate the increased metabolism related to ATP and GTP production for energy and for co-factor production.

An increase in enzyme and metabolite production related to sphingolipid metabolism have been observed in the transciptome and proteome in first trimester maternal and placental tissue (Kaneko-Tarui et al. 2007). It has been implied that these molecules act in lipid signaling cascades in the maternal–embryo interface. Disruption of sphingosine kinase genes cause defective decidualization, maternal artery instability and early pregnancy loss (Mizugishi et al. 2007). One sphingolipid, sphingosine-1-phosphate (S1P), is a known bioactive lipid mediator and acts as a signalling molecule to provide regulation of vascular and immune systems and angiogenesis by suppressing apoptosis (Spiegel and Milstien 2002). S1P has been associated with preeclampsia (Kenny et al. 2010; Romanowicz and Bankowski 2009). The role of ceramides, a subclass of sphingolipids, has also been implicated in initiating mitochondria-mediated apoptosis through the formation of ceramide channels in the mitochondrial outer membrane which release proapoptotic intermembrane space proteins from mitochondria to the cytoplasm (Colombini 2010; Siskind 2005).

Table 2 Chemically identified metabolites which show a statistically significant differences ($P < 0.05$) in their relative concentration between placental tissue collected from early and late first trimester pregnancies

Metabolites	*P* value	Ratio (late/early)	Platform
Acyl carnitines			
Palmitoylcarnitine	0.0104	1.79 (1.22, 2.84)	UPLC–MS+
Hexanoylcarnitine	0.0176	0.33 (0.13, 0.95)	UPLC–MS+
Heptadecanoylcarnitine	0.0330	1.77 (1.18, 2.86)	UPLC–MS+
Diglycerides			
DG (41:2)	0.0039	1.66 (1.27, 2.30)	UPLC–MS−
DG (35:0)	0.0039	1.96 (1.23, 2.90)	UPLC–MS+
DG (32:0)	0.0104	1.61 (1.24, 2.18)	UPLC–MS+
DG (37:1)	0.0104	1.63 (0.99, 2.32)	UPLC–MS+
DG (35:2)	0.0163	0.65 (0.45, 0.95)	UPLC–MS+
DG (39:4)	0.0163	1.48 (1.12, 1.94)	UPLC–MS+
DG (34:1)	0.0250	1.29 (1.02, 1.60)	UPLC–MS+
DG (38:4)	0.0250	1.41 (0.99, 1.91)	UPLC–MS+
DG (39:2)	0.0250	1.57 (1.19, 2.16)	UPLC–MS−
DG (34:2)	0.0374	0.66 (0.46, 0.95)	UPLC–MS+
DG (36:4)	0.0374	1.35 (0.99, 1.81)	UPLC–MS+
Fatty acids and related metabolites			
Ethyl-hexenoic acid and/or methyl-heptenoic acid and/or octenoic acid	0.0039	0.37 (0.25, 0.69)	UPLC–MS−
Pentadecenoic acid	0.0062	2.56 (1.19, 4.87)	UPLC–MS+
6-Hydroxymethylpterin and/or ethyl-hexenoic acid and/or Methyl-heptenoic acid and/or octenoic acid	0.0065	0.39 (0.24, 0.86)	UPLC–MS+
Sebacic acid and/or 2-amyl 3-butenoic acid and/or nonenoic acid	0.0143	0.28 (0.18, 0.54)	UPLC–MS−
Tetratriacontanoic acid	0.0163	1.68 (1.01, 2.36)	UPLC–MS+
7,8-dihydro-7,7-dimethyl-6-hydroxypterin and/or methyl-octanoic acid and/or nonanoic acid	0.0176	1.38 (0.94, 1.85)	UPLC–MS+
Hydroxy-dodecanoic acid	0.0330	0.61 (0.33, 0.98)	UPLC–MS−
Dodecenoic acid and/or undecanedicarboxylic acid and/or methyl-dodecanedioic acid	0.0339	0.36 (0.22, 0.82)	UPLC–MS−
Dodecatrienol	0.0339	1.71 (0.91, 2.60)	UPLC–MS+
Hexadecenoic acid and/or aminohexadecanoic acid	0.0374	0.62 (0.40, 1.05)	UPLC–MS+
1,2-eicosanediol and/or 13-methyl-1,2-nonadecanediol	0.0374	1.28 (0.99, 1.64)	UPLC–MS+
Hydroxy-dodecenoic acid and/or Oxo-dodecanoic acid	0.0374	1.78 (0.98, 2.68)	UPLC–MS+
Icosatetraenoic acid and/or Eicosatetraenoic acid	0.0374	1.93 (0.93, 4.15)	UPLC–MS−
Methyl-nonadecanoic acid and/or 2,14-dimethyl-octadecanoic acid and/or phytanic acid	0.0455	0.39 (0.21, 0.85)	UPLC–MS+
Lysophosphotidylethanolamines			
LysoPE (22:6)	0.0039	2.12 (1.18, 3.25)	UPLC–MS+
LysoPE (18:1)	0.0374	1.30 (1.03, 1.66)	UPLC–MS−
Nucleotides			
3′,5′-cyclic diguanosine monophosphate and/or guanosine triphosphate adenosine	0.0039	0.62 (0.51, 0.78)	UPLC–MS+
*N*4-acetylcytidine and/or 2′,3′-cyclic uridine monophosphate	0.0105	2.81 (1.02, 5.10)	UPLC–MS−
3′,5′-Cyclic inosine monophosphate and/or 2′-deoxyguanosine-5′-monophosphate and/or 7-alpha-D-ribosyladenine 5′-phosphate and/or adenosine monophosphate	0.0330	3.34 (1.10, 7.12)	UPLC–MS+
8-Oxo-2′-deoxy-guanosine-5′-monophosphate and/or guanosine monophosphate	0.0433	3.84 (0.97, 11.54)	UPLC–MS+
Other metabolites and mixed classifications			
SM (d18:1/22:1) and/or PC (38:2)	0.0039	1.74 (1.32, 2.22)	UPLC–MS−
PC (29:1) and/or PE (32:1) and/or PS (16:0/16:0)	0.0039	1.79 (1.30, 2.39)	UPLC–MS−

