Magnetic Resonance Imaging (MRI) Biomarkers of Placental Structure and Function in Normal and Growth Restricted Pregnancy

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Abstract

Fetal growth restriction (FGR) is a serious complication of human pregnancy where the fetus fails to reach its genetically pre-determined growth potential. It is a common condition, affecting 5 - 15 % of all pregnancies (Gardosi 2009) and is linked to a third of all antepartum deaths (CEMACH 2008). An ongoing problem for obstetricians is the difficulty in diagnosing and predicting FGR and those at highest risk of poor outcomes. Placental insufficiency is a major cause of FGR and specific abnormalities in placental morphology and function occur in this condition; constituting an abnormal FGR placental phenotype (Sibley, Turner et al. 2005). Magnetic Resonance Imaging (MRI) is a powerful tool that allows quantitative analysis of several indices relating to tissue structure and function and, therefore, is of potential use in identifying this phenotype. We hypothesised that a range of MR indices would be feasible in the placenta at 1.5 T, that these indices would be altered in FGR and that there would be correlations with relevant parameters of placental morphology. Ultimately, we aimed to assess whether these indices could be used in the assessment of FGR in utero.

Using MRI we estimated placental volume, widths, length and depths in groups of women with normal and FGR pregnancies. We also measured placental relaxation times, T₁ and T₂, which relate to tissue composition and assessed parameters relating to blood flow using Intra-Voxel Incoherent Motion (IVIM) and Arterial Spin Labelling (ASL). We demonstrated an FGR placental phenotype that was reduced in volume but increased in depth, by around 10mm, with a shorter T₂ relaxation time and lower values of D (the diffusion coefficient) measured by IVIM. A trend for reduced perfusion measured by ASL was observed in pregnancies with birthweights less than 10th centile (Gardosi, Chang et al. 1992). T₂ and D also correlated with stereological indices of placental morphology.

In conclusion, the studies in this thesis illustrate these MRI indices show great potential as biomarkers for identifying the FGR placenta
Declaration

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A note on Alternative Format Theses

The Alternative Format thesis allows a postgraduate doctoral to incorporate sections that are in a format suitable for submission for publication in a peer-reviewed journal. Apart from the inclusion of such materials, the alternative format thesis must conform to the same standards expected for a standard thesis. Materials included in the alternative format thesis may include those which are solely and/or partly authored by the student and may be already published, accepted for publication, or submitted for publication in externally refereed contexts such as journals and conference proceedings. The work must constitute a body of publication tending towards a coherent and continuous thesis, rather than a series of disconnected publications. As such, any publications should be adapted and integrated within the structure of the thesis. Any sections of the thesis which are published or in publishable format should be clearly identified. The incorporation of publication-style chapters in the thesis will inevitably lead to some duplication since each publication-style chapter will have self-contained components that will overlap with parts of the other sections of the thesis.

My reasons for wishing to submit in this format were that the body of work naturally falls into chapters that are also transferable to original research papers. Therefore, the thesis will be written in a very similar way in an alternative format as it would have been in a classical format and the readability is maintained. I also felt that aiming to have each chapter written as a research paper would improve my writing skills and those chapters submitted as papers prior to thesis submission would benefit from the feedback of peer review, particularly as much as the work is novel and exploratory in nature. Completing article submission to peer reviewed journals has been a great learning experience and helped me focus on which aspects of the work are of greatest interest to the reader and researcher.
Chapter 1

Introduction to Fetal Growth Restriction (FGR), the Placenta and Magnetic Resonance Imaging (MRI)
1.1 Fetal Growth Restriction

Fetal growth restriction (FGR) is a serious complication of human pregnancy where the fetus fails to reach its genetically pre-determined growth potential. It is a common condition, affecting 5 -15 % of all pregnancies (depending on definition used) (Gardosi 2009) and is linked to a third of all antepartum deaths (CEMACH 2008). Growth restricted infants are at increased risk of a number of neonatal morbidities (Gilbert and Danielsen 2003), often worsened by iatrogenic prematurity. In addition, serious neurological sequelae, such as cerebral palsy, are more common (Jarvis, Glinianaia et al. 2003) along with behavioral and emotional problems in childhood (Zubrick, Kurinczuk et al. 2000). Small size at birth also predisposes the infant to major diseases in adult life, such as diabetes, hypertension and cardiovascular disease (Hales, Barker et al. 1991; Leon, Lithell et al. 1998).

