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Novel Use of Proton Magnetic Resonance Spectroscopy (1HMRS) to Non-Invasively Assess Placental Metabolism

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Abstract

Background: Placental insufficiency is a major cause of antepartum stillbirth and fetal growth restriction (FGR). In affected pregnancies, delivery is expedited when the risks of ongoing pregnancy outweigh those of prematurity. Current tests are unable to assess placental function and determine optimal timing for delivery. An accurate, non-invasive test that clearly defines the failing placenta would address a major unmet clinical need. Proton magnetic resonance spectroscopy (1H MRS) can be used to assess the metabolic profile of tissue in-vivo. In FGR pregnancies, a reduction in N-acetylaspartate (NAA)/choline ratio and detection of lactate methyl are emerging as biomarkers of impaired neuronal metabolism and fetal hypoxia, respectively. However, fetal brain hypoxia is a late and sometimes fatal event in placental compromise, limiting clinical utility of brain 1H MRS to prevent stillbirth. We hypothesised that abnormal placental 1H MRS may be an earlier biomarker of intrauterine hypoxia, affording the opportunity to optimise timing of delivery in at-risk fetuses.

Methods and Findings: We recruited three women with severe placental insufficiency/FGR and three matched controls. Using a 3T MR system and a combination of phased-array coils, a 20×20×40 mm1H MRS voxel was selected along the ‘long-axis’ of the placenta with saturation bands placed around the voxel to prevent contaminant signals. A significant choline peak (choline/lipid ratio 1.35–1.79) was detected in all healthy placentae. In contrast, in pregnancies complicated by FGR, the choline/lipid ratio was ≤0.02 in all placentae, despite preservation of the lipid peak (p<0.001).

Conclusions: This novel proof-of-concept study suggests that in severe placental insufficiency/FGR, the observed 60-fold reduction in the choline/lipid ratio by 1H MRS may represent an early biomarker of critical placental insufficiency. Further studies will determine performance of this test and the potential role of 1H-MRS in the in-vivo assessment of placental function to inform timing of delivery.

Introduction

Placental insufficiency is one of the commonest causes of fetal growth restriction (FGR) and antepartum stillbirth. When placental insufficiency is diagnosed antenatally, the only effective treatment is delivery which, if preterm is itself associated with increased morbidity and mortality and considerable financial costs. [1] If placental insufficiency remains undiagnosed and results in stillbirth [2], this can have profound and long lasting consequences for parents and their extended family. [3] One of the main challenges in current obstetric practice is therefore our inability to accurately and non invasively diagnose placental insufficiency, quantify its severity and predict its clinical sequelae. Better diagnosis would improve the timing of clinical interventions and potentially improve perinatal outcome.

In current clinical practice, diagnosis of placental insufficiency and fetal compromise is largely based on Doppler assessment of umbilical artery blood flow, fetal arterial and venous Dopplers (e.g. ductus venosus and middle cerebral artery Doppler waveforms) or ultrasound biometry. [4] Although abnormal Dopplers correlate with cord pH, fetal hypoxia and lactate generation [5], and are associated with the presence of gross placental lesions detectable by ultrasound [6,7], neither Doppler nor conventional ultrasound is able to directly measure placental function. Sibley et al [8] recently proposed that the constellation of physiological and morphological changes may constitute a placental phenotype in some pregnancies complicated with FGR with an abnormal phenotype being associated with poor perinatal outcome. Development of non-invasive tools capable of assessing placental metabolism and cell turnover directly may allow placental phenotyping to occur, thus enabling clinical interventions to be targeted to those pregnancies at highest risk of adverse outcome and timely delivery to be effected.

Magnetic resonance imaging (MRI) is a non-invasive imaging technique which is safe in pregnancy. The technique has the ability to acquire a combination of anatomical information with high spatial resolution, and directly assess a variety of physiological function. MRI is non-invasive and does not involve the use of...
We studied 3 women with a singleton pregnancy complicated
by severe FGR and suspected fetal compromise and 3 gestation
matched controls. Gestation was calculated from the last
menstrual period and confirmed by routine ultrasonography at
11–13 weeks gestation. All participants had a structurally normal
fetal anomaly scan at 20 weeks gestation. Severe FGR was defined
as an abdominal circumference by ultrasound <3rd centile. [15]
Suspected fetal compromise was defined as absent or reversed end-
diastolic flow on umbilical artery Doppler. Exclusion criteria for
study participation included significant co-existing maternal
systemic disease including gestational diabetes, microvascular
disease, multiple pregnancy, or contraindication to MRI.

Magnetic Resonance Studies
All magnetic resonance (MR) studies were performed using a
wide-bore dedicated clinical research 3 tesla MR Verio system
(Siemens Medical, Germany). Women were scanned in a left-
lateral tilt to avoid compression of veno-cava with blood pressure
constantly monitored using a Veris MR Vital Signs Monitor
(Medrad, UK). Total MR acquisition times were limited to 40–
45 minutes per participant. No fetal sedation was used. A
combination of body and spine matrix phased-array coils was
used to obtain all images and 1H MRS data. Prior to acquisition of
1H MRS data, a series of 2D HASTE slices was acquired in three
orthogonal planes centred on the placenta. Multiple 6 mm slices
were acquired with no inter-slice gap in all three planes, in order
to localise the extent of the placenta. Each HASTE image took
approximately 1 second to acquire, so all images were acquired
with the mother free-breathing.

MR Spectroscopy Studies
For 1H MRS acquisition, a 20×20×40 mm PRESS voxel was
selected along the ‘long-axis’ of the placenta (TE/TR 144/
1500 ms, 96 averages). The voxel was selected approximately 2 cm from the cord insertion in all cases and voxel selection was
confirmed to be limited to within the placenta using the range of
orthogonal HASTE slices (Figure 1). Six saturation bands were
placed around the voxel to further prevent any non-placenta
contaminant signals and a second-order semi-automatic shim was
applied over the selected voxel to counter any local inhomogene-
ities in the magnetic field. The voxel dimensions were selected to
maximise sampling of the placental unit, thereby increasing signal-
to-noise values of the resulting spectra. 1H MRS data was acquired
with the mother free-breathing. In our experience, the placental
unit does not significantly move outwith our selected voxel
dimensions during either maternal breathing, or fetal motion. The
resulting raw spectral data was exported to an external workstation
and MRS analysis to assess placental metabolism (Lipid/choline
ratio) was quantified using the Java-based MRS analysis tool
JMRUI (http://www.mrui.uab.es/mrui).

Data Analysis
The 1H MRS quantification process was performed using the
nonlinear least-squares quantitation algorithm AMARES [A-
vanced Method for Accurate, Robust and Efficient Spectral fitting]
with peak fitting performed assuming a Lorentzian line shape.
Since only two peaks were clearly identified, each peak was
identified manually according to its frequency and the line widths
and areas under the curves semi-automatically estimated. Birth
percentiles were calculated using centile charts for birthweight for
gestational age for Scottish singleton births. [16] Data were
analysed by GraphPad Prism (Version 5.0).

Methods

Ethics Statement
The study was approved by Lothian Research Ethics Commit-
tee (10/S1105/36) 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 imaging (MRI) studies were performed at the Clinical
Research Imaging Centre in the Queen’s Medical Research
Institute, University of Edinburgh, Edinburgh, UK.
Results

Demographics of Study Population

Maternal age ranged between 20 and 37 (mean, 28 ± 6.8 years) and maternal body mass index (BMI) between 18.4 and 32.8 (mean, 25.7 ± 4.8 kg/m²). The demographics of the study population at the time of MRS scan and delivery are demonstrated in Tables 1 and 2. Neonatal outcome and time between 1H MRS and delivery are demonstrated in Table 3.

MRI Results

In utero 1H MRS of the placenta was obtained in all participants. A choline and lipid peak were easily detectable, centred at 3.2 ppm and 1.2 ppm, respectively from placentae in all healthy controls (Figure 2). In the healthy controls a significant choline signal was obtained, resulting in a choline/lipid ratio of 1.35–1.79. In contrast, despite preservation of the lipid peak, there was severe attenuation or absence of detectable choline peak in placentae from pregnancies complicated by severe FGR and suspected fetal compromise (Figure 3). The choline/lipid ratio was reduced to ≤0.02, a reduction of more than 60-fold in pregnancies

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Parity</th>
<th>BMI</th>
<th>Umbilical artery</th>
<th>Liquor volume</th>
<th>Gestational age at 1H MRS (wks ± days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy 1</td>
<td>33</td>
<td>0±0</td>
<td>18.4</td>
<td>25th–50th</td>
<td>Normal</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>Healthy 2</td>
<td>31</td>
<td>1±1</td>
<td>23.1</td>
<td>50th–95th</td>
<td>Normal</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Healthy 3</td>
<td>37</td>
<td>2±1</td>
<td>25.3</td>
<td>50th–95th</td>
<td>Normal</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Compromised 1</td>
<td>30</td>
<td>0±0</td>
<td>23.1</td>
<td>&lt;5th</td>
<td>AEDF</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Compromised 2</td>
<td>20</td>
<td>0±1</td>
<td>26.4</td>
<td>&lt;5th</td>
<td>AEDF</td>
<td>25 ± 0</td>
</tr>
<tr>
<td>Compromised 3</td>
<td>23</td>
<td>0±1</td>
<td>32.8</td>
<td>&lt;5th</td>
<td>AEDF</td>
<td>27 ± 1</td>
</tr>
</tbody>
</table>

AEDF (absent end diastolic flow).

Table 1. Maternal demographics, gestational age and antenatal Dopplers at the time of 1H MRS.
complicated by severe FGR compared to the gestation matched healthy controls (Table 3) (p < 0.001).

Discussion

To our knowledge, this is the first report of placental 1H MRS in vivo in normal and FGR pregnancies. We demonstrate that in healthy pregnancies, choline and lipid spectral peaks were clearly detected in all placentae using 1H MRS. In contrast, in pregnancies complicated by FGR, despite preservation of the lipid peak, the choline peak was severely attenuated or absent from all placentae. We speculate that a reduction in the choline/lipid ratio by 1H MRS may provide a novel biomarker of critical placental failure, indicative of reduction in cell turnover, which predates fetal hypoxia and antepartum stillbirth in pregnancies with severe FGR.

To date, knowledge about placental function in FGR pregnancies has been largely extrapolated from in vitro and ex vivo studies. Placental weight, total volume, villous volumes and surface area are significantly reduced in FGR pregnancies. [17] At a histological level, there is evidence of increased apoptosis, a thickened basal lamina and reduction in cytotrophoblastic nuclei and cell-turnover. [14,18] The marked impairment of nutrient transport [13,19] and placental perfusion which occurs results in global placental dysfunction and altered metabolism [20,21]. Cetin et al suggested that such alterations in placental metabolism and function may precede the onset of placental and intrauterine hypoxia in affected pregnancies. [13] If it were possible to detect altered placental metabolism prior to the onset of critical placental failure, this might afford an opportunity for more timely clinical intervention (including delivery) thus preventing adverse perinatal outcome.

A variety of non-invasive methods have attempted to assess placental function in vivo to predict the functional capacity of the pregnancy and/or pregnancy outcome. The ultrasound based ‘Grannum grading’ of placenta, which was originally developed as a biomarker for fetal lung maturity, has been assessed as a predictive tool for fetal growth restriction [22] and placental function [23]. However, although there is a relationship between Grannum III grade and FGR, the positive predictive and sensitivity value of this “test” is low (62% and 66%, respectively) [22] and Grannum grading at 31–34 weeks of gestation is unable to reliably predict the functional capacity of the term placenta as expressed by the surrogate measure, morphometric diffusive conductance. [23] More recently, near infrared spectroscopy has been explored as potential method of assessing tissue oxygenation and placental function. [24–26] To date, results have been conflicting in FGR pregnancies with tissue oxygenation indexes exhibiting both increase and decrease in the presence of FGR depending on its cause. [24–25] Furthermore, due to technical limitations, the latter technique is only able to assess placental oxygenation within a narrow range and in women with an anterior placentae and a thin layer of subcutaneous fat. [24] Until further method development occurs, these techniques are therefore unlikely to have a clinical utility in quantifying placental oxygenation and function in vivo.

Several groups have used MRI as a tool for assessing fetal and placental structure and function in vivo. At the macroscopic level, placental volume measured by MRI during the second trimester

Table 2. Characteristics of population at delivery.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gestational age at delivery, wks±days</th>
<th>Mode delivery</th>
<th>Sex</th>
<th>Fetal weight (g)</th>
<th>Percentile at birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy 1</td>
<td>40±6</td>
<td>SVD</td>
<td>Male</td>
<td>3060</td>
<td>25th</td>
</tr>
<tr>
<td>Healthy 2</td>
<td>40±3</td>
<td>SVD</td>
<td>Male</td>
<td>3760</td>
<td>50th</td>
</tr>
<tr>
<td>Healthy 3</td>
<td>39±5</td>
<td>SVD</td>
<td>Male</td>
<td>3630</td>
<td>50th</td>
</tr>
<tr>
<td>Compromised 1</td>
<td>30±4</td>
<td>SVD</td>
<td>Male</td>
<td>750</td>
<td>&lt;0.4th</td>
</tr>
<tr>
<td>Compromised 2</td>
<td>25±4</td>
<td>EmCS</td>
<td>Male</td>
<td>670</td>
<td>9th</td>
</tr>
<tr>
<td>Compromised 3</td>
<td>28±4</td>
<td>EmCS</td>
<td>Male</td>
<td>530</td>
<td>&lt;0.4th</td>
</tr>
</tbody>
</table>

SVD (spontaneous vertex delivery), EmCS (emergency caesarean section).
doi:10.1371/journal.pone.0042926.t002

Table 3. Neonatal outcome and choline/lipid integral 1H MRS ratio.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Outcome</th>
<th>Time between 1H MRS and delivery (days)</th>
<th>Choline/lipid integral ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy 1</td>
<td>Alive and well</td>
<td>108</td>
<td>1.35</td>
</tr>
<tr>
<td>Healthy 2</td>
<td>Alive and well</td>
<td>70</td>
<td>1.79</td>
</tr>
<tr>
<td>Healthy 3</td>
<td>Alive and well</td>
<td>82</td>
<td>1.36</td>
</tr>
<tr>
<td>Compromised 1</td>
<td>Stillbirth1</td>
<td>13</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Compromised 2</td>
<td>Neonatal death at 42 days</td>
<td>4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Compromised 3</td>
<td>Discharged from NNU with BPD and ROP on supplemental oxygen at 42 weeks corrected gestation</td>
<td>12</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1Compromised 1 pregnancy was expectantly managed until antenatal stillbirth occurred. NNU (neonatal unit), BPD (bronchopulmonary dysplasia), ROP (retinopathy of prematurity).
doi:10.1371/journal.pone.0042926.t003
correlates with uterine artery perfusion and is reduced in pregnancies that subsequently delivered FGR infants. [27] Furthermore, the severity of FGR and incidence of fetal or neonatal mortality has been shown to correlate with the MR volume of placenta affected by pathology. [28] More recently Wright et al demonstrated that placental relaxation times (T1 and T2) were negatively correlated with gestation. [29] However, when the relaxation times were compared to postnatal examination, T2 only correlated with placental fibrin deposition if the scan and delivery were within one week of each other.

Studies using MRI to assess placental function are more limited. Bonel et al report that reduced apparent diffusion coefficient (ADC) as measured by diffusion-weighted MRI is exhibited in placental dysfunction associated with FGR. The authors hypothesised that placenta dysmaturity and focal disruption of the placental barrier which occur in FGR was responsible for the altered diffusion [30]. Using the technique of intravoxel incoherent motion and perfusion fraction mapping, Moore et al identified differences in function within the normal placenta in vivo, and between the placentae of normal and IUGR pregnancies. [31] Finally, using gadoterate melamine for contrast enhancement, Brunelli et al demonstrated that intervillous circulation was severely compromised in pregnancies with severe FGR. [32] However, this study was undertaken only a few hours prior to...
delivery by caesarean section due to concerns about fetal toxicity of gadolinium-based contrast agents, which are not licenced for use in pregnancy. None of these MRI techniques assess placental metabolism and function directly.

$^1$H MRS by comparison to MRI is able to dynamically assess levels of specific metabolites in the region of interest selected. To date, the use of $^1$H MRS during pregnancy has been restricted to assessment of the fetal brain in health and disease. In pregnancies complicated by FGR, a reduction in NAA/choline in the fetal brain is thought to be indicative of impaired neuronal metabolism and reduced cell-turn over [12], and the presence of lactate methyl [33] to be indicative of established fetal hypoxia. However, both of these ‘biomarkers’ are likely to develop relatively late in the evolution of fetal compromise and hypoxia, limiting opportunities for therapeutic intervention.

To our knowledge, $^1$H MRS has not been previously undertaken in the placenta in vivo. We demonstrate that in FGR pregnancies with suspected compromise, despite preservation of the lipid peak, there is a severe reduction or absence of a placental choline peak. This is in contrast to healthy pregnancy where choline and lipid peaks are readily detectable. The presence of choline by $^1$H MRS in organs including the fetal brain is thought to indicate cell-turnover and growth. [34] In contrast, a reduction in the choline peak (compared to baseline) occurs when cell

Figure 3. $2\times2\times4$ cm voxel MRS acquired at 144 ms from placenta from compromised participant 2. Lipid spectral peak demonstrated at frequency of 1.42 ppm. Choline peak below level of reliable detection.
doi:10.1371/journal.pone.0042926.g003
turnover is significantly reduced and in the presence of apoptosis. [35] Using ex vivo and in vitro models, Heazell et al [36] and others [19] [14] have demonstrated a reduction in cell-turnover and increase in apoptosis in placentae from pregnancies with FGR. We therefore propose that the significant reduction in choline/lipid ratio which we demonstrate in FGR, placenta may be a novel biomarker of reduced cell turnover before apoptosis resulting in impaired placental function and critical organ failure.

Acquiring the 1H MRS data at 3T had the added benefit of the increased signal to noise available at this higher clinical field strength. This meant that less averages were required to obtain an acceptable signal-to-noise ratio for our 1H MRS data. Modern clinical 3T systems also allow rapid and accurate magnet shimming to correct for static field inhomogeneities. This meant that an entire MRI and 1H MRS placental examination could be obtained with a 40-minutes total acquisition time.

Although our study is limited by small numbers, we were able to detect a reproducible spectral output from placentae from all the women that were scanned, regardless of placental site and size, fetal motion and maternal habitus. However, for this proof-of-concept study we specifically recruited women with severe FGR who had evidence of fetal compromise and growth measurements <3rd centile. All babies with severe FGR had poor outcomes. To assess the clinical utility of 1H MRS as a diagnostic tool for placental failure, future studies should recruit women with less severe FGR to assess whether there is a lipid/choline ratio below which risk of adverse perinatal outcome increases.

In conclusion, our proof-of-concept study demonstrates that the MRS spectra of placentae in pregnancies complicated by severe FGR are significantly different from those from healthy pregnancies. Future studies should explore whether the absence of a choline peak represents a biomarker of critical placental failure and the consequence of this for perinatal outcome.

Acknowledgments

We would also like to thank Isabel Crawford, Mary Simpson and Jennifer Rowan for recruiting women to take part in this study. Finally, we would like to thank the radiographers at the Clinical Research Imaging Centre for facilitating and performing the MRI scans.

Author Contributions

Conceived and designed the experiments: FCD SIS JEN IM JW. Performed the experiments: FCD SIS IM JW. Analyzed the data: FCD SIS JEN IM. Contributed reagents/materials/analysis tools: FCD SIS JEN IM JW. Wrote the paper: FCD SIS JEN IM JW SJ.

References