Table 2 continued

Metabolites	*P* value	Ratio (late/early)	Platform
Azelaic acid	0.0062	0.33 (0.22, 0.68)	UPLC–MS−
Eicosenoic acid and/or *N,N*-dimethylsphing-4-enine	0.0065	0.51 (0.35, 0.77)	UPLC–MS+
LysoPC (18:2) and/or PC (18:2)	0.0104	0.61 (0.44, 0.85)	UPLC–MS−
PC (34:1) and/or PC (36:4)	0.0104	0.84 (0.74, 0.94)	UPLC–MS−
SM (d18:0/22:0) and/or PC (40:3)	0.0104	1.53 (1.12, 2.03)	UPLC–MS−
Cis-zeatin and/or trans-zeatin and/or hydroxy-11-dodecenoic acid and/or oxo-dodecanoic acid	0.0106	1.46 (1.02, 1.98)	UPLC–MS+
Eicosenol And/or thromboxane	0.0106	1.47 (1.15, 1.92)	UPLC–MS+
4-aminobutanoic acid	0.0143	2.24 (1.33, 5.17)	GC–MS
PC (O-12:0/O-20:0) and/or PC (O-14:0/O-18:0) and/or PC (O-16:0/O-16:0) and/or PC (O-20:0/O-12:0) and/or PC (35:1) and/or PE (14:0/38:1)	0.0163	1.44 (1.09, 1.85)	UPLC–MS−
PC (34:4) and/or PE (37:4)	0.0163	1.60 (1.03, 2.25)	UPLC–MS−
Octadecadienol and/or oxo-nonadecanoic acid and/or docosahexaenoic acid and/or methylpregn-4-ene-3,20-dione and/or methylprogesterone	0.0163	2.40 (0.90, 4.37)	UPLC–MS+
Docosapentaenoic acid and/or 17beta-hydroxy-4,4,17-trimethylandrost-5-en-3-one and/or 1alpha-hydroxy-23,24,25,26,27-pentanorvitamin D3 and/or 22-hydroxy-23,24,25,26,27-pentanorvitamin D3	0.0163	3.08 (1.12, 9.59)	UPLC–MS−
GlcCer (d18:0/26:1) and/or TG (50:0) and/or TG (52:3)	0.0176	0.60 (0.32, 0.93)	UPLC–MS+
11-hydroxy-9,10-dihydrojasmonic acid 11-beta-D-glucoside	0.0176	0.73 (0.52, 0.96)	UPLC–MS+
CE (12:0)	0.0176	1.85 (1.21, 2.79)	UPLC–MS+
Arabinosylhypoxanthine and/or hydroxynicotine and/or nicotine-1′-*N*-oxide	0.0190	1.37 (1.12, 1.68)	UPLC–MS−
PC (32:0) and/or PE (35:0)	0.0250	0.87 (0.78, 0.97)	UPLC–MS−
PC (33:1) and/or PE (36:1)	0.0250	1.25 (1.03, 1.52)	UPLC–MS−
PC (O-14:0/15:0) and/or PE (O-16:0/16:0) and/or PE (38:7)	0.0250	1.31 (1.02, 1.66)	UPLC–MS−
SM (d18:1/22:0) and/or PC (40:4)	0.0250	1.41 (1.08, 1.84)	UPLC–MS−
Dehydroprogesterone and/or pregna-4,11-diene-3,20-dione and/or 3-Methoxy-19-nor-17alpha-pregna-1,3,5 (10),20-tetraen-17-ol	0.0250	1.79 (1.10, 2.60)	UPLC–MS+
Oxaloglutarate	0.0253	0.66 (0.49, 1.02)	UPLC–MS−
1-(O-alpha-D-glucopyranosyl)-29-keto-dotriacontanetriol and/or 1-(O-alpha-D-mannopyranosyl)-29-keto-dotriacontanetriol and/or 2,3-Bis-O-(geranylgeranyl)glycerol 1-phosphate	0.0285	1.42 (1.09, 1.94)	UPLC–MS+
Dimethyl-pentenoic acid and/or methyl-hexenoic acid and/or Dimethyl adipate and/or ethyladipic acid and/or suberic acid	0.0330	0.42 (0.18, 1.00)	UPLC–MS+
CerP (d18:1/20:0) and/or PA (36:1) and/or PC (31:0) and/or PE (34:0)	0.0374	1.43 (1.06, 2.13)	UPLC–MS−
Dynorphin A (1–5) and/or enkephalin L	0.0374	1.59 (1.03, 2.37)	UPLC–MS+
MG (18:2) and/or tetradecanoylcarnitine	0.0374	1.59 (1.03, 2.86)	UPLC–MS+
CerP (d18:1/18:0) and/or PA (34:1) and/or PC (29:0) and/or PE (32:0)	0.0374	1.63 (1.12, 2.38)	UPLC–MS−
2,3-dinor-6-keto-prostaglandin F1 a and/or 2,3-dinor-8-iso prostaglandin F2alpha and/or 2,3-dinor-PGE1 and/or 2R-hydroperoxy-9Z,12Z,15Z-octadecatrienoic acid	0.0374	2.60 (1.16, 5.35)	UPLC–MS+
Docosahexaenoic acid and/or methylpregn-4-ene-3,20-dione and/or 6alpha-methylprogesterone	0.0446	2.27 (1.00, 4.50)	UPLC–MS+
Leucine phosphonate and/or norleucine phosphonate and/or *S*-carboxymethyl-L-cysteine	0.0495	0.41 (0.26, 0.62)	UPLC–MS−
Phospholipids			
PC (30:0) and/or PE (33:0)	0.0039	1.26 (1.12, 1.42)	UPLC–MS+
PC (14:0/dm18:1) and/or PC (14:1/dm18:0) and/or PC (16:1/dm16:0) and/or			
PC (O-14:0/18:2) and/or PC (P-14:0/18:1)	0.0039	1.43 (1.26, 1.64)	UPLC–MS−
PC (35:3) and/or PE (38:3) and/or PE (40:6)	0.0039	1.46 (1.25, 1.72)	UPLC–MS+