An ongoing problem for obstetricians is the difficulty in predicting, detecting and diagnosing FGR, as well as predicting fetal outcome. Ultrasound biometry of fetal size may not always be able to distinguish the fetus that is growth restricted, from one that is constitutionally small and may also miss those whose growth is failing but fetal size remains within normal limits. Although the use of ultrasound Doppler in the uterine and umbilical circulations has been described in over 200 investigations of uteroplacental disease, no sufficiently predictive test for FGR has been established.

1.1.1 Consequences of FGR

The association of FGR with stillbirth and neonatal death is well established. A fetus with growth restriction is at risk for sudden unexplained intrauterine death [odds ratio 7.0] (Froen, Gardosi et al. 2004). The severity of growth restriction is directly related to an
increased risk of fetal death (Kramer, Olivier et al. 1990), a relationship that holds true regardless of gestational age (Piper, Xenakis et al. 1996).

The perinatal mortality rate is higher among both term and preterm FGR infants, Lackman et al. (Lackman, Capewell et al. 2001) reported a 5 to 6-fold increased rate of death among both term and preterm infants with FGR, McIntire et al. (McIntire, Bloom et al. 1999) reported up to a 10-fold increase rate of death in growth-restricted infants at term. SGA infants are also at increased risk of morbidity, including low Apgar scores, seizures, hypoxic ischaemic encephalopathy, hypothermia, hypoglycaemia, hypocalcaemia, polycythaemia, sepsis, coagulopathy and hepatocellular dysfunction (Pallotto and Kilbride 2006). These are often secondary to prematurity (Lackman, Capewell et al. 2001), as early delivery may be required for maternal or fetal health reasons. Premature infants that are small, as opposed to average for gestational age, appear to be most at risk, with higher rates of serious complications such as respiratory distress syndrome and chronic lung disease (Tyson, Kennedy et al. 1995; Regev, Lusky et al. 2003).

Long term follow up of SGA infants suggests there are health implications lasting into childhood, with poor growth (Karlberg and Albertsson-Wikland 1995) and academic under-achievement (Strauss 2000), although this does not outweigh the more important influence of socioeconomic status (Goldenberg, Hoffman et al. 1998). Serious neurological sequelae, such as cerebral palsy are seen more commonly in SGA infants (Jarvis, Glinianaia et al. 2003) and there are less conclusive associations with behavioral and emotional problems in childhood (Zubrick, Kurinczuk et al. 2000).

A growing body of evidence has indicated that many major diseases of adulthood e.g. coronary heart disease, hypertension and type 2 diabetes - are associated with low birthweight (Barker, Osmond et al. 1989; Hales, Barker et al. 1991; Leon, Lithell et al.
1998). These associations extend across into the normal range of birthweights and depend on low birth weight in relation to gestation rather than prematurity (Barker, Gluckman et al. 1993). Such findings have led to the ‘developmental origins of adult disease’ hypothesis, which states that our susceptibility to such diseases is programmed in utero as a response to the fetal environment (Barker 1995), thought to occur through processes of developmental plasticity and epigenetic modification. A concept of predictive adaptation has also been developed to explain the relationship between early life events and the risk of later disease. The model suggests that a mismatch between fetal expectation of its postnatal environment and actual postnatal environment contribute to later adult disease risk (Gluckman, Hanson et al. 2005). Both concepts are important in our understanding of how FGR impacts upon health well beyond the perinatal period.

1.1.2 Definitions

Normal fetal growth is determined by a genetically predetermined growth potential and modulated by maternal, fetal, placental, and external factors. Fetal Growth Restriction (FGR) is defined as a condition in which the fetus fails to achieve its own genetically determined growth potential and may be secondary to a number of causes. However, as the genetic growth potential is often not known, FGR can in practice be difficult to diagnose, which has led to a variety of alternative terms and definitions being used in the literature. Small for gestational age or SGA (usually defined as a birthweight less than the 10th percentile for gestational age) is often used as a proxy, but it is important to stress that the terms are not synonymous. Although FGR and SGA are often diagnosed antenatally, indirect assessments of fetal size, usually made using ultrasound, are subject to a range of errors and confirmation after delivery is required. Antenatal definitions are,
nevertheless, necessary for clinicians, to guide management decisions that must be made prior to delivery. Correct assessment of fetal growth is also reliant upon accurate pregnancy dating, where first trimester ultrasound is a better predictor of gestational age than using the last menstrual period dates alone (Campbell and Thoms 1977).

Common terms and definitions used in the literature to define FGR include;

Determined antenatally;

1) Estimated fetal weight (EFW) generated by ultrasound biometric measurements of less than the 3rd, 5th, 10th or 15th percentile for gestational age or 2 standard deviations below the mean for gestational age.

2) Fetal abdominal circumference less than two standard deviations below the mean for gestational age determined by ultrasound.

Determined after birth;

1) Small for gestational age (SGA) - a newborn with a birthweight below the 3rd, 5th, 15th but generally 10th percentile for gestational age.

2) Low birthweight infants – a birthweight regardless of gestation of less than 2500g (very low birthweight less than 1500g or extremely low birthweight less that 1000g).

3) Individualised birthweight ratio (IBR) or centile of less than the 5th or 10th percentile. This is relative to predicted birthweight (calculated using gestational age to delivery, parity, ethnic origin, height, booking weight and fetal sex).

4) Ponderal index (weight divided by cube of length) of below the 10th percentile for gestational age.
The situation is further complicated as the percentile given may be based upon a birthweight reference, representative of an entire population, or a birthweight standard, which limits the population to those births that have optimal outcomes. More recently, it has been recognised that additional factors such as maternal height, weight, ethnicity, parity and fetal gender should perhaps also be accounted for, rather than gestational age alone (whole population based). This has led to the introduction of ‘customised growth charts’ in clinical practice and the increased use of the IBR in research practice. Both have an improved correlation with fetuses at risk of poor perinatal outcomes (Wilcox, Johnson et al. 1993; Clausson, Gardosi et al. 2001). Compared to whole population based charts, customised growth charts have better sensitivities for identifying SGA fetuses (Gardosi, Chang et al. 1992), identifying morphometric evidence of FGR (Sanderson, Wilcox et al. 1994) and have lower false-positive rates (Mongelli and Gardosi 1996). The use of customised growth charts has raised some controversies regarding improvement of outcome, however, they are now recommended in the UK by the Royal College of Obstetricians and Gynaecologists (Gynaecologists 2002). Post-delivery, definitions involving the IBR probably relate most closely to true FGR. In a study of 616 infants, an IBR less than the 10th percentile reclassified 25% of those less than the 10th percentile of birthweight for gestation as normal (Sanderson, Wilcox et al. 1994). In the infants that remained classified as small, significantly more infants had abnormal ponderal indices and reduced skin fold thicknesses, suffered abnormal fetal heart rate patterns, underwent operative delivery for fetal distress and required neonatal resuscitation than those who were reclassified as normally grown.

