The placenta is a temporary organ that is essential for a healthy pregnancy. It performs several important functions, including the transport of nutrients, the removal of waste products and the metabolism of certain substances. Placental disorders have been found to account for over 50% of stillbirths. Despite this, there are currently no methods available to directly and non-invasively assess placental function in utero. The primary aim of this pilot study was to investigate the use of $^1$H MRS for this purpose. $^1$H MRS offers the possibility to detect several placental metabolites, including choline, lipids and the amino acids glutamine and glutamate (Glx), which are vital to fetal development and placental function. Here, in utero placental spectra were acquired from nine small for gestational age (SGA) pregnancies, a cohort who are at increased risk of perinatal morbidity and mortality, and from nine healthy gestation-matched pregnancies. All subjects were between 26 and 39 weeks of gestation. Placenta Glx, choline and lipids at 1.3 and 0.9 ppm were quantified as amplitude ratios to that of intrinsic H$_2$O. Wilcoxon signed rank tests indicated a significant difference in Glx/H$_2$O ($p = 0.024$) between the two groups, but not in choline/H$_2$O ($p = 0.722$) or in either lipid/H$_2$O ratio (1.3 ppm, $p = 0.813$; 0.9 ppm, $p = 0.058$). This study has demonstrated that $^1$H MRS has potential for the detection of placental metabolites in utero. This warrants further investigation as a tool for the monitoring of placental function.

Keywords: MRS; spectroscopic quantification; glutamine; glutamate; fetal growth restriction; placenta

INTRODUCTION

Disorders of the placenta are associated with over 50% of stillbirths (1). Despite this, there are currently no methods available to directly and non-invasively assess placental function in utero. Most information on this temporary, yet crucially important, organ has been gathered from post-delivery assessments, an exercise that has been likened to a post-mortem examination (2). There is an urgent clinical need to be able to monitor the function of the living placenta, as it supports the developing fetus, to both improve our understanding of this organ and enable the effective identification and management of the at-risk fetus.

There is potential value in the direct detection of placental choline, lipids and the amino acids glutamine and glutamate, as they are all vital to placental metabolism and fetal development. These metabolites can be detected using $^1$H MRS, but its application to the in utero placenta is novel. The primary aim of this pilot study was to investigate the use of $^1$H MRS for this purpose.

Choline is essential for the normal function of cells, ensuring both structural integrity and signal functions of cell membranes, and for influencing stem cell proliferation and apoptosis (3). The placenta delivers choline directly to the fetus, but is also known to store large amounts of choline as acetylcholine (3). Lipids provide fuel for oxidative metabolism and are precursors for other compounds (4). They are delivered to the fetus in different ways, e.g. via fatty acid-binding proteins in the placenta (5). Glutamine and glutamate are important for fetal and placental nutrition, and many other products are derived from them (6,7). For example, glutamine is involved in the synthesis of the nucleotides and amino sugars required for the proliferation and differentiation of cells (6), and glutamate is a precursor of glutamine. The mother, placenta and fetus are all involved in a delicate balance of the delivery and synthesis of glutamine and glutamate. The fetus receives glutamine from two sources: direct transplacental transport from mother to fetus and glutamine synthesised within the placenta (7,8). Glutamine is transported from the placenta...

Abbreviations used: AMARES, advanced method for accurate, robust and efficient spectral fitting; BMI, body mass index; choline/H$_2$O, amplitude ratio of choline to water; EmCS, emergency caesarean section; FGR, fetal growth restriction; Glx, combined spectral contribution for glutamine and glutamate; Glx/H$_2$O, amplitude ratio of Glx to water; HPFD, Haag Ferguson forceps delivery; HLSVD, Hankel Lanczos Singular Values Decomposition Filter; lipid 1.3 ppm/H$_2$O, amplitude ratio of the lipid peak at 1.3 ppm to water; lipid 0.9 ppm/H$_2$O, amplitude ratio of the lipid peak at 0.9 ppm to water; MVM, microvillous plasma membrane; NNU, neonatal unit; PRESS, point-resolved spectroscopy; RDS, respiratory distress; REDF, reversed end-diastolic flow; SGA, small for gestational age; SVD, spontaneous vertex delivery.

† These authors contributed equally.
to the fetus, where it is used to synthesise glutamate in the fetal liver. Glutamate is then transported back to the placenta, where it is converted back to glutamine (7). Day et al. (8) have suggested that placental glutamine and glutamate may also play a role in facilitating the placental uptake of other amino acids and in passing waste products from the fetus to the mother. Therefore, choline, lipids, glutamine and glutamate are known to be present in the placenta, and their detection may have merit in the assessment of placental function.

Small for gestational age (SGA) fetuses (defined as a birthweight less than the 10th centile) are at increased risk of perinatal morbidity and mortality (9). In SGA pregnancies, current obstetric management comprises fetal surveillance to try to predict fetal acidemia, thereby allowing the timely delivery of SGA fetuses prior to irreversible end-organ damage and intrauterine fetal death. A wide range of tests, including fetal biometry, biophysical profile (10), cardiotocography (11), ultrasound assessment of amniotic fluid volume (12), and umbilical artery (13,14), middle cerebral artery (15), ductus venosus (16) and umbilical venous Doppler blood flow measurements (17), are used with varying efficacy. Despite reports in the literature indicating that placental function may be compromised in SGA pregnancies (18,19), none of the currently available tests directly assess placental function in utero. Here, $^1$H MR spectra were acquired from the placenta of nine SGA pregnancies and nine healthy gestation-matched subjects. The aim was to assess the ability of this technique to detect choline, lipids and the combined spectral contribution of glutamine and glutamate (Glx) in the placenta of these two groups.

METHODS

Ethics statement

This study was approved by Lothian Research Ethics Committee (11/SS/0031) and all participants gave written informed consent.

Study population

Patients were enrolled at the Simpson Centre for Reproductive Health at the Royal Infirmary, Edinburgh, UK. Magnetic resonance studies were performed at the Clinical Research Imaging Centre in the Queen’s Medical Research Institute, University of Edinburgh, UK.

Nine women with a pregnancy complicated by SGA and nine matched healthy controls were recruited prospectively. This was a pilot study and subjects were not derived from a larger cohort. Gestation was calculated from routine dating ultrasonography at 11–13 weeks of gestation. To avoid bias, controls were prospectively recruited to match each recruited SGA pregnancy in turn for gestation (within 6 days) and smoking status. The inclusion criteria were a singleton pregnancy and >20 weeks of gestation. The exclusion criteria were significant co-existing maternal systemic disease, including microvascular disease and gestational diabetes, or contraindication to MRI. SGA was defined using ultrasound as an abdominal circumference less than the 10th centile (20) in accordance with the Royal College of Obstetricians and Gynaecologists guidelines (9,21). SGA status was subsequently confirmed by a birthweight of less than the 10th centile.

Doppler ultrasound scan

All participants had a transabdominal ultrasound scan performed using a Toshiba Apio XG system (Toshiba Medical Systems Ltd., Crawley, West Sussex, UK) to assess fetal size, liquor volume and umbilical artery Doppler blood flow. Fetal size was assessed by measuring the head and abdominal circumference. Liquor volume was assessed subjectively, with the amniotic fluid index being calculated if subjective assessment was abnormal. A free loop of the umbilical cord was interrogated with colour flow and then pulsed Doppler in order to determine the resistance index. Centile charts for gestational age for Scottish singleton births were used to calculate birthweight percentiles (22).

$^1$H MRS acquisition

All MR spectra were acquired using a wide-bore 3-T MR Verio system (Siemens Healthcare Sector, Siemens AG, Erlangen, Germany). To avoid vena cava compression, women were placed in a left lateral tilt, with the blood pressure being constantly monitored using a Veris MR Vital Signs Monitor (Medrad, Newbury, Berkshire, UK). No fetal sedation was used and each participant was limited to spending 45 min in the scanner. Placental location and orientation were confirmed using standard half-Fourier rapid MRI acquisitions and used to spatially select the MRS voxel. No other structural MR scans were acquired. A single-voxel point-resolved spectroscopy (PRESS) technique was applied with TR/TE = 1500 ms/30 ms, 96 signal averages, bandwidth of 2000 Hz and a water suppression bandwidth of 50 Hz. Each spectral acquisition took 2 min 30 s. Signal was received from selected elements of the spine matrix coil and body matrix surface coils positioned to allow adequate coverage of the placenta. Following the acquisition of scout images, the scanner bed was moved to ensure that the placenta was positioned at the isocentre, and great care was taken to position the $20 \times 20 \times 40$-mm$^3$ voxel within the placenta and to avoid any contaminant signal from surrounding tissue. An example of voxel positioning for MRS acquisition is shown in Fig. 1. An optimised semi-automated spectroscopy method was systematically applied until the full width at half-maximum of the water peak was less than 30 Hz. Significant movement of the placenta is not expected during spectral acquisition, and data were acquired with the mother free breathing throughout.

Spectral analysis

All spectral analysis was carried out using jMRUI (23). Water peak amplitudes were first estimated for each spectrum using the advanced method for accurate, robust and efficient spectral fitting (AMARES) a semi-automatic quantification method (24). Analysis of overlapping in utero placental spectral components was then

![Figure 1](http://example.com/figure1.png) An example of the positioning of the $20 \times 20 \times 40$-mm$^3$ voxel on coronal (left) and transverse (right) images prior to placental $^1$H MRS acquisition.
carried out using the Quest algorithm (23,25). The Quest algorithm is a time domain spectral analysis tool which estimates metabolite amplitudes using a non-linear least-squares fit of a weighted combination of simulated metabolite signals to the acquired spectrum. As recommended in the literature (25), the intrinsic water peak was removed using the Hankel Lanczos Singular Values Decomposition Filter (HLSVD) prior to metabolite quantification. For this purpose, a metabolite basis set was generated using the NMR-Scope function also available in JMRUI (25), and included contributions from the overlapping resonances of glutamine and glutamate (Glx) between 2.06 and 2.44 ppm and between 3.76 and 3.78 ppm, from choline (3.2, 3.53 and 4.08 ppm) and fatty acids (1.3 and 0.9 ppm). Separate simulations of glutamine and glutamate were generated and then summed using the JMRUI pre-processing tool to create a simulation of Glx. The Quest algorithm calculates errors associated with the estimated metabolite amplitudes using an extended version of the Cramer–Rao lower bounds calculation (25). The errors for each of the calculated metabolite ratios were derived through error propagation of this JMRUI output. The Quest algorithm carries out a reproducible estimation and subtraction of the baseline, and has been subjected previously to extensive Monte Carlo studies that have validated its reliability with respect to bias and variance (25).

An example of the Quest algorithm output for SGA subject 5 is shown in Fig. 2. This shows the acquired spectrum (Fig. 2a), estimated spectrum (Fig. 2b), simulations of individual metabolites (Fig. 2c) and residue function (Fig. 2d) following the fitting of the estimated spectrum to the acquired spectrum. Glx, choline and both lipid peaks at 1.3 and 0.9 ppm were quantified in terms of ratios to the amplitude of the intrinsic water peak (i.e. Glx/H₂O, choline/H₂O, lipid 1.3 ppm/H₂O and lipid 0.9 ppm/H₂O).

**Statistical tests**

The null hypotheses are that no differences exist between the following metabolite ratios of matched SGA and control placentae: (i) Glx/H₂O; (ii) choline/H₂O; (iii) lipid contribution at 1.3 ppm (lipid 1.3 ppm/H₂O); and (iv) lipid contribution at 0.9 ppm (lipid 0.9 ppm/H₂O). Not all metabolite ratio differences conformed to a normal distribution. Therefore, separate Wilcoxon signed rank tests were carried out on paired differences of each of the four metabolite amplitude ratios using Minitab® 16 Statistical Software (Minitab, Inc.; www.minitab.com). Significance was defined as \( p < 0.05 \).

**RESULTS**

**Demographics of study population**

The mean maternal age range was 28.5 ± 7.6 years and the mean maternal body mass index (BMI) was 23.3 ± 6.0 kg/m². The maternal demographics and population characteristics at delivery are shown in Table 1.

**Perinatal outcome**

Perinatal outcomes are shown in Table 2. All SGA subjects were confirmed by a birthweight of <10th centile. Five of the nine SGA subjects delivered preterm (defined as <37 weeks of gestation), whereas all controls delivered at term. All controls and seven of nine SGA subjects were discharged home alive and well. One SGA subject was stillborn and one had a neonatal death following surgery for gastroschisis. Three SGA subjects were admitted to the neonatal unit (NNU), two following surgery for gastroschisis and one as a result of low temperature and hypoglycaemia secondary to low birthweight. Two controls were admitted to the NNU, one because of suspected sepsis and the other following an unplanned home delivery. Other than the two SGA subjects with gastroschisis, all other controls and subjects had no genetic or structural abnormalities.

**¹H MRS**

*In utero* ¹H MRS of the placenta was obtained in all nine SGA pregnancies and in all nine healthy control subjects. The spectrum acquired from control 6 is shown in Fig. 3. In Fig. 3 (left), the water peak is dominant at 4.7 ppm and, in Fig. 3 (right), the water peak has been filtered out to reveal the smaller amplitude metabolite peaks. Spectral peaks corresponding to choline, Glx and lipids are indicated. The amplitude ratios of choline, Glx and the lipid peaks at 1.3 and 0.9 ppm to the intrinsic water peak at 4.7 ppm (i.e. choline/H₂O, Glx/H₂O, lipid 1.3 ppm/H₂O, lipid 0.9 ppm/H₂O) are shown in Table 3.

No significant difference in paired SGA–control placental choline/H₂O ratios was found (\( p = 0.722 \)). This suggests that placental choline metabolism is preserved in this SGA cohort. A significant difference (\( p = 0.024 \)) was found between paired SGA–control placental Glx/H₂O ratios, suggesting that Glx metabolism is altered in this cohort. Finally, the amplitude ratios of lipid 1.3 ppm/H₂O and lipid 0.9 ppm/H₂O did not demonstrate a significant difference between matched SGA–control pairs (\( p = 0.813 \) and \( p = 0.058 \), respectively).
Table 1. Maternal demographics

| Subject | Gestational age at \(^1\text{H MRS (weeks + days)}\) | Smoker (Yes/No) | Age (years) | Parity | BMI (kg/m\(^2\)) | Abdominal circumference ultrasound centile | Umbilical artery flow | Liquor volume |
|---------|----------------------------------|-----------------|-------------|--------|----------------|------------------------------------------|----------------------|------------|
| SGA 1   | 33\(^{+3}\)                      | No              | 36          | 1 + 0  | 19            | 5th                                      | Normal               | Reduced    |
| Healthy 1 | 33\(^{+3}\)                  | No              | 33          | 0 + 0  | 24.2          | 25th–50th                               | Normal               | Normal     |
| SGA 2   | 33\(^{+2}\)                      | Yes             | 32          | 1 + 0  | 18.3          | <5th                                    | Normal               | Normal     |
| Healthy 2 | 33\(^{+5}\)                   | Yes             | 18          | 0 + 0  | 18.7          | 50th–95th                               | Normal               | Normal     |
| SGA 3   | 29\(^{+2}\)                      | Yes             | 26          | 2 + 3  | 23.6          | 5th                                    | Normal               | Reduced    |
| Healthy 3 | 29\(^{+3}\)                  | Yes             | 20          | 0 + 0  | 19.5          | 75th                                    | Normal               | Normal     |
| SGA 4   | 26\(^{+0}\)                      | No              | 41          | 0 + 0  | 39.7          | <5th                                    | NORMAL                | Normal     |
| Healthy 4 | 26\(^{+6}\)                  | No              | 24          | 1 + 1  | 30.5          | 75th                                    | Normal               | Normal     |
| SGA 5   | 36\(^{+6}\)                      | No              | 26          | 1 + 1  | 23.1          | 5th                                    | Normal               | Normal     |
| Healthy 5 | 36\(^{+1}\)                   | No              | 38          | 0 + 2  | 18.8          | 95th                                    | Normal               | Normal     |
| SGA 6   | 31\(^{+4}\)                      | No              | 27          | 0 + 0  | 19.8          | 5th–10th                                | Normal               | Normal     |
| Healthy 6 | 32\(^{+0}\)                  | No              | 39          | 2 + 0  | 20.9          | 95th                                    | Normal               | Normal     |
| SGA 7   | 37\(^{+3}\)                      | Yes             | 28          | 2 + 1  | 30.2          | <5th                                    | Raised               | Normal     |
| Healthy 7 | 38\(^{+3}\)                  | Yes             | 22          | 2 + 0  | 23.7          | 25th                                    | Normal               | Normal     |
| SGA 8   | 33\(^{+5}\)                      | No              | 18          | 0 + 0  | 19.2          | <5th                                    | Normal               | Normal     |
| Healthy 8 | 33\(^{+3}\)                  | No              | 28          | 1 + 0  | 20.7          | 95th                                    | Normal               | Normal     |
| SGA 9   | 28\(^{+5}\)                      | No              | 33          | 0 + 0  | 23.0          | 5th–10th                                | Raised               | Normal     |
| Healthy 9 | 29\(^{+2}\)                  | No              | 35          | 1 + 1  | 20.3          | 95th                                    | Normal               | Normal     |

REDF, reversed end-diastolic flow.

**DISCUSSION**

To our knowledge, this is the first in utero placental \(^1\text{H MRS study to assess choline and lipid using the internal water peak as an independent reference and to additionally assess placental glutamine and glutamate as it supports the developing fetus. This study showed that the Glx/H\(_2\)O ratios of the SGA placenta and gestation-matched healthy control placentae were significantly different, but that their choline/H\(_2\)O and lipid/H\(_2\)O ratios were not. This suggests that, in this cohort of SGA placentae, the metabolism of Glx is altered, but that choline and lipid metabolism is preserved.**

The finding that placental Glx is significantly reduced in a cohort of nine SGA subjects is consistent with other studies in the literature. First, there are general reports of a reduction in most essential amino acid concentrations in the SGA fetus prior to delivery (26,27). There are also reports of a reduction in the amino acid transport mechanisms of the placenta in SGA pregnancies (18,19). More specifically, in an ovine fetal growth-restricted model, Regnault et al. (28) found that there was a significant reduction in the umbilical uptake of glutamine and in the placental uptake of fetal glutamate. Hill et al. (29) identified three different transport mechanisms in vitro that allow glutamine uptake across the microvillous plasma membrane (MVM) of the placenta. They reported that the majority of Na\(^+\)-dependent and Na\(^+\)-independent transport of glutamine across the MVM was mediated by mechanisms that are known to be reduced in fetal growth restriction (FGR) pregnancies, a severely compromised subset of SGA pregnancies.

As described previously (7), the mother, placenta and fetus all form part of a delicate glutamine–glutamate shuttle. Glutamine is transported across the placenta from the mother and is also manufactured in the placenta (6–8) using glutamate which is synthesised in the fetal liver. Therefore, any disruption in the supply or transport mechanism of either amino acid from any one source could potentially disrupt this fine balance of placental Glx metabolism. It is known from studies in the porcine placenta (6) that both glutamine and glutamate vary with gestational age. However, by limiting analysis to comparisons of SGA–control pairs matched to gestational age, the influence of gestation on glutamine and glutamate is removed.

The Glx/H\(_2\)O ratio was found to be reduced in seven of the SGA subjects (Table 3) compared with their matched controls, but was increased in two subjects (SGA subjects 4 and 7) compared with their matched controls. These subjects also had reversed end-diastolic flow (REDF) and raised flow in their umbilical artery, respectively. It is beyond the scope of this article to make conclusions regarding clinical outcomes, but it should be noted that SGA subject 4 was stillborn 10 days after MRS acquisition. Therefore, it is likely that placental metabolism was significantly altered in this case. Regnault et al. (28) reported that, even in the most severe SGA cases in which FGR is confirmed, the fetus is not consistently in a state of constant hypoxia, and so may have access to higher concentrations of amino acids at different times. Their work indicates that, in severe SGA with FGR, there is a reduction in placental O\(_2\) transport which reduces fetal metabolism. They suggest that this reduction in metabolic rate may be greater than the reduction in placental amino transport capacity, leading to normal or higher than normal amino acid concentrations. It is possible that this mechanism may account for the relative increase in Glx/H\(_2\)O ratios in SGA subjects 4 and 7 compared with their respective control subjects.

This study found that there were no significant differences in choline/H\(_2\)O ratios between SGA placentae and matched control placentae. Choline is vital for normal brain development, including the prevention of neural tube defects (3). Choline passes from mother to fetus via the placenta against a concentration gradient, and both fetal tissue and amniotic fluid have very high choline concentrations compared with maternal levels (3). Despite this, there is evidence that reproductive age women are relatively resistant to choline deficiency as a result of an oestrogen–

wileyonlinelibrary.com/journal/nbm Copyright © 2015 John Wiley & Sons, Ltd. NMR Biomed. 2015; 28: 1275–1282
Table 2. Perinatal outcome

| Subject | Time between MRI and delivery (days) | Gestational age at delivery (weeks + days) | Mode of delivery | Sex | Birthweight (g) | Percentile at birth | Outcome |
|---------|--------------------------------------|------------------------------------------|------------------|-----|-----------------|---------------------|---------|
| SGA 1   | 46                                   | 40 +1                                    | SVD              | Female | 2610            | <2nd                 | Alive and well NNU admission for 5 days with suspected sepsis. Alive and well Gastrochisis, surgery, neonatal death 11 days of age |
| Healthy 1 | 44                                     | 39 +6                                    | HFFD             | Male  | 2960            | 9th–25th             | Alive and well |
| SGA 2   | 23                                   | 36 +4                                    | SVD              | Male  | 1610            | <2nd                 | NNU admission for 2 days with RDS following unplanned home delivery. Alive and well Gastrochisis surgery, neonatal death 11 days of age |
| Healthy 2 | 45                                     | 40 +1                                    | SVD              | Male  | 3420            | 25th–50th            | Alive and well |
| SGA 3   | 44                                   | 35 +4                                    | EmCS             | Female | 1690            | 2nd                  | NNU admission for 5 days with low temperature and hypoglycaemia. Alive and well Gastrochisis surgery, alive and well |
| Healthy 3 | 83                                     | 41 +1                                    | SVD              | Female | 3640            | 50th–75th            | Alive and well Stillbirth 10 days after MRI scan |
| SGA 4   | 10                                   | 27 +3                                    | EmCS             | Female | 500             | <2nd                 | Alive and well |
| Healthy 4 | 102                                    | 40 +3                                    | SVD              | Female | 3650            | 50th–75th            | Alive and well |
| SGA 5   | 2                                    | 37 +10                                   | EmCS             | Female | 1840            | <2nd                 | Alive and well |
| Healthy 5 | 24                                     | 39 +4                                    | SVD              | Male  | 4020            | 91st                 | Alive and well |
| SGA 6   | 40                                   | 37 +2                                    | EmCS             | Female | 1710            | <2nd                 | NNU admission for 5 days with low temperature and hypoglycaemia. Alive and well Gastrochisis surgery, alive and well |
| Healthy 6 | 53                                     | 39 +4                                    | SVD              | Female | 3670            | 75th–91st            | Alive and well |
| SGA 7   | 4                                    | 38 +1                                   | EmCS             | Female | 1980            | <2nd                 | Alive and well |
| Healthy 7 | 9                                     | 39 +4                                    | SVD              | Male  | 3170            | 25th                 | Alive and well |
| SGA 8   | 3                                    | 34 +1                                    | EmCS             | Male  | 1670            | <9th                 | Alive and well |
| Healthy 8 | 56                                     | 41 +3                                    | SVD              | Female | 3500            | 25th–50th            | Alive and well |
| SGA 9   | 42                                   | 34 +5                                    | EmCS             | Male  | 1570            | <2nd                 | Admitted to NNU, RDS for 21 days. Alive and well |
| Healthy 9 | 81                                     | 39 +6                                    | SVD              | Male  | 3890            | 75th–91st            | Alive and well |

EmCS, emergency caesarean section; HFFD, Haag Ferguson forceps delivery; NNU, neonatal unit; RDS, respiratory distress; SVD, spontaneous vertex delivery.

Figure 3. $^1$H MR placental spectra acquired in utero from a healthy control (subject 6). Left: the water peak is shown at 4.7 ppm. Right: the water peak has been removed in preparation for the subsequent spectral analysis of other metabolites. Spectral contributions from Glx, choline and lipid are indicated. Glx, combined spectral contribution for glutamine and glutamate.
Table 3. Placenta amplitude ratios of Glx/H$_2$O, choline/H$_2$O, lipid 1.3 ppm/H$_2$O and lipid 0.9 ppm/H$_2$O for small for gestational age (SGA) and gestation-matched healthy controls quantified from placental $^1$H MR spectra using jMRUI. Standard deviations (SDs), derived through error propagation of those estimated by jMRUI, are also quoted. H$_2$O refers to the intrinsic water peak of each individual spectrum observed at 4.7 ppm. N/A indicates that the metabolite was not detected and * indicates that the amplitude ratio for the SGA placenta is higher than that of the matched control. Wilcoxon signed rank test $p$ values (significance < 0.05) are quoted for SGA–healthy subject comparisons for each ratio.

| Metabolite amplitude ratios ± SD | Glx/H$_2$O ($\times 10^{-3}$) | Choline/H$_2$O ($\times 10^{-3}$) |
|---------------------------------|-----------------------------|----------------------------------|
| **Glx/H$_2$O**                  | SGA                         | Healthy                          | SGA                             | Healthy                          |
| SGA 1/Healthy 1                 | 1.42 ± 0.02                 | 2.85 ± 0.04                      | 0.50 ± 0.01                     | 0.80 ± 0.08                      |
| SGA 2/Healthy 2                 | 0.68 ± 0.02                 | 1.83 ± 0.05                      | 0.18 ± 0.05                     | 0.31 ± 0.13                      |
| SGA 3/Healthy 3                 | 0.47 ± 0.01                 | 3.66 ± 0.04                      | 0.52 ± 0.01                     | 0.69 ± 0.10                      |
| SGA 4/Healthy 4                 | 2.67 ± 0.03*                | 2.38 ± 0.06                      | 0.63 ± 0.02*                    | 0.36 ± 0.10                      |
| SGA 5/Healthy 5                 | 6.48 ± 0.07                 | 9.76 ± 0.10                      | 1.36 ± 0.17*                    | 0.81 ± 0.18                      |
| SGA 6/Healthy 6                 | 0.89 ± 0.03                 | 3.08 ± 0.06                      | 0.53 ± 0.11                     | 0.83 ± 0.04                      |
| SGA 7/Healthy 7                 | 2.44 ± 0.09*                | 1.38 ± 0.03                      | 0.83 ± 0.14*                    | 0.38 ± 0.08                      |
| SGA 8/Healthy 8                 | 0.28 ± 0.08                 | 2.12 ± 0.04                      | 0.61 ± 0.22                     | 0.74 ± 0.03                      |
| SGA 9/Healthy 9                 | 4.00 ± 0.08                 | 5.39 ± 0.06                      | 1.07 ± 0.22                     | 0.90 ± 0.03                      |
| **p value**                     |                             | 0.024                            | 0.722                           |
| **Lipid 1.3 ppm/H$_2$O**        | SGA                         | Healthy                          | SGA                             | Healthy                          |
| SGA 1/Healthy 1                 | 3.27 ± 0.41                 | 7.59 ± 0.53                      | 3.84 ± 0.10                     | 5.43 ± 0.25                      |
| SGA 2/Healthy 2                 | 0.96 ± 0.27                 | 8.17 ± 0.59                      | 0.44 ± 0.08                     | 5.99 ± 0.23                      |
| SGA 3/Healthy 3                 | 3.50 ± 0.37                 | 8.51 ± 0.68                      | 3.87 ± 0.08                     | 5.08 ± 0.20                      |
| SGA 4/Healthy 4                 | 0.68 ± 0.39                 | 2.78 ± 0.63                      | 2.52 ± 0.15                     | 2.61 ± 0.88                      |
| SGA 5/Healthy 5                 | 44.9 ± 1.35*                | 16.5 ± 1.34                      | 11.9 ± 0.37                     | 36.6 ± 1.37                      |
| SGA 6/Healthy 6                 | 5.69 ± 0.74                 | 15.1 ± 0.91                      | 5.63 ± 0.19                     | 7.69 ± 0.22                      |
| SGA 7/Healthy 7                 | 43.8 ± 1.46*                | 6.83 ± 0.40                      | 6.70 ± 0.48*                    | 2.54 ± 0.13                      |
| SGA 8/Healthy 8                 | 4.62 ± 1.58                 | 4.32 ± 0.62                      | N/A                             | 3.33 ± 0.20                      |
| SGA 9/Healthy 9                 | 23.1 ± 1.82*                | 8.72 ± 0.87                      | 2.94 ± 0.34                     | 9.83 ± 0.29                      |
| **p value**                     |                             | 0.813                            | 0.058                           |

In the liver (30,31). The importance of choline to fetal neurodevelopment and the different reserves that the mother can use to supply choline to the fetus could perhaps explain the preserved choline levels in SGA placenta compared with controls found in this study.

Individual SGA subjects 4, 5 and 7 showed increased choline compared with their matched control subjects. As discussed above, SGA subjects 4 and 7 had REDF and raised flow in the umbilical artery, respectively. Subject 5 also had raised umbilical artery flow. This anecdotal finding is consistent with previous larger studies (31,32) which measured choline concentrations in venous umbilical cord blood samples in SGA and normal pregnancies. Venous blood samples reflect maternal choline concentrations, maternal–placental choline transfer and placental choline metabolism, as opposed to arterial blood samples that reflect fetal metabolism (31). Although both studies hypothesised that a status of SGA and subsequent low birthweight would result in low choline levels, in fact they observed high choline concentrations in the umbilical cord blood samples of these neonates. Hogeveen et al. (31) have suggested two reasons for this. The first is that high choline concentrations reflect low choline consumption by the fetus, and the second is the potential that slow fetal growth triggers an increased placental transport of choline.

Assessment of the lipid 1.3 ppm/H$_2$O ratios, the dominant lipid peak, of individual SGA–control pairs showed that there was an increase for SGA subjects 5, 7 and 9 compared with their control subjects. These subjects all had raised umbilical artery Doppler ultrasound measurements. Interestingly, two of these SGA subjects also had increased placental choline/H$_2$O ratios. Choline is a precursor for the phospholipid phosphatidylcholine, a main constituent of membranes, lipoproteins, bile and surfactants (33). Therefore, it is possible that placental choline and lipid metabolism are related.

A previous study (34) has demonstrated a reduction in the placental choline/lipid ratio using in vivo $^1$H MRS, acquired with TE = 144 ms, in three pregnancies complicated by severe SGA with FGR. All subjects in that study showed reduced liquor volume and absent end-diastolic flow in the umbilical artery. That study speculated that this may be a biomarker of critical placental failure indicated by reduced cell turnover. These subjects were recruited as their placental function was known to be severely compromised and were confirmed to be suffering from placental-mediated FGR. Conversely, the present study concerns pregnancies not confirmed to be suffering from FGR. Spectra were acquired using a lower TE of 30 ms, allowing the detection of Glx in addition to choline and lipid. Significant differences were not detected between the choline/lipid 1.3 ppm ratios (Wilcoxon signed rank test $p = 0.286$; level of significance $p < 0.05$) or the choline/lipid 0.9 ppm ratios ($p = 0.155$) of SGA placentae and those of matched controls (data not shown). This was expected following confirmation that no significant differences were observed in choline/H$_2$O ratios or either of the lipid/H$_2$O ratios between these groups. It was also expected that,
as these two studies considered subjects with different clinical characteristics, their choline and lipid metabolism would be different. In the future, it would be of interest to assess placental Glx/H2O ratios in known cases of severe FGR.

McKelvey and Kay (35) carried out a review of MRS studies of the placenta. Of these studies, only two applied 1H MRS to study ex vivo placental tissue samples. Serkova et al. (36) calculated absolute placental concentrations of major cellular metabolites, including glutamine, glutamate, phosphocholine, glycerophosphocholine and lipids, in extracts of normal placenta collected at different time points following delivery. Pulkkinen et al. (37) found that normal ex vivo placental samples had higher phospholipid content than normal myometrium tissue samples. The only in vivo study reported was by Weindling et al. (38), who acquired 31P MR spectra on a 1.5-T scanner from an anteriorly placed placenta in seven women with normal pregnancies. Therefore, there is currently no opportunity to directly compare the results from the present study with any other published data.

We accept that the acquired in utero placental spectra may contain overlapping contributions from different metabolites. Therefore, it is to be expected that spectral resolution will be lower than is possible in ex vivo studies. Previous ex vivo and in vivo studies have attributed metabolite peaks detected at approximately 2 ppm to Glx (39,40). However, one possible contaminant may simply be polysaturated fatty acids (41). In addition, Takeuchi et al. (42) suggested that a peak detected at approximately 2 ppm in a 1H MRS study of ovarian tumours could be attributed to N-acetyl mucinous compounds (i.e. those containing the glycosylated protein mucin). There is evidence in the literature that mucin-15 is expressed in the human placenta to varying degrees at different stages of gestation (43), but equally there is evidence that glutamine is present in ovarian tumours (44). Takeuchi et al. (42) conceded that their results also suggest contamination from other sources. The use of a powerful spectral analysis tool, such as the Quest algorithm, is imperative to allow contributions from overlapping spectral metabolites to be quantified.

This study is limited by its small sample size and heterogeneity in demographics and clinical characteristics of the study participants. There is no evidence that factors such as maternal age, BMI and parity can influence the results. All but one of the control subjects had an abdominal circumference above the 50th centile. Although control subjects were not selectively recruited to have abdominal circumferences above the 50th centile, this may potentially influence the observed placental metabolite differences between SGA and control subjects. Two of the fetuses had gastrochisis. There is evidence that placental dysfunction is an important causative factor in cases of SGA with gastrochisis (45). However, we accept that other factors will also play a role in causing SGA in fetuses with gastrochisis, and that this may be a potential limitation in the SGA group. Four SGA subjects had abnormal umbilical artery flow measurements. It is known that umbilical artery Doppler ultrasound measurements are often normal in the SGA fetus (46,47). This study did not aim to and was not powered to detect differences in placental metabolites in SGA pregnancies with and without evidence of abnormal Doppler measurements. However, in future studies, it would be preferable to subclassify subjects into groups of normal and abnormal Doppler measurements. The application of 1H MRS to study the in utero placenta requires specialist knowledge and so may not be immediately transferrable to all centres or all patients. Future studies would benefit from the acquisition of placental volume scans, which could then be assessed for correlation with metabolite ratios.

### CONCLUSION

This study suggests that 1H MRS can be used to detect placental metabolites in utero. We have demonstrated an ability to detect significant differences in Glx in the placenta of SGA pregnancies in utero, and have shown that choline and lipid levels are maintained in these pregnancies. Although these findings cannot currently be used to inform clinical decisions, these encouraging initial results indicate that 1H MRS warrants further investigation in the assessment of in utero placental function. This is a new application of an established technique and, in the first instance, further tests in larger populations are required to confirm these results.

### Acknowledgements

We would like to acknowledge funding support from Action Medical Research (ref: SP4626) and Tommy's (ref: 1060508). We would also like to thank the radiographers at the Clinical Research Imaging Centre (University of Edinburgh, Edinburgh, UK) for facilitating and performing the MRI scans. Finally, we would like to thank Dr Rob Elton (Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK) for his statistical advice during the course of this study.

### REFERENCES

1. Heazell H. Abnormalities of the placenta. BMC Pregnancy Childbirth 2012; 12(Suppl. 1): A2.
2. Guttmacher AE, Maddox YT, Spong CY. The human placenta project: placental structure, development and function in real time. Placenta 2014; 35: 303–304.
3. Ziesel SH. Choline: critical role during fetal development and dietary requirements in adults. Annu. Rev. Nutr. 2006; 26: 229–250.
4. McNamley T, Woods J. Placental Physiology. Global Library of Women's Medicine (ISSN: 1756–2228) 2008; DOI 10.3843/GLWOM.10195.
5. Herrera E, Amusquivar E, Lopez-Soldado I, Ortega H. Maternal lipid metabolism and placental lipid transfer. Horm. Res. 2006; 65(Suppl. 3): 59–64.
6. Self JT, Spencer TE, Johnson GA, Hu J, Bazer FW, Wu G. Glutamine synthesis in the developing porcine placenta. Biol. Reprod. 2004; 70: 1444–1451.
7. Neu J, Shenyi V, Chakraborti R. Glutamine nutrition and metabolism: where do we go from here? FASEB J. 1996; 10: 829–837.
8. Day PE, Cleal JK, Lofthouse EM, Goss V, Koster G, Postle A, Jackson JM, Hanson MA, Jackson AA, Lewis RM. Partitioning of glutamine synthesised by the isolated perfused human placenta between the maternal and fetal circulations. Placenta, 2013; 34: 1223–1231.
9. Robson RC, Martin WL, Morris RK. The Investigation and Management of the Small-for-Gestational-Age Fetus. Royal College of Obstetricians and Gynaecologists Green-top Guideline No. 31, 2nd edn. Royal College of Obstetricians and Gynaecologists: London; 2013.
10. Walkinshaw S, Cameron H, MacPhail S, Robson S. The prediction of fetal compromise and acidosis by biological profile scoring in the small for gestational age fetus. J. Perinat. Med. 1992; 20: 227–232.
11. Grivell RM, Alfirevic Z, Gyte GM, Devane D. Antenatal cardiotocography for fetal assessment Cochrane Database. Syst Rev. 2010; 1: CD007863.
12. Naban AN, Abdelmoula YA. Amniotic fluid index versus single deepest vertical pocket as a screening test for preventing adverse pregnancy outcome Cochrane Database. Syst Rev. 2008; 3: CD006593.
13. Morris K. Shershad Syed: improving maternal care in Pakistan. The Lancet 2011; 377: 1309.