Table 2 continued

Metabolites	*P* value	Ratio (late/early)	Platform
PC (33:4) and/or PE (36:4)	0.0039	1.52 (1.23, 1.94)	UPLC–MS+
PE (20:5/dm18:1) and/or PE (22:6/dm16:0) and/or PE (O-16:1(1Z)/22:6)	0.0039	1.52 (1.31, 1.78)	UPLC–MS+
PC (34:0) and/or PE (37:0)	0.0039	1.55 (1.15, 2.08)	UPLC–MS+
PS (32:0)	0.0039	1.66 (1.32, 2.14)	UPLC–MS−
PC (35:1) and/or PE (38:1)	0.0039	1.76 (1.42, 2.20)	UPLC–MS+
PC (35:4) and/or PE (36:4) and/or PE (38:4) and/or PE (18:3/40:7)	0.0062	1.89 (1.12, 2.79)	UPLC–MS+
PC (O-14:0/16:0)	0.0062	3.48 (2.20, 4.81)	UPLC–MS+
PC (42:10)	0.0065	0.53 (0.28, 0.81)	UPLC–MS−
PC (32:2) and/or PC (34:5) and/or PE (37:5)	0.0065	1.41 (1.18, 1.66)	UPLC–MS+
PE (16:0/dm18:1) and/or PE (16:1/dm18:0) and/or PE (18:1/dm16:0)	0.0065	1.47 (1.26, 1.75)	UPLC–MS+
PC (O-14:0/16:0) and/or PC (34:4) and/or PE (37:4) and/or PC (36:7)	0.0065	2.06 (1.16, 3.06)	UPLC–MS+
PC (18:0/dm18:0) and/or PC (20:0/dm16:0) and/or PC (O-16:0/20:1) and/or PC (20:2/dm18:1) and/or PC (20:3/dm18:0) and/or PC (O-16:0/22:4) and/or PC (O-18:0/20:4)	0.0065	2.87 (1.04, 5.30)	UPLC–MS+
PC (36:2)	0.0104	0.71 (0.56, 0.87)	UPLC–MS−
PC (32:2) and/or PE (35:2)	0.0104	1.24 (1.08, 1.43)	UPLC–MS+
PC (31:0) and/or PE (34:0)	0.0104	1.34 (1.14, 1.61)	UPLC–MS+
PC (36:1)	0.0104	1.36 (1.12, 1.66)	UPLC–MS−
PC (16:0/dm18:0) and/or PC (O-16:0/18:1) and/or PC (O-18:0/16:1) and/or PC (18:2/dm18:1) and/or PC (18:3/dm18:0) and/or PC (20:3/dm16:0) and/or PC (O-16:0/20:4)	0.0104	1.50 (1.12, 2.01)	UPLC–MS−
PC (20:0/dm18:1) and/or PC (20:1/dm18:0) and/or PC (22:1/dm16:0) and/or PC (22:4/dm18:0) and/or PC (O-18:0/22:5)	0.0104	1.64 (1.19, 2.45)	UPLC–MS+
PC (O-15:0/O-1:0) and/or PC (O-16:0/0:0) and/or PC (O-8:0/O-8:0)	0.0104	1.96 (1.20, 3.26)	UPLC–MS−
PS (34:1)	0.0105	1.25 (1.11, 1.41)	UPLC–MS+
PC (16:0/O-16:0) and/or PC (O-14:0/18:0)	0.0106	1.43 (1.15, 1.77)	UPLC–MS+
PE (16:1/dm18:1) and/or PE (18:2/dm16:0)	0.0106	1.45 (1.14, 1.86)	UPLC–MS+
PC (18:2/dm18:1) and/or PC (18:3/dm18:0) and/or PC (20:3/dm16:0) and/or PC (O-16:0/20:4)	0.0163	0.50 (0.28, 0.77)	UPLC–MS+
PC (16:0/O-16:0) and/or PC (O-14:0/18:0) and/or PC (O-16:0/16:0) and/or PC (16:1/dm18:1) and/or PC (18:2/dm16:0) and/or PC (O-16:0/18:3) and/or PC (P-16:0/18:2)	0.0163	0.58 (0.40, 0.85)	UPLC–MS−
PC (20:4/dm18:1) and/or PC (20:5/dm18:0) and/or PC (22:5/dm16:0) and/or PC (O-16:0/22:6)	0.0163	0.60 (0.34, 0.91)	UPLC–MS+
PC (36:4) and/or PC (38:7)	0.0163	0.68 (0.47, 0.91)	UPLC–MS+
PC (38:4)	0.0163	1.31 (1.07, 1.62)	UPLC–MS+
PC (35:4) and/or PE (38:4)	0.0163	1.35 (1.10, 1.67)	UPLC–MS+
PS (38:4)	0.0163	1.42 (1.05, 1.93)	UPLC–MS+
PC (36:2) and/or PC (38:5)	0.0163	1.43 (1.07, 1.91)	UPLC–MS+
PC (38:1) and/or PC (40:5)	0.0163	1.46 (1.10, 1.92)	UPLC–MS+
PC (O-16:0/O-18:0) and/or PC (18:0/dm18:0) and/or PC (20:0/dm16:0) and/or PC (O-16:0/20:1) and/or PC (20:2/dm18:1) and/or PC (20:3/dm18:0) and/or PC (O-16:0/22:4) and/or PC (O-18:0/20:4)	0.0163	1.59 (1.19, 2.23)	UPLC–MS+
PC (36:4) and/or PE (22:5/dm18:1)	0.0176	0.66 (0.40, 0.95)	UPLC–MS+
PE (44:2)	0.0176	1.80 (1.21, 2.63)	UPLC–MS−
PC (37:2) and/or PE (40:2)and/or DG (46:3)	0.0201	0.70 (0.60, 0.83)	UPLC–MS+
PC (16:1/dm18:1) and/or PC (18:2/dm16:0) and/or PC (O-16:0/18:3) and/or PC (P-16:0/18:2)	0.0250	0.57 (0.32, 0.95)	UPLC–MS+