Doppler flow studies, most commonly of umbilical blood flow, are also often included in research definitions of FGR. FGR may, however, occur in the absence of abnormal umbilical artery Dopplers. SGA infants without abnormal umbilical artery Dopplers remain at risk of poor perinatal and neurodevelopmental outcomes (Figueras, Eixarch et
al. 2007) although numerous studies confirm that groups with abnormal Dopplers are at highest risk of adverse perinatal outcomes, particularly perinatal death (Thornton and Lilford 1993). A significant association also exists between abnormal Doppler indices and fetal acid-base compromise in FGR. One study reported that 67% to 80% of the fetuses were hypoxic and 45% were acidotic when absent end-diastolic flow was present (Nicolaides, Bilardo et al. 1988). The absence or reversal of end-diastolic flow is known to be associated with very poor perinatal outcomes and a high perinatal mortality rate (Nicolaides, Bilardo et al. 1988) (Karsdorp, van Vugt et al. 1994). Doppler studies of umbilical flow therefore provide an indication of the severity of FGR, but are not a compulsory component of the definition.

An appreciation of the difficulties in defining and diagnosing FGR is important when considering the literature surrounding this condition, as study groups often comprise of relatively heterogenous populations. More rigorous definitions of FGR, perhaps based on the abnormalities of placental structure and function seen, would offer advantages from both clinical and research viewpoints.

1.1.3 Causes of FGR

When discussing causes of FGR, the definition used is relevant. An infant that is small for gestational age may be constitutionally small or have failed to reach its genetic growth potential i.e. true FGR. By using any of the definitions given in the previous section, it is virtually impossible to completely separate the two, leading to heterogeneous groups that include small but healthy infants.
For truly growth restricted infants, who have failed to reach their genetically determined growth potential, placental insufficiency is the major cause. Specific placental abnormalities, such as a single (rather than double) uterine artery, are found in the minority (Catanzarite, Hendricks et al. 1995). Generally, the diagnosis is made on the basis of exclusion of other causes or antenatal tests suggesting poor placental function e.g. abnormal uterine artery Dopplers or oligohydramnios. Causes often regarded as ‘non-placental’ may also indirectly affect placental function, for example trisomy 18 babies frequently exhibit asymmetrical growth restriction with abnormal uteroplacental Dopplers (Kennerknecht and Terinde 1990).

Causes and associations can be broadly categorised into three groups (adapted from Sabogal 2007):

**Fetal:**

- Genetic diseases (5% to 20%) (e.g., aneuploidy, uniparental disomy, growth factor mutations, etc.)
- Infection (5% to 10%) (e.g., CMV, toxoplasmosis, malaria, rubella)
- Malformations (1% to 2%)
- Multiple gestation (3%)

**Uteroplacental:**

- Placental insufficiency
- Placental /cord abnormalities (3%) (abruption, mosaicism, chorioangiomas, 2 vessel cord, velamentous insertion, etc.)
- Uterine abnormalities

**Maternal:**
• Hypertensive disorders (20% to 30%)

• Pre-gestational diabetes

• Autoimmune disease (e.g., APS, SLE)

• Cardiac disease (e.g., complex cyanotic congenital heart diseases)

• Other maternal diseases (especially if poorly controlled)

• Toxic exposure (smoking, alcohol, cocaine, drugs)

• Malnutrition

• Living at high altitudes

• Living in developing country

• Low socio-economic status

• Race (e.g., Afro-American)

• Family or prior history of FGR

• Extremes of maternal age

• Short inter-pregnancy interval

### 1.1.4 Predicting FGR

FGR is a serious condition with poor outcomes. Currently, a sufficiently predictive test for the condition is not available. Although many epidemiological associations are described, few are particularly strong. In a study of 8030 deliveries, 2788 women with risk factors
and 292 representative women from the group with no risk factors were analysed in detail (Galbraith, Karchmar et al. 1979). One third of FGR infants came from a population with no risk factors and only 9.8% of women with risk factors delivered an SGA infant, leaving the vast majority with an average sized infant. Pre-eclampsia and smoking remain major risk factors for SGA; pre-eclampsia is associated with a 4-fold increase in the risk of having a small for gestational age infant (Odegard, Vatten et al. 2000), whilst maternal cigarette smoking leads to an average birth weight reduction of 6% when continued throughout gestation (Cliver, Goldenberg et al. 1995).

It is of course important that clinicians are aware of the risk factors, so that they may advise women on risk reduction and look for warning signs of disease. However, given that so many cases of FGR occur in groups with no risk factors and most women with risk factors will have a normal outcome, such factors cannot be used as a predictive test in practice.

There are over 150 publications examining the role of Doppler assessment of blood flow in the uteroplacental vessels, primarily the uterine arteries, in the prediction of FGR and pre-eclampsia. A recent meta-analysis of 61 studies (in which Doppler assessment of the uterine arteries was used to predict FGR), evaluated the accuracy of 15 Doppler indices, using well-described and robust statistical methods (Cnossen, Morris et al. 2008). FGR in low-risk patients was best predicted in the second trimester by an increased pulsatility index with notching (positive likelihood ratio 9.1, 95% CI 5.0–16.7; negative likelihood ratio 0.89, 95% CI 0.85–0.93). Severe FGR in low-risk patients was best predicted in the second trimester by an increased pulsatility index (positive likelihood ratio 13.7, 95% CI 10.3–16.9; negative likelihood ratio 0.34, 95% CI 0.23–0.48) or an increased pulsatility index with notching (positive likelihood ratio 14.6, 95% CI 7.8–26.3; negative likelihood ratio 0.78, 95% CI 0.68–0.87). An increased resistance index (> 0.58 or > 90th percentile)
in the second trimester best predicted severe fetal growth restriction in high-risk patients (positive likelihood ratio 10.9, 95% CI 10.4–11.4; negative likelihood ratio 0.20, 95% CI 0.14–0.26). One of the challenges of conducting a review in this area is the variability in definitions of FGR, aetiologies included and varying approaches to management. In addition, the studies are heterogeneous in their timing of Doppler assessment and inclusion of other screening methods. This said, although the positive likelihood ratios of these data are reasonable, the negative likelihood ratios are poor, therefore we cannot be reassured that women with negative results are less likely to develop FGR. Although improvements are seen in higher risk populations, this negates the use of uterine artery Doppler as a screening test for FGR in a low-risk population.

In addition to uterine artery Doppler ultrasonography, a variety of proteins and hormones have been studied as potential early biomarkers of FGR. Low first trimester PAPP-A levels are associated with low birth weight, but studies of first and second trimester AFP, hCG and inhibin-A have shown a less consistent picture (Yaron, Cherry et al. 1999; Spencer, Yu et al. 2005). Second-trimester maternal serum screening markers for aneuploidy (the presence of an abnormal number of chromosomes), β human chorionic gonadotropin (β-hCG) and α-fetoprotein have been shown to be associated with increased risk (Raty, Koskinen et al. 1999; Yaron, Cherry et al. 1999). Multiple combinations of these biochemical tests and uterine artery Dopplers have been attempted, but as yet no sufficiently predictive test for use in low-risk pregnancy has been established. A profile of placental function may, however, be of value to screen pregnancies that are clinically judged to be at increased risk of preeclampsia, fetal death, and FGR (Whittle, Chaddha et al. 2006). Kingdom et al. have adopted this approach, finding that in 212 high-risk pregnancies assessed using a placental profile (combining a)16- to 18-week maternal serum screening, b)18- to 23-week uterine artery Doppler imaging and c) ultrasound assessment of placental morphologic condition), the development of adverse outcomes,
such as SGA, severe early onset FGR and intrauterine fetal death, were significantly less in women with all normal test results. Combining groups of women with two or 3 abnormal test results together predicted 14 of 19 severe FGR and 15 of 22 IUFD cases (Toal, Chan et al. 2007). These results combining tests of placental function, rather than relying on ultrasound alone is useful in stratifying risk in FGR. The investigation of other tools, such as placental MRI, in important in this concept. The SCOPE (Screening of Pregnancy Endpoints) Consortium has fully adapted the approach that using a combination of various clinical, imaging and chemical markers is likely to provide the most effective screening for conditions such as FGR. In 2009 they published on patterns of uterine artery Doppler and pregnancy outcome (Groom, North et al. 2009) and in 2010 again published on risk factors relating to FGR including a number of lifestyle factors and the previously reported associations with abnormal uterine artery Dopplers at 20 weeks gestation (McCowan, Roberts et al.). Both studies concluded that a range of markers, including biochemical parameters not included in these studies, were most likely to provide effective predictive testing for FGR. The SCOPE consortium as a whole takes a novel approach, recruiting large numbers of women from a group of centres across the globe. Using this approach, they aim to build on current knowledge by investigating more novel markers identified by use of proteomic, metabolomic and genomic techniques. Although the full outcomes of this research in relation to FGR are yet to be reported, a number of important associations have already been identified in other related diseases, such as pre-eclampsia (Atkinson, Blumenstein et al. 2009; Blumenstein, McMaster et al. 2009).

1.1.5 Diagnosis of FGR

Clinical examination is most commonly by symphysis-fundal height (SFH) measurement, although the technique is associated poor intra and inter-observer reproducibility and
lack of account for physical build and abnormalities such as fibroids or abnormal fetal lie. Studies conducted in the 1980s suggested that reduced SFH measurements correctly identified only 25-50% of fetuses whose birthweight was <10<sup>th</sup> percentile; a frightening statistic when considering that for most women, this is their route of entry to ultrasound surveillance (Pearce and Campbell 1987).

Again, it is important to stress that accurate pregnancy dating is a pre-requisite for accurate growth assessment and can lead to difficulties in ‘late-bookers’ who miss the window (late first or early second trimester) for sonographic assessment of gestation. Growth parameters, primarily biparietal diameter (BPD), head circumference (HC), femur length (FL) and abdominal circumference (AC) are plotted on charts which delineate the normal range of growth within a population. These values can also be combined to plot an estimated fetal weight (EFW), where the predicted weight will fall within 15 -18% of the actual weight in 95% of cases (Hadlock, Harrist et al. 1985). As discussed earlier, attempts to improve identification of true FGR has led to the introduction of customised growth charts of EFW.

Even using the best available charts, there are other difficulties in sonographic surveillance of growth. Fetal growth is a dynamic process, whereas ultrasound examines the fetus at isolated time points. While a single ultrasound measurement may be an indication of a problem, this does not itself diagnose a growth abnormality. Successive measurements are required, however depending upon the start and end-points used and frequency of assessment, FGR could still be missed. False reassurance may also be gained from isolated measurements where the parameters plot within the normal range, but in fact growth is failing. This is illustrated in Chapter 1 Figure 1.

Furthermore, a degree of error lies within the measurements themselves. Abdominal
circumference is commonly the most abnormal in FGR but also the most difficult to reliably obtain, due to its lack of bony rigidity and shift in liver position with the onset of fetal breathing. Calculation of estimated weight is subject to error in all measurements used in the calculation, with discrepancies of up to 25% between actual and predicted weight, particularly when estimating weight in macrosomia or FGR (Chauhan, Hendrix et al. 1998). Amniotic fluid volume is also measured routinely in clinical practice however, despite observed lower amniotic fluid indices in SGA, sonographic estimation of amniotic fluid volume is not a reliable predictor of suboptimal growth (Chauhan, Magann et al. 2008).

The most recent meta-analysis of the use of fetal and umbilical Dopplers in low risk pregnancies included five trials involving 14,185 women (Alfirevic, Stampalija et al.). The methodological quality of several of the studies was deemed to be unclear because of insufficient data included in the reports, though several conclusions were drawn. Importantly, routine fetal and umbilical Doppler ultrasound examination in low-risk or unselected populations did not result in increased obstetric interventions but equally no benefits were detected for substantive short term clinical outcomes such as perinatal mortality. The authors found that there was insufficient evidence to assess the effect on substantive long term outcomes such as childhood neurodevelopment. Despite these findings, some units do offer screening at 32 weeks (for example, Kings College Hospital, London, where I am currently placed. Although there is no published data on psychological benefits, for most women these scans seem to be very much wanted and well received, if perhaps the medically indications remain unclear.

In conclusion, diagnosis of FGR can be difficult and subject to error. Although umbilical Dopplers are very useful in stratifying fetal risk in suspected FGR, even when the Doppler is normal the fetus remains at some risk. Additionally, change in umbilical Dopplers is
often late in the disease, so that ongoing fetal surveillance is required through the pregnancy. Ideally, a more predictive test of FGR and fetal outcome would be available. Until this is established, it is important that research continues into other potential candidates, including biochemical tests and other imaging modalities, such as MRI.
Chapter 1 Figure 1. Graph showing theoretical pitfalls of ultrasonographic biometry and estimation of fetal weight. Gestation is plotted in weeks on the x axis against estimated fetal weight in grams. AGA is average for gestational age in size, SGA is small for gestational age in size. AGP is average for gestational age in phenotype FGRP is FGR phenotype. Average sized infants whose growth is failing may not be picked up, whereas small but normal infants may face unnecessary intervention.
1.1.6 Management of FGR

Our methods identifying the growth restricted fetus antenatally are not ideal. Using standard care, only 26% of SGA babies were suspected to be small before birth in an unselected hospital population (Hepburn and Rosenberg 1986). In a low-risk population in Nottingham, only 16% of SGA infants were detected with standard methods of antenatal assessment (Hepburn and Rosenberg 1986; Kean, Liu et al. 1996). Identifying FGR is crucial to the management of disease. As no intervention has yet been demonstrated to alter the growth pattern of the fetus, management is reliant upon surveillance and appropriate delivery in terms of time, mode and location. Timing of delivery is individualized, as yet no randomized controlled trial has fully elucidated the optimal time frame and no national or international guidelines are in place, however, from the literature available a system of best care can be derived.

The Growth Restriction Intervention Trial (2003) was a prospective randomized multicenter study of over 500 women with pregnancies (24 to 36 weeks gestation) complicated by FGR. Patients were randomly assigned to immediate versus delayed delivery based on physician discomfort. The median time-to-delivery intervals were 0.9 days in the immediate groups and only 4.9 days in the delay group. Total deaths before discharge were 10% in the immediate group and 9% in the delay group. Essentiall, no differences in short-term outcomes were observed but immediate delivery was associated with higher neonatal mortality while delayed delivery carried the price of a higher stillbirth rate. However, follow up studies demonstrated there may be further impact on long term neurodevelopment outcomes, assessed at 2 years of age or beyond (Thornton, Hornbuckle et al. 2004). The greatest difference in disability was seen in babies randomised before 31 weeks gestation; 14% in the immediate group versus 5% in the delayed group. Based on these studies it was concluded that safe prolongation of
pregnancy offers the best combination of decreased perinatal mortality, morbidity, and improved neurodevelopment. In terms of mode of delivery, one third of FGR pregnancies requires caesarean delivery (Group 2003), but again choice is individualized. Elective caesarean did not show statistically significant differences in poor outcomes such as respiratory distress syndrome and seizures although trends were towards this (Grant 2003). Delivery in a tertiary level neonatal unit is often indicated, so that staff and facilities for appropriate resuscitation and stabilisation are available.

The DIGITAT trial (Disproportionate Intrauterine Growth Intervention Trial at Term) was designed to compare the effect of induction of labour with expectant monitoring on neonatal outcome and operative delivery rates in suspected growth restriction at term (beyond 36 weeks) (Boers, Vijgen et al.). FGR was defined as abdominal circumference <10th centile, estimated fetal weight <10th centile or a tailing off across the centiles. 321 were randomized to induction and 329 to expectant monitoring, which led to delivery around 10 days later. Birthