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Brain development in fetuses of mothers with diabetes: a case-control magnetic resonance imaging study

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Abstract

Background and Purpose: Offspring exposed to maternal diabetes are at increased risk of neurocognitive impairment but origins of this are unknown. Using 3 tesla (T) MRI, we investigated the feasibility of comprehensive assessment of brain metabolism (1HMRS), micro- (DWI) and macro-structure (sMRI) in the third trimester fetus in women with diabetes and to determine normal ranges for the MRI parameters measured.

Materials and Methods: Women with singleton pregnancy with diabetes (n=26) and healthy controls (n=26) were recruited prospectively for MRI studies between 34-38 weeks gestation.

Results: Data suitable for post-processing was obtained from 79%, 71% and 46% of women for 1HMRS, DWI and sMRI, respectively. There was no difference in the NAA/Cho and NAA/Cre ratios in the fetal brain in women with diabetes compared to controls (1.74 (0.79) vs 1.79 (0.64) p=0.81, and 0.78 (0.28) vs 0.94 (0.36) p=0.12, respectively) but the Cho/Cre ratio was marginally lower (0.46 (0.11) vs 0.53 (0.10) p=0.04). There was no difference in mean anterior white, posterior white and deep grey matter ADC between cases and controls (1.16 (0.12) vs 1.16 (0.08) p=0.96, 1.54 (0.16) vs 1.59 (0.20) p=0.56 and 1.49 (0.23) vs 1.52 (0.23) p=0.89, respectively) or volume of the cerebrum (cc^3) (243.0 (22.7) vs 253.8 (31.6), p=0.38).

Conclusion: Acquiring multi-modal MRI of fetal brain at 3T from pregnant women with diabetes is feasible. Further study of fetal brain metabolism in maternal diabetes is warranted.

Abbreviations:

T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
GDM	Gestational diabetes
DWI	diffusion weighted imaging
sMRI	structural magnetic resonance imaging
IQR	interquartile range

Introduction

Diabetes is the most common medical disorder of pregnancy with the prevalence of type 1 (T1DM), type 2 (T2DM) and gestational (GDM) diabetes all increasing among women of childbearing age in resource rich settings. The perinatal complications of maternal diabetes, which reflect altered metabolic function *in utero,* include major congenital malformations, macrosomia, and stillbirth [1]. Long term, children born to mothers with diabetes are at increased risk for cognitive impairment [2, 3], inattentiveness [4], impaired working memory [5], and altered language development [6]. These adverse outcomes are not fully explained by postnatal events, which focuses research attention on vulnerability of the developing brain during fetal life. Identification of the nature and timing of alterations to brain structure and function that underlie neurocognitive impairment could help the development of strategies to designed to improve the long-term outcome of children of diabetic mothers.

During fetal life the predominant source of brain energy is glucose, which crosses the placenta by facilitated diffusion [7]. While severe perturbations in glucose homeostasis after birth are associated with neonatal brain injury, the effect of chronic fluctuant glucose concentration experienced by fetsuses of women with diabetes on in utero brain development has not been investigated. Maternal diabetes is also associated with disturbances in fatty acid metabolism: umbilical venous blood docosahexaenoic acid concentration is reduced, which reflects lower docosahexaenoic acid transfer to the fetus [8]. Docosahexaenoic acid accumulates in the brain in abundance from the third trimester and is essential for neurogenesis, neurotransmission and protection from oxidative stress. Reduced bioavailability of this key metabolite has been suggested as a putative mechanism for programming altered neurodevelopment [8, 9].

Advances in proton magnetic resonance spectroscopy (1HMRS), and diffusion weighted and structural magnetic resonance imaging (DWI, sMRI) have led to the development of objective and sensitive measures of fetal brain structure and metabolism. Use of these technologies has revealed alterations in cerebral NAA:choline ratio and gyrification in fetuses with congenital heart disease [10], temporal lobe volumes in fetuses with congenital cytomegalovirus infection [11], and ADC values and parenchymal volume in antenatal ventriculomegaly [12, 13]. Historically, the majority of fetal imaging studies have been undertaken at 1.5T.

However, although an increasing number of studies have been performed at 3T field strength [14-20] which has benefits over 1.5 T due to improved signal-to-noise and is likely to be advantageous for depicting fetal anatomy [21], to date there have been no studies assessing the feasibility of recruiting women with diabetes for fetal neuroimaging.

Early life metrics derived from 1HMRS, DWI and sMRI are associated with function in childhood. After preterm birth, NAA/Cho and Cho/Cr ratios are associated with neurodevelopmental outcome at age 2 [22], lactate/NAA predicts outcome following hypoxic ischaemic encephalopathy [23] and abnormalities in the NAA/Cre and Cho/Cre ratios in neonates [24] and older children [25] predict developmental delay. Increased ADC values in white matter are associated with diffuse white matter injury following preterm birth [26] and with poor outcome after hypoxic ischaemic encephalopathy in term infants [27, 28]. Finally, reduced regional and whole brain volumes, are associated with specific preterm comorbidities [29, 30] and structural alteration predicts long term impairment after preterm birth [31, 32]

Based on disturbances to fetal glucose and fatty acid metabolism associated with maternal diabetes and the neurocognitive profile of offspring, we aimed to investigate the feasibility of comprehensive fetal brain assessment by acquiring measurements of NAA/Cho, NAA/Cre and Cho/Cre ratios, regional apparent diffusion coefficient (ADC) measurements and volume of the cerebrum during the third trimester of pregnancy from women with diabetes, and from healthy controls using 3T MRI. The secondary aim was to determine normal values for these measures for future studies designed to investigate the effect of maternal disease of fetal brain development, and *in utero* origins of neurodevelopmental impairment.

Methods

Study population

Ethical approval was obtained from the National Research Ethics Committee (South East Scotland Research Ethics Committee) and written informed consent was obtained. Women with a pregnancy complicated by diabetes (n=26) and healthy controls (n=26) were recruited prospectively from antenatal diabetes clinics at the

Simpson Centre for Reproductive Health at the Royal Infirmary, Edinburgh, UK. The inclusion criteria were a singleton pregnancy and normal fetal anomaly scan at 20 weeks gestation. Women with diabetes were eligible to participate if they had gestational diabetes, diagnosed using the Scottish Intercollegiate Guideline Network diagnostic criteria [33] as a fasting venous plasma glucose of ≥ 5.1mmol/l or two hour glucose of ≥ 8.5mmol/l after a 75 g oral glucose tolerance test or pre-gestational type 1 or type 2 diabetes. Exclusion criteria were: significant co-existing maternal systemic disease other than maternal diabetes, and women with any contra-indications to MRI including metal implants and pacemakers.

MR image acquisition

Magnetic resonance studies were performed at the Clinical Research Imaging Centre in the Queen's Medical Research Institute, University of Edinburgh, UK using a Siemens Magnetom Verio 3T MRI clinical scanner (Siemens Healthcare GmbH, Erlangen, Germany). To avoid vena-cava compression, women were placed in a left-lateral tilt, with blood pressure being constantly monitored using a Veris MRI Vital Signs Monitor (Medrad, Bayer, UK). No fetal sedation was used, women were limited to spending 45 minutes in the scanner and data were acquired with women free breathing throughout. MRI scans were performed between 34 – 38 weeks gestation. A radiologist with experience in MRI reported all images.

T_2 weighted half-Fourier acquisition single-shot turbo spin-echo images were acquired of the fetal brain in sagittal, coronal and transverse orientations (HASTE: TR/TE = 1800/86ms, FOV = 400 x 400mm, matrix = 192 (phase) x 256 (frequency), slice thickness = 8mm, acquisition time = 18 s). These images were used to plan the position of the single 20 mm^3 spectroscopy voxel within the fetal brain. The scanner bed was moved to ensure that the fetal brain was positioned at the isocentre and the voxel was positioned within one hemisphere of the fetal brain, avoiding ventricles and contaminant signal from surrounding tissue. An optimised semi-automated shimming protocol was systematically applied until the full width at half-maximum of the water peak was less than 20 Hz. A single-voxel point-resolved spectroscopy 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. The 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 fetal brain. A post-spectroscopy 3-plane HASTE acquisition was then compared with the pre-spectroscopy HASTE images to allow visual assessment of fetal movement during the spectral acquisition. If the expert operator observed evidence of significant movement between HASTE acquisitions then the spectroscopy voxel was repositioned and the spectral acquisition was repeated. No additional filtering or quality-control limiting of data was applied during the processing stage. We therefore processed all of the MRS data that was acquired. An example of voxel positioning for MRS acquisition is shown in Fig. 1a.

Transverse DWI of the whole fetal brain (TR/TE =7300/106ms, FOV=400 × 400mm, matrix = 128 × 128, slice thickness = 3mm, b-values = 0, 500 and 1000 s/mm^2) were acquired. DWI were checked at point of acquisition for obvious signs of fetal motion, and repeated if required. ADC maps were generated automatically from the diffusion weighted images.

Finally, additional transverse HASTE images were acquired with identical coverage to the DW images to aid subsequent ROI analysis and to enable construction of the 3D motion-corrected brain volumes.

Data analysis: 1HMRS

Spectral analysis was carried out using the QUEST algorithm available in jMRUI [34]. This technique estimates metabolite amplitudes using a non-linear least squares fit of simulated metabolite signals to the acquired spectrum. A metabolite basis set was generated using the NMR-Scope function available in jMRUI [35] and included contributions from NAA (2.01, 2.49 and 2.70 ppm), Cho (3.2, 3.53 and 4.08ppm) and Cre (3.04 and 3.93 ppm). The following ratios were then calculated: NAA/Cho, NAA/Cre and Cho/Cre [36, 37]. The Quest algorithm calculates errors associated with the estimated metabolite amplitudes using an extended version of the Cramor-Rao lower bounds calculation [35]. The errors for each of the calculated metabolite ratios were derived through error propagation of the jMRUI output.

Data analysis: diffusion and structural MRI

(i) Apparent Diffusion Coefficients

Region of interest (ROI) analysis was carried out on ADC maps using standard software on the 3 T MR Siemens Magnetom Verio system. First, ROIs within white matter and grey matter were identified from the HASTE images acquired in the same plane and with the same coverage as the diffusion weighted images. A slice above the ventricles was identified as white matter and a slice at the level of the thalami was identified as deep grey matter using landmarks described in Boardman et al [38]. The identical slices were then identified on the corresponding ADC map; 4 ROIs were positioned in the white matter (2 posterior and 2 anterior) and 2 were positioned in the grey matter. Due to differences in fetal brain volume an anatomically appropriate ROI size was used for each individual brain, taking care to avoid partial volume effects from adjacent structures and artefacts. The mean (standard deviation, SD) ADC value for each ROI was recorded. The mean (SD) white matter ROI size was 0.30±0.12 and mean grey matter ROI size was 0.32±0.13. Example ROI placements for white and grey matter are shown in Figure 1b. Inter-rater agreement was checked by two independent investigators (DA, GM).

(ii) Structural MRI

For each participant, a single 3D motion-corrected brain volume was reconstructed using a slice-to-volume registration method [39] (Figure 1c). The fetal brain was extracted from surrounding fetal and maternal tissue using an atlas-based approach [40]. All reconstructed images were non-linearly aligned to the closest age-matched template from a publically available 4D fetal brain atlas [41]. Then, an automatic method based on an Expectation-Maximisation framework for brain tissue segmentation was used, where the priors of brain tissues were propagated using prior probabilities provided by the 4D atlas. Finally, binary masks of the cerebrum (intracranial contents excluding intraventricular CSF, extra-axial CSF, choroid plexus, brainstem, cerebellum and pons structures) and the intracranial volume (GM, WM and CSF) were deformed to the subject's native space, and volumes were calculated.

178

179 Statistical analysis

180 This was a feasibility study so a formal power calculation for sample size was not required [42, 43]. For
181 normally distributed data, mean and SD are reported and for non-normally distributed data, the median and
182 interquartile range (IQR) are reported. For group-wise comparisons of normally distributed variables
183 independent sample t-test was used, and for skewed data the Mann-Whitney U test was used. To analyse
184 regional ADC values, we first tested for evidence of laterality in anterior and posterior white matter, and deep
185 grey matter values using paired samples t-test, and if there were no significant difference between left and
186 right the values were averaged to compute mean anterior white matter ADC, mean posterior white ADC and
187 mean deep grey matter ADC per individual. The distributions were assessed for normality, and independent
188 samples t-test was used for group-wise comparisons of regional ADC. Inter-observer agreement in ADC
189 measurements was assessed for each region in a randomly selected subset of 20 participants using Bland-
190 Altman statistics. For group-wise analysis of NAA/Cho, NAA/Cre and Cho/Cre ratios, cerebrum volume and
191 intracranial volume, independent samples t-test was used after assessing for equality of variance between
192 groups. Statistical analyses were performed using SPSS 21 (SPSS Inc, Chicago, IL) with statistical significant
193 defined as $p<0.05$.

194

195 **Results**

196

197 Participants

198 The maternal demographics and delivery outcomes of the study population are demonstrated in Table 1. All
199 women tolerated the MRI scan well and no scan had to be abandoned due to maternal discomfort or
200 claustrophobia. Of the women with diabetes, thirteen were diagnosed with GDM during pregnancy, twelve had
201 T1DM and one had T2DM. In women with GDM, the median (range) gestation at diagnosis and diagnosis to
202 scan interval was 27.1 weeks (12.0 - 31.0) and 8.9 weeks (4.4 – 23.6), respectively. Only one woman with
203 GDM was treated with diet alone. The other twelve were treated with metformin (n=9) or metformin and insulin

(n=3) to achieve glycaemic control. All women with T1DM were insulin-treated and the one woman with T2DM was treated with insulin and metformin. The HbA1c (glycolated haemoglobin) at booking for women with T1DM and T2DM was 51.9 (16.6) mmol/mol. Two women with GDM, four women with T1DM and one control had antenatal steroids for fetal lung maturation prior to MRI. Three babies of women with T1DM were admitted to the neonatal unit for less than 72 hours. The reasons for admission were suspected sepsis (culture negative) and transient low blood glucose (n=1), a fractured clavicle sustained during a forceps delivery with shoulder dystocia and a duplication cyst that was not diagnosed antenatally. No babies born to healthy controls required admission. All babies were discharged home alive and well.

There was no difference in the gestation in weeks at MRI between women with diabetes and healthy controls (36.0 (0.8) vs 36.1 (0.9), p=0.69). No adjustment was therefore made for gestational age in the statistical analysis. No congenital anomalies, acquired brain injuries or incidental findings were detected by MRI.

MR spectroscopy

In utero 1HMRS of the fetal brain of suitable quality for analysis was obtained in 41/52 (79%) of the study population [22/26 (85%) women with diabetes, 19/26 (73%) healthy controls. There was no difference in the clinical characteristics of women in whom interpretable data was acquired compared to those in whom it was not (data not shown). There was no difference in the NAA/Cho and NAA/Cre ratios in the fetal brain in women with diabetes compared to controls (1.74 (0.70) vs 1.79 (0.64) p=0.81, and 0.78 (0.28) vs 0.94 (0.36) p=0.12, respectively). The Cho/Cre ratio was marginally lower in the fetal brain in women with diabetes compared to controls (0.46 (0.11) vs 0.53 (0.10) p=0.04) (Figure 2).

Diffusion weighted imaging - ADC

DWI amenable to ADC computation were available for 37/52 (71%) of the study population (18/26 (69%) women with diabetes, 19/26 (73%) healthy controls). Fetal motion or maternal size prevented interpretable data being obtained from 9/52 (17%) of the study population. There was no difference in the clinical

characteristics of women in whom interpretable data was acquired compared to those in whom it was not (data not shown).

There was no evidence of laterality in the anterior white matter, posterior white matter or deep grey matter ADC values (all p>0.05). Data were therefore combined to three variables – mean anterior white matter, mean posterior white matter and mean deep grey matter ADC. There was no difference in mean (SD) ADC values for anterior white matter, posterior white matter and deep grey matter in women with DM compared to controls (1.16 (0.12) vs 1.16 (0.08) p=0.96, 1.54 (0.16) vs 1.59 (0.20) p=0.56 and 1.49 (0.23) vs 1.52 (0.23) p=0.89, respectively) (Figure 3).

There was good inter-rater agreement between the two independent investigators for ADC values. The mean difference and 95% confidence intervals between investigators for anterior white matter, posterior white matter and deep grey matter measurements are reported in Table 2.

Brain volumes

Tissue segmentation data suitable for analysis was used to assess the macrostructure of the fetal brain in 24/52 (46%) of the study population [9/26 (35%) women with diabetes, 15/26 (58%) healthy controls]. Fetal motion or data quality prevented interpretable data being obtained from 28/52 (54%) of the study population. There was no difference in cerebrum volume /cc^3 (sd) in women with diabetes compared to controls (243.0cc^3 (22.7) vs 253.8cc^3 (31.6), p=0.39). There was no difference in intracranial volume in fetuses of women with diabetes compared to controls (265.0cc^3 (22.5) vs 274.5cc^3 (32.3), p=0.47)

Discussion

In this study we demonstrated that it is feasible to recruit pregnant women with diabetes to undergo MRI at 3T during the third trimester of pregnancy for measurements of NAA/Cho, NAA/Cre and Cho/Cre ratios, regional

ADC measurements and cerebrum and intracranial volumes. We chose to acquire 1HMRS, DWI and sMRI because of their use as markers of tissue injury / altered metabolism in the newborn period and their relationships with long term outcome. The values we acquired contribute useful normative data for future fetal brain studies carried out using 3T systems.

Although this feasibility study was not powered to detect group differences, we observed a marginal but significant reduction in Cho/Cre in the brains of fetuses of diabetic mothers during the third trimester. The MR spectroscopy choline peak includes free choline, phosphocholine, and glycerophosphocholine, so these data raise the possibility that brain metabolism and neuronal membrane phospholipid turn-over are altered in pregnancies with diabetes. While this finding requires confirmation in a larger study, it is notable that alterations in the Cho/Cre ratio in brains of adults with Type 2 diabetes have been reported [44].

A strength of our study is that we recruited a cohort of women with well-characterized diabetes with all participants being scanned within a four-week time window and gestation matched to our control group. This is important because 1HMRS spectra and ADC values are dynamic during this period of brain development [45-47]. We also acquired sMRI suitable for conventional clinical reporting was available for all participants. A limitation of our study is that we were unable to acquire data amenable to quantitative analysis from on all fetus' scanned. Despite ensuring comfort of the women in a large bore scanner, data could not be processed from 1HMRS in 21% of cases, DWI in 29% of cases and sMRI in 54% of cases. The low data yield for sMRI was partly because acquisition of 1HMRS and DWI was prioritized over sMRI. For future study designs that require fetal brain segmentation, yield may be increased by modifications to the acquisition protocol such as increasing the number of stacks per plane, accepting that time constraints required for safety may curtail other acquisitions (we capped imaging at 45 minutes). Of note, sMRI suitable for conventional clinical reporting was available for all participants.

We chose to recruit a heterogeneous population of women with diabetes to assess the feasibility of dissecting the effect of different *in utero* exposure to T1DM, T2DM and GDM in a future study. Recruitment of women with T1DM and GDM was relatively easy, thus recruitment to a future study assessing the effect of *in utero* exposure of T1DM and GDM on the fetal brain would be feasible. In contrast, we were only able to recruit one woman with T2DM, due to the lower prevalence of this condition. Thus, targeting recruitment of women with T2DM to a future study will not be practical unless recruitment occurred across multiple sites.

Our data were acquired using a 3 T system as opposed to a 1.5 T. For the advanced imaging techniques used in this study, there are advantages of acquiring data using the higher field strength of 3T [48]. Compared to lower field strengths, imaging at higher field strengths increases the signal-to-noise ratio. This improves the spectral quality obtained in 1HMRS and the ability to differentiate between closely located metabolites, particularly at short echo times. Inability to complete data acquisition within the time available due to fetal movement is a major limitation of MRI in pregnancy. Acquiring data more rapidly by using more advanced imaging methodologies, employing methods of motion correction to compensate for fetal movement and using alternative sampling techniques such as compressed sensing are likely to significantly increase data yield in the future. Finally, one advantage of 3 T is the ability to acquire images with higher spatial resolution (depending on the imaging coil used), potentially increasing diagnostic accuracy [49].

Perinatal image metrics are sensitive to tissue injury and neuroprotective treatment strategies. They are therefore increasingly used to address the 'gap in translation' in perinatal neuroscience to assess therapies that show promise in pre-clinical studies at lower economic and opportunity costs than randomised controlled trials powered on clinical outcomes [50]. The normative data provided here may inform the development of fetal brain biomarkers for use in interventional perinatal neuroprotective outcome studies.

Conclusions

In conclusion, the data provide proof-of-concept that comprehensive assessment of fetal brain using measures derived from images acquired at 3T from women with diabetes and healthy controls is achievable. In addition they suggest that fetal brain MRS may provide a promising image marker of altered brain development in maternal diabetes. Finally, although we studied fetuses of mothers with diabetes, this research pipeline and the normative values obtained could be applied to any paradigm in which fetal origins of brain development are being investigated using 3T MRI.

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Figure Legends:

Figure 1:

Examples of: MRS voxel placement in fetal brain (A - C), Regions of Interest for DWI in anterior white matter and posterior white matter (right and left) (E) and deep grey matter (right and left) (F), tissue segmentation in the brain with the brain highlighted in green (G - H).

Figure 2:

Metabolite ratios for NAA/Cho, NAA/Cr and Cho/Cr in the fetal brain in women with diabetes and healthy controls. Data presented as mean +/- standard deviation.

Figure 3:

ADC values in the anterior white matter, posterior white matter and deep grey matter the fetal brain in women with diabetes and healthy controls. Data presented as mean +/- standard deviation.

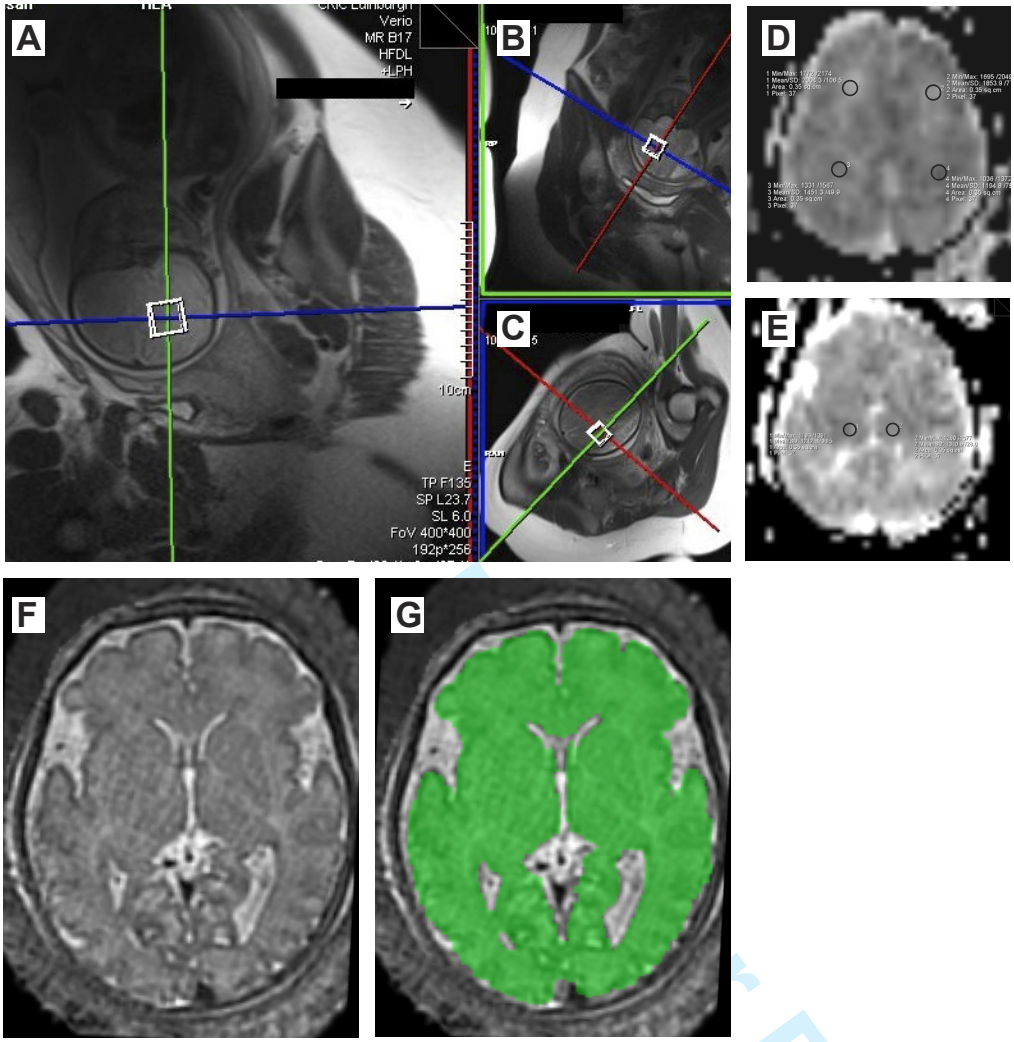
Table 1: Demographics, MRI details and delivery outcomes

		Diabetes			
	Control (n=26)	All (n=26)	GDM (n=13)	T1DM (n=12)	T2DM (n=1)
Maternal Demographics					
Maternal age (years)[1]	31 (5)	31 (5)	32 (5)	30 (6)	34
Parity[2]	0 (0-3)	0 (0-3)	1 (0-2)	0 (0-3)	0
Current smoker[3]	1 (4)	3 (12)	1 (8)	2 (17)	
Deprivation[3]					
SIMD 1-3	13 (50)	13 (50)	6 (46)	6 (50)	1
SIMD 4-5	13 (50)	13 (50)	7 (54)	6 (50)	
MRI details					
Gestation at MRI (weeks)[1]	36.1 (0.9)	36.0 (0.8)	36.0 (0.8)	36.0 (0.9)	36.7
MRI to delivery interval (weeks)[1]	3.6 (1.6)	2.1 (1.2)	2.6 (1.2)	1.6 (1.1)	15
Neonatal outcome					
Gestation delivery (weeks)[1]	39.7 (1.5)	38.1 (1.4)	38.6 (1.1)	37.6 (1.5)	38.9
Birthweight (g)[1]	3372 (467)	3551 (627)	3629 (483)	3508 (780)	3040
Sex (male: female)	13:13	9:17	6:7	2:10	Male
Occipito-frontal circumference (cm)[1]	34.4 (1.4)	34.8 (1.8)	35 (1.6)	35 (2.2)	36

[1] Mean (SD), [2] Median (range), [3] n (%), [4] SIMD Scottish Index of Multiple Deprivation, SIMD 1 most deprived, SIMD 5 most affluent

Table 2: Bland Altman statistics for ADC measurements recorded by two observers.

	Mean difference	Mean + (1.96*SD)	Mean - (1.96*SD)
Grey Matter ADC	-0.073×10^{-3} mm²/s	0.108×10^{-3} mm²/s	-0.253×10^{-3} mm²/s
Anterior White Matter ADC	-0.033×10^{-3} mm²/s	0.175×10^{-3} mm²/s	-0.241×10^{-3} mm²/s
Posterior White Matter ADC	-0.028×10^{-3} mm²/s	0.225×10^{-3} mm²/s	-0.281×10^{-3} mm²/s


A
B
C
D
E
F
G
Verio
MR B17
HFDL
+LPH
10cm
TP F135
SP L23.7
SL 6.0
FoV 400*400
192p*256