Table 2 continued

Metabolites	*P* value	Ratio (late/early)	Platform
PE (24:0/dm18:1) and/or PE (24:0/dm18:1) and/or PE (24:1/dm18:0)	0.0250	0.64 (0.44, 0.93)	UPLC–MS+
PE (16:1/dm18:1) and/or PE (18:2(9Z, 12Z)/dm16:0)	0.0250	0.71 (0.51, 0.94)	UPLC–MS−
PC (34:2) and/or PE (37:2)	0.0250	0.77 (0.63, 0.94)	UPLC–MS+
PC (32:1) and/or PC (34:4)	0.0250	1.27 (1.00, 1.71)	UPLC–MS−
PC (33:0) and/or PE (36:0)	0.0250	1.30 (1.07, 1.58)	UPLC–MS+
PE (44:4)	0.0250	1.36 (1.06, 1.77)	UPLC–MS+
PC (38:1) and/or PC (40:4)	0.0250	1.41 (1.07, 1.91)	UPLC–MS−
PC (34:1) and/or PE (37:1)	0.0374	1.14 (1.02, 1.27)	UPLC–MS+
PE (18:3/dm18:1) and/or PE (18:4/dm18:0) and/or PE (20:4/dm16:0)	0.0374	1.21 (1.01, 1.46)	UPLC–MS+
PC (O-14:0/22:0) and/or PC (O-16:0/20:0) and/or PC (O-18:0/18:0) and/or PC (O-20:0/16:0) and/or PC (40:4) and/or PC (42:7)	0.0374	1.47 (1.08, 2.13)	UPLC–MS+
PC (42:9)	0.0433	0.69 (0.53, 0.93)	UPLC–MS−
PC (36:1) and/or PE (39:1)	0.0446	1.27 (1.02, 1.54)	UPLC–MS+
Sphingolipids			
4-hydroxysphinganine and/or phytosphingosine	0.0039	0.41 (0.31, 0.55)	UPLC–MS+
SM (d18:1/24:1) and/or PC (40:2)	0.0039	1.58 (1.25, 2.00)	UPLC–MS−
Cer (d18:1/24:1)	0.0039	1.62 (1.37, 1.90)	UPLC–MS+
SM (d18:1/24:1)	0.0039	1.86 (1.45, 2.41)	UPLC–MS+
Cer (d18:1/22:1)	0.0039	2.20 (1.27, 3.22)	UPLC–MS+
SM (d18:1/22:1)	0.0039	2.27 (1.51, 3.18)	UPLC–MS+
SM (d18:1/18:1)	0.0062	1.85 (1.45, 2.34)	UPLC–MS+
Sphinganine	0.0065	0.45 (0.31, 0.67)	UPLC–MS+
Cer (d18:1/25:0)	0.0065	1.81 (1.29, 2.67)	UPLC–MS+
SM (d18:1/23:0)	0.0065	1.91 (1.33, 2.80)	UPLC–MS+
N-(24-hydroxytetracosanyl)sphinganine and/or *N*-tetracosanylphytosphingosine	0.0104	0.53 (0.34, 0.77)	UPLC–MS−
Cer (d18:0/24:1) and/or Cer (d18:1/24:0) and/or *N*-lignoceroylsphingosine	0.0104	1.32 (1.13, 1.57)	UPLC–MS−
Dehydrosphinganine and/or 5-hydroxysphingosine and/or sphingosine	0.0163	0.63 (0.44, 0.89)	UPLC–MS+
SM (d18:1/14:0)	0.0163	1.56 (1.18, 1.98)	UPLC–MS+
Tricosanamide	0.0163	1.64 (1.17, 2.34)	UPLC–MS−
SM (d18:1/16:0)	0.0250	1.23 (1.05, 1.46)	UPLC–MS+
Etn-1-P-Cer (d14:1/18:0)	0.0250	1.53 (1.15, 2.04)	UPLC–MS+
SM (d18:0/24:1) and/or SM (d18:1/24:0)	0.0250	1.69 (1.12, 2.89)	UPLC–MS+
SM (d18:1/22:0)	0.0250	1.70 (1.09, 2.63)	UPLC–MS+
GlcCer (d18:0/24:1)	0.0275	1.56 (1.09, 2.14)	UPLC–MS−
Cer (d18:1/22:0)	0.0374	1.47 (1.03, 2.09)	UPLC–MS+
Triglycerides			
TG (52:4)	0.0039	0.49 (0.38, 0.66)	UPLC–MS+
TG (50:2)	0.0039	0.78 (0.69, 0.88)	UPLC–MS+
TG (50:3) and/or TG (52:6)	0.0065	0.49 (0.36, 0.77)	UPLC–MS+
TG (52:4) and/or TG(54:7)	0.0104	0.54 (0.38, 0.78)	UPLC–MS+
TG(54:4)	0.0104	0.63 (0.53, 0.78)	UPLC–MS+
TG(56:7)	0.0143	0.61 (0.46, 0.82)	UPLC–MS+
TG(54:6)	0.0163	0.51 (0.32, 0.76)	UPLC–MS+
TG(49:1)	0.0163	0.56 (0.40, 0.79)	UPLC–MS−
TG(53:1)	0.0163	0.66 (0.51, 0.85)	UPLC–MS+
TG(54:6) and/or TG(56:9)	0.0250	0.54 (0.28, 0.91)	UPLC–MS+
TG(54:5) and/or TG(56:8)	0.0250	0.55 (0.32, 0.85)	UPLC–MS+
TG(58:7) and/or TG(60:10)	0.0285	0.60 (0.42, 0.90)	UPLC–MS+

Table 2 continued

Metabolites	*P* value	Ratio (late/early)	Platform
TG(53:0)	0.0285	0.62 (0.34, 0.95)	UPLC–MS+
TG(55:1)	0.0374	0.67 (0.40, 0.97)	UPLC–MS+
Vitamin D metabolites			
Vitamin D metabolite (greater than 6 hits)	0.0065	1.29 (0.97, 1.63)	UPLC–MS+
Vitamin D metabolite (greater than 6 hits)	0.0104	1.28 (1.00, 1.59)	UPLC–MS+
Vitamin D metabolite (greater than 6 hits)	0.0106	1.28 (1.06, 1.52)	UPLC–MS+
(6R)-6,19-ethano-25-hydroxy-6,19-dihydrovitamin D3 and/or (6S)-6,19-ethano-25-hydroxy-6,19-dihydrovitamin D3/and/or 1 1-alpha-hydroxy-26,27-dimethylvitamin D3 and/or 1-hydroxyvitamin D5 and/or 25-hydroxy-26,27-dimethylvitamin D3	0.0374	2.36 (1.00, 4.08)	UPLC–MS+

Cer ceramide, *DG* diglyceride, *GlcCer* glycosphingolipids, *MG* monoglyceride, *PC* glycerophosphatidycholine, *PE* glycerophosphatidylethanolamine, *PS* glycerophosphatidylserine, *SM* sphingomyelin, *TG* triglyceride

All metabolites are putatively identified. The ratio of responses for late versus early first trimester tissue are shown with 95% confidence limits. The metabolites are grouped by metabolite class or chemical similarity and are subsequently sorted by *P*-value and then ratio of median peak areas of late-to-early first trimester tissue classes

3.2 Placental tissue from uncomplicated and preeclamptic pregnancies

This study was designed to fulfill three objectives; (i) to determine whether the position of sampling of tissue from the placenta (edge, centre, middle distance between edge and centre) influences the metabolic profile observed, (ii) to determine whether the type of delivery influences the tissue metabolic profile and (iii) to determine whether the type of pregnancy (preeclamptic or uncomplicated) from which the tissue was sampled influences the metabolic profile. 3334, 1324 and 167 metabolic features were detected by the UPLC–MS (+), UPLC–MS (−) and GC–ToF–MS analytical platforms, respectively, after signal correction and/or QA processes.

3.2.1 Analytical versus biological variation

The objective of the first investigation was to assess the variation in the data related to the operation of the analytical platform over time (technical variability) and to compare this to the variability observed from studying different biological samples (Zelena et al. 2009). To achieve this objective the relative standard deviation (RSD) for each metabolic feature was calculated to determine four levels of variability; variability observed for injection of the same QC sample multiple times, variability observed from sampling a single placenta at different positions, the variability observed from sampling multiple placenta and the variability observed from sampling placentas from two different kinds of pregnancies (uncomplicated and PE). This was performed separately for each of the three analytical platforms. The median and inter-quartile ranges are reported in Table 3.

Technical variability was calculated from all QC samples analysed from injection 4 (GC–ToF–MS, $n = 15$) and injection 9 (UPLC–MS, $n = 14$). This assesses the variability (or reproducibility) of multiple injections of the same QC sample. These data indicate that technical variability is low, the variability is related to the analytical platform applied, and the median RSD was less than 14% for all analytical platforms.

The variance observed from sampling a single placenta at three different positions ($n = 3$) was greater than the

Table 3 The median relative standard deviation and interquartile ranges determined for multiple sources of variation present in the study of placenta from uncomplicated and preeclamptic pregnancies on three separate analytical platforms

Platform/comparison	GC–ToF–MS	UPLC–MS (+)	UPLC–MS (−)
Technical variability ($n = 14$ or 15)	13.5 (7.6–30.8)	4.7 (2.6–8.2)	12.1 (6.2–20.2)
Biological within-placenta variability ($n = 3$)	44.8 (28.7–72.0)	15.6 (10.0–22.3)	16.1 (10.5–23.9)
Biological within-class variability ($n = 6$)	61.5 (45.6–79.9)	22.8 (10.7–40.3)	23.0 (12.8–37.5)
Biological between-class variability ($n = 6$)	69.4 (56.5–89.7)	30.4 (17.5–47.6)	31.3 (19.6–44.0)

technical variability but lower than the variability from tissue samples collected from multiple different placenta ($n = 6$). The biological variability observed from sampling multiple placenta is the highest with within class variability (uncomplicated or preeclamptic, $n = 6$) being slightly lower than between class variability (the biological variability associated with comparing uncomplicated and preeclamptic tissue). Note that n is different for each comparison.

3.2.2 *Comparison of the tissue metabolome related to position of sampling of the placenta*

Three separate univariate comparisons were performed to determine whether the metabolomes of tissue sampled from different positions of the same placenta are different. The three separate comparisons performed were from applying data separately from (i) the uncomplicated vaginal delivery samples, (ii) the uncomplicated caesarean delivery samples and (iii) the preeclamptic vaginal delivery samples. For GC–ToF–MS, no metabolic features were consistently observed to be statistically different ($P < 0.05$) in 2 or 3 of the comparisons described above. For UPLC–MS (−) four metabolic features were statistically significant ($P < 0.05$) in 2 of the 3 comparisons described; a phosphatidylinositol (PI 38:4), nitrate or peroxynitrite and 2 unidentified features. For UPLC–MS (+) 2 metabolic features were statistically significant ($P < 0.05$) in 2 of the 3 comparisons described; both were unidentified features.

3.2.3 *Comparison of the placental tissue metabolome related to mode of delivery*

Three separate univariate comparisons were performed to determine whether the metabolome of tissue collected from different modes of delivery (vaginal and caesarean section) for uncomplicated pregnancies is statistically different. The three separate comparisons performed were from comparing data separately from (i) the tissue samples collected from the edge of the placenta, (ii) the tissue samples collected from the centre of the placenta and (iii) the tissue samples collected from a middle position between the edge and centre of the placenta. For GC–ToF–MS, seven metabolic features were consistently observed to be statistically different ($P < 0.05$) in 2 or 3 of the comparisons described; lysine, methyl acetoacetate, phosphoenolpyruvate, two sugars and two unidentified features. For UPLC–MS (−) five metabolic features were statistically significant ($P < 0.05$) in 2 of the 3 comparisons described; sphingosine or ketosphingosine or 4,8-sphingadienine, dodecanoylcarnitine, a monoglyceride (MG 16:0) and two unidentified features. For UPLC–MS (+) 19 metabolic features were statistically significant ($P < 0.05$) in 2 of the 3 comparisons described; a vitamin D metabolite, a cholesteryl ester (CE 15:0), three oxidised or methylated fatty acids, two diglycerides, dodecanamide and 11 unidentified features.

The number of metabolic features showing a statistically significant difference in both of the comparisons described above (3.2.2 and 3.2.3) highlight that metabolic variations are observed at different areas of the placenta and that changes in metabolism are observed for different types of deliveries. This might be expected as differences in cell turnover and mRNA expression have been noted in labouring versus non-labouring placentas (Tissot van Patot et al. 2010). However, compared to the number of changes observed in placental tissue from early and late first-trimester pregnancies (above) and from the comparison of preeclamptic and uncomplicated pregnancies (below) the number of changes are much lower when comparing samples from different areas of the placenta or from different modes of delivery. Nevertheless, to minimise variation it is recommended to collect and analyse samples from multiple positions on a placenta and from multiple placentas for experimental robustness and to ensure samples analysed were collected from the same delivery type.

3.2.4 *Comparison of term placental tissue metabolomes from uncomplicated and preeclamptic pregnancies*

Following data pre-processing and assessment of technical and biological variability, PCA was performed with all available data. The scores plots for GC–ToF–MS showed no clear separation of tissue from uncomplicated and preeclamptic pregnancies (data not shown). The scores plots (PC1 vs. PC2) for data acquired on UPLC–MS (+) and UPLC–MS (−) platforms are shown in Fig. 2. The QC samples are tightly clustered in relation to the clustering of the placental tissue samples showing good technical reproducibility. UPLC–MS (+) and UPLC–MS (−) data shows clear separation of tissue collected from preeclamptic and uncomplicated pregnancies.

In relation to the previous section which assessed the metabolic changes related to mode of delivery, the samples acquired from uncomplicated pregnancies delivered by caesarean section are positioned in PCA space between uncomplicated and preeclamptic pregnancies with vaginal deliveries in UPLC–MS (+). These data suggest that in gross terms the phenotype of this tissue falls between the two other tissue types. As preeclamptic tissue is proposed to have a hypoxic phenotype (other non-metabolomic studies have shown this (Genbacev et al. 1996; Granger et al. 2002; Rajakumar et al. 2001; Rampersad and Nelson 2007; Vaiman et al. 2005; Soleymanlou et al. 2005) as well as a previous metabolic footprinting study (Dunn et al.

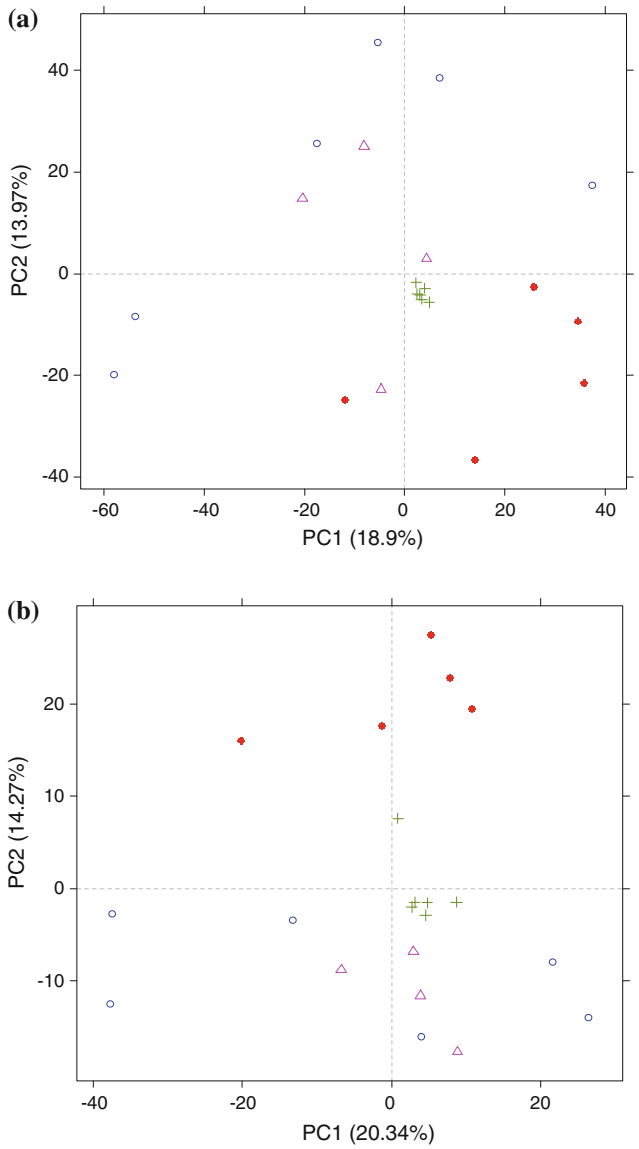

Fig. 2 PCA scores plots for all data acquired in the study of placental tissue from uncomplicated and preeclamptic pregnancies. Data are shown for (**a**) UPLC–MS (+) and (**b**) UPLC–MS (−). Preeclamptic tissue and vaginal delivery (*red infilled circle*); uncomplicated pregnancy and vaginal delivery (*blue empty circle*); uncomplicated pregnancy and caesarean section delivery (*pink triangle*) and quality control sample (*green cross*) (Color figure online)

2009)), this suggests that delivery by caesarean section may provide a higher oxidative stress compared to vaginal delivery. Previous data suggest that there is increased apoptosis and related gene expression in placentas from labour which are not evident in those from caesarean section (Tissot van Patot et al. 2010).

Univariate analysis was applied to define statistically significant differences ($P < 0.05$) between preeclamptic tissues and tissues collected from uncomplicated pregnancies, all with vaginal deliveries only. 326, 83 and 13 metabolic features were statistically different at $P < 0.05$ for the three analytical platforms of UPLC–MS (+), UPLC–MS (−) and GC–ToF–MS, respectively. When taking into account the fact that multiple metabolic features can be detected for a single metabolite and that not all metabolic features can be assigned a putative identification a lower number of putatively identified and unique metabolites can be reported. 10, 46 and 30 unique metabolites were putatively chemically identified. The metabolites showing a statistically significant difference are shown in Table 4.

The relative concentrations of metabolites as grouped into seven classes were observed to change when comparing placental tissue between uncomplicated and preeclamptic pregnancies with vaginal delivery. These are, acyl glycerides (mono- and di-, 8), phospholipids (19), fatty acids and related metabolites (14), amino acid related metabolites (3), vitamin D-related metabolites (3), isoprenoids (2), and steroids (2). The majority of these classes show a decrease in the relative concentration of metabolites in preeclamptic tissue. These included 8 of 14 fatty acids and related metabolites, 7 of 8 acyl glycerides, all 3 amino acid related metabolites and 2 of 3 vitamin D-related metabolites. 14 of 19 phospholipids show an increase in the relative concentration of metabolites in preeclamptic tissue.

Recent research has highlighted potential changes in vitamin D metabolism in preeclampsia (Robinson et al. 2010), and it is interesting to note (i) that supplementation with calcium ions is one of the only effective preventative strategies for pre-eclampsia, and (ii) that women with high levels of vitamin D appear to have a lower incidence of preeclampsia (Haugen et al. 2009). However, the results here are not straightforward to interpret, in that they show a higher placental tissue concentration of vitamin D-related metabolites, but not of vitamin D itself, suggesting a complex perturbation in this area of placental metabolism that bears further study.

Elevated concentrations of hexanoylglycine, caproylglycine and suberylglycine are all indicative of mitochondrial fatty acid beta oxidation disorders and are used for diagnosis in the newborn screening of metabolic disorders (Rinaldo et al. 1988). In this study we observed decreases in the concentration of these acylglycines in preeclamptic tissue. This indicates a potential decrease in mitochondrial fatty acid beta oxidation highlighted by the build-up of these intermediates in preeclamptic tissue and therefore these specific acyl glycines indicate a potential decrease in the first step of beta-oxidation. An increase in two acylglycines was also observed in plasma at 15 weeks of preeclamptic pregnancies (Kenny et al. 2010). This may reflect the active transport of these molecules from placenta to maternal blood supply, assuming at least some of the

Table 4 Metabolic features observed to be statistically significantly different ($P < 0.05$) in their relative concentration when comparing placental tissue collected from uncomplicated and preeclamptic pregnancies

Metabolites	*P* value	Ratio (PE/Control)	Platform
Amino acids and related metabolites			
Hexanoylglycine and/or isovalerylalanine and/or isovalerylsarcosine	0.0285	0.34 (0.21, 0.66)	UPLC–MS−
Capryloylglycine	0.0446	0.45 (0.28, 0.85)	UPLC–MS−
Isovalerylglutamic acid and/or suberylglycine	0.0446	0.63 (0.24, 1.09)	UPLC–MS+
Fatty acids, fatty alcohols and related metabolites			
Hydroxy-dodecenoic acid	0.0062	1.33 (1.10, 1.61)	UPLC–MS−
Amino-hexadecanoic acid	0.0062	2.09 (1.53, 3.04)	UPLC–MS+
Palmitic amide	0.0062	4.31 (3.21, 6.21)	UPLC–MS+
Heneicosadienoic acid and/or methyl-eicosadienoic acid	0.0176	0.74 (0.63, 0.89)	UPLC–MS+
Amino-hexadecenoic acid	0.0176	2.19 (1.30, 4.03)	UPLC–MS+
1-tetratriacontanol	0.0176	2.74 (1.32, 7.84)	UPLC–MS+
N-arachidonoyl taurine	0.0283	1.47 (1.05, 2.22)	UPLC–MS+
Hydroxy-decanoic acid	0.0285	0.50 (0.11, 1.00)	UPLC–MS+
Hydroxy-dodecanoic acid	0.0285	1.20 (1.02, 1.40)	UPLC–MS−
1-dotriacontanol	0.0285	1.78 (1.07, 3.16)	UPLC–MS+
Hexadecatrienediynoic acid	0.0330	0.27 (0.08, 0.82)	UPLC–MS+
Octadecadienol and/or oxo-nonadecanoic acid	0.0446	0.32 (0.06, 0.92)	UPLC–MS−
Oxo-heneicosanoic acid	0.0446	0.41 (0.05, 1.17)	UPLC–MS−
7-hydroxy-5-heptynoic acid	0.0446	0.78 (0.60, 0.99)	UPLC–MS+
Acyl glycerides			
MG (16:0)	0.0062	1.69 (1.37, 2.17)	UPLC–MS−
DG (36:8/18:4)	0.0062	5.67 (2.46, 32.58)	UPLC–MS+
MG (16:1)	0.0143	4.73 (0.54, 9.73)	UPLC–MS+
MG (18:1)	0.0163	2.34 (1.25, 3.88)	UPLC–MS−
DG (33:0)	0.0283	1.43 (1.03, 2.05)	UPLC–MS+
DG (35:0) and/or DG (37:3) and/or DG (39:6)	0.0285	1.70 (1.07, 3.09)	UPLC–MS−
DG (30:2)	0.0339	0.36 (0.18, 1.21)	UPLC–MS+
MG (18:0)	0.0446	1.43 (1.02, 2.13)	UPLC–MS−
Isoprenoids			
C25:1 Monocyclic highly branched isoprenoid and/or C25:2 highly branched isoprenoid	0.0176	0.60 (0.42, 0.96)	UPLC–MS+
C25:0 Monocyclic highly branched isoprenoid and/or C25:1 highly branched isoprenoid	0.0176	2.65 (1.31, 5.00)	UPLC–MS−
Other metabolites			
Stearoylcarnitine	0.0062	0.71 (0.60, 0.85)	UPLC–MS+
LysoPC (20:3) and/or coproporphyrin	0.0062	1.33 (1.15, 1.58)	UPLC–MS−
Leucyl-leucine and/or *N*-(6-aminohexanoyl)-6-aminohexanoate	0.0062	2.45 (1.82, 3.69)	UPLC–MS+
Nitrophenol	0.0106	0.70 (0.54, 0.89)	UPLC–MS−
Succinylcholine	0.0106	2.49 (1.58, 4.35)	UPLC–MS+
Nitrate and/or peroxynitrite	0.0143	1.52 (1.23, 1.86)	UPLC–MS−
Octadecenoic acid and/or MG (16:1) and/or HDoHE	0.0176	1.29 (1.10, 1.51)	UPLC–MS+
Sphingosyl-phosphocholine and/or 1alpha,25-dihydroxy-24a-homo-22-thia-20-epivitamin D3 and/or 1alpha,25-dihydroxy-24a-homo-22-thiavitamin D3 and/or 24a,24b-epoxy-23-tetradehydro-24a,24b-dihomo-1alpha,25-dihydroxyvitamin D3	0.0209	1.38 (1.23, 1.55)	UPLC–MS+
Acetic acid	0.0285	0.35 (0.02, 0.79)	GC–MS
S-Succinylglutathione	0.0285	1.31 (1.07, 1.63)	UPLC–MS+
Prenol and/or choline	0.0285	1.39 (1.04, 1.88)	UPLC–MS+
N-acetylglucosamine	0.0285	1.92 (1.07, 3.21)	GC–MS

Table 4 continued

Metabolites	*P* value	Ratio (PE/Control)	Platform
C6 sugar	0.0285	2.12 (0.61, 3.98)	GC–MS
Succinic acid	0.0285	2.30 (1.11, 9.05)	GC–MS
Molybdopterin-AMP	0.0433	0.75 (0.58, 0.96)	UPLC–MS+
Canavaninosuccinate	0.0433	0.85 (0.73, 0.99)	UPLC–MS+
3-ethylcatechol and/or methoxybenzyl alcohol and/or hydroxyphenylethanol	0.0446	0.47 (0.33, 0.77)	UPLC–MS+
Beta-aspartyl-leucine and/or L-gamma-glutamyl-L-valine	0.0446	0.52 (0.15, 0.97)	UPLC–MS+
Pseudouridine and/or uridine	0.0446	0.82 (0.69, 0.98)	UPLC–MS+
PC (O-16:0/O-18:0) and/or *N*-(2-hydroxyhexacosanoyl)-4,8-sphingadienine	0.0446	0.84 (0.72, 1.01)	UPLC–MS+
Hydroxykynurenamine and/or tyrosinamide and/or aminophenylalanine	0.0446	1.11 (1.02, 1.21)	UPLC–MS+
Methylguanine	0.0495	1.38 (1.05, 1.80)	UPLC–MS+
Phospholipids			
PC (O-16:0/3:1) and/or LysoPC (21:4)	0.0062	0.66 (0.58, 0.77)	UPLC–MS+
LysoPC (16:0) and/or PC (O-14:0/2:0)	0.0062	1.53 (1.25, 1.92)	UPLC–MS+
PC (18:0/O-16:0) and/or PC (O-17:0/17:0) and/or PC (O-18:0/16:0) and/or PC (18:1/dm18:1) and/or PC (18:2/dm18:0) and/or PC (20:2/dm16:0)	0.0106	1.18 (1.06, 1.33)	UPLC–MS−
PS(38:0)	0.0106	1.26 (1.03, 1.61)	UPLC–MS+
PC (18:0/dm18:1) and/or PC (18:1/dm18:0) and/or PC (18:2/O-18:0) and/or PC (20:1/dm16:0) and/or PC (P-18:0/18:1)	0.0106	1.40 (1.11, 1.89)	UPLC–MS−
GlcCer (d18:0/20:0)	0.0190	1.80 (1.15, 2.93)	UPLC–MS+
PC (38:0) and/or PE (41:0) and/or PC (40:3)	0.0190	1.91 (1.26, 2.93)	UPLC–MS−
PE (34:2)	0.0285	1.31 (1.06, 1.64)	UPLC–MS+
PE (40:9)	0.0330	0.38 (0.22, 0.77)	UPLC–MS+
Cer (d18:1/22:0)	0.0339	0.67 (0.57, 0.80)	UPLC–MS+
PC (36:1) and/or PC (36:2) and/or PC (38:4)	0.0446	0.89 (0.81, 0.98)	UPLC–MS−
PE (16:0/dm18:1) and/or PE (16:1/dm18:0) and/or PE (18:1/dm16:0)	0.0446	1.10 (1.01, 1.21)	UPLC–MS−
PI (18:0/18:1)	0.0446	1.19 (1.00, 1.43)	UPLC–MS−
Cer (d18:0/26:1) and/or ceramide (d18:1/26:0)	0.0446	1.21 (1.01, 1.45)	UPLC–MS+
Sphingosine and/or ketosphingosine and/or 4,8-sphingadienine	0.0446	1.48 (0.95, 2.60)	UPLC–MS−
PG (28:0)	0.0455	2.09 (1.48, 3.59)	UPLC–MS+
PA (36:4) and/or PE (34:3)	0.0472	1.40 (1.06, 1.79)	UPLC–MS−
PG (38:5) and/or PG (40:8)	0.0472	1.62 (1.10, 2.46)	UPLC–MS−
Steroids and related metabolites			
17beta-amino-5alpha-androstan-11beta-ol	0.0090	2.47 (1.74, 3.51)	UPLC–MS−
3alpha,7alpha,12alpha-trihydroxy-5beta-cholestane or isomer	0.0176	0.28 (0.17, 0.64)	UPLC–MS+
Vitamin D and related metabolites			
(20S)-1alpha,25-dihydroxy-20-ethoxy-26,27-dimethyl-24a-homovitamin D3 and/or (20S)-1alpha,25-dihydroxy-20-methoxy-26,27-diethylvitamin D3 and/or 1alpha,25-dihydroxy-21-(3-hydroxy-3-methylbutyl)vitamin D(3) and/or 1alpha-hydroxy-2beta-(5-hydroxypentoxy)vitamin D3 and/or 26,27-diethyl-1alpha,25-dihydroxy-24a,24b-dihomo-23-oxa-20-epivitamin D3	0.0285	0.72 (0.58, 0.93)	UPLC–MS+
1alpha-hydroxy-24-(dimethylphosphoryl)-25,26,27-trinorvitamin D3 and/or isovitamin D2 and/or 4alpha-methyl-5alpha-cholesta-8,24-dien-3-one or isomer and/or 5,7,24(28)-ergostatrienol or isomer and/or dehydroepisterol;5 and/or ergocalciferol and/or ergosterol	0.0446	1.63 (0.99, 2.63)	UPLC–MS+
1alpha,25-dihydroxy-19-nor-22-oxavitamin D3	0.0455	2.36 (1.15, 4.01)	UPLC–MS+

Cer ceramide, *DG* diglyceride, *GlcCer* glycosphingolipids, *HDoHE* hydroxydocosahexaenoic acid, *MG* monoglyceride, *PA* monoacylglycerophosphates, *PC* glycerophosphatidylcholine, *PG* phosphotidylglycerol, *PI* glycerophosphotidylinositol, *PS* glycerophosphotidylserine

Only tissues collected from pregnancies with vaginal deliveries were compared. All metabolites are putatively identified. The ratio of preeclamptic versus uncomplicated pregnancies are shown with 95% confidence limits. The metabolites are grouped by metabolite class or chemical similarity and are subsequently sorted by p-value and then ratio of median peak areas of preeclamptic-uncomplicated pregnancies

pathophysiological mechanisms of preeclampsia are similar at 15 weeks and at term, and given the interest is seeking early biomarkers of preeclampsia this bears further analysis.

Three acyl amino acids (isovalerylalanine, isovalerylglutamate and isovalerylsarcosine) which are related to a separate inborn error of metabolism, isovaleric acidemia (Tanaka et al. 1966), were shown to change in their relative concentration. Again these metabolites are observed at increased concentrations in blood for diagnosis but are at lower concentrations in placental tissue. This indicates the potential increased consumption of leucine in preeclamptic tissue. A study of cord blood (artery and vein) showed lower concentrations of branched chain amino acids in IUGR (of which preeclampsia is related) compared to uncomplicated pregnancies (Cetin et al. 1988).

Nitrate and/or peroxynitrite are increased in preeclamptic tissue, as has been shown previously (Speake et al. 2003). Nitrate is a final product of the arginine-nitric oxide (NO) pathway, specifically an end-product of NO metabolism. Metabolites in this pathway have been shown to play an important role in placental tissue and the progress of pregnancies. Nitrate has also been shown to be lower in biofluids of preeclamptic pregnancies (Gupta et al. 2003; Schiessl et al. 2006). Arginine supplementation has been shown to be beneficial in reducing blood pressure in stress-induced preeclamptic rats (Altun et al. 2008) and a recent study has assessed the influence of arginine supplementation in reducing the prevalence of preeclamptic pregnancies (Vadillo-Ortega et al. 2011). An increased activity of cationic amino acid transporters in preeclampsia may reflect alterations in the delivery of arginine to syncytiotrophoblast eNOS (Speake et al. 2003). The increased concentration of nitrate may reflect this difference. There is a greater level of protein nitration in the placenta in preeclampsia (Webster et al. 2008). *S*-nitrosylation (NO post-translation modification) of proteins has been shown to modulate protein function and lead to changes in the regulation of biological functions in the placenta (Zhang et al. 2011). Peroxynitrite has also been shown to cause nitrative stress and modifications of proteins and DNA including nitrosylation which may be associated with alteration of protein function (Kell 2009, 2010; Myatt 2010). Peroxynitrite has been observed at higher concentrations in erythrocytes of preeclamptic women (Dordevic et al. 2008) and is capable of attenuating vascular responses in the human placenta (Kossenjans et al. 2000).

4 Concluding remarks

The placenta plays an essential role in pregnancy. Studies described here have shown changes in the relative concentrations of metabolites in preeclamptic compared to uncomplicated pregnancies and have also shown a large number of changes in lipid metabolism during the early development of the placenta. The studies presented here show that it is important to study placental tissue to define specific changes in pregnancy complications and we suggest that these changes should be integrated with data from biofluids including serum/plasma, urine, cord blood and foetal blood.

The results here highlight the great potential of applying metabolomics for the understanding of pregnancy disorders, as also indicated previously (Dunn et al. 2009; Graca et al. 2007; Heazell et al. 2008; Kenny et al. 2010). The integration of data from different studies (biofluids including urine, maternal and cord blood plasma or serum, amniotic fluid, tissue metabolic footprint and placental tissue) will undoubtedly provide a greater understanding of the complex interactions between mother, placenta and fetus during normal and complicated pregnancies. However caution should be exercised with regard to the low sample sizes applied in the studies described here (generally $n = 6$ per class) and the absence of application of corrections for multiple testing (Broadhurst and Kell 2006). However, the observation of several metabolites from the same metabolite class which showed statistically significant changes provides extra confidence in the validity of the results.

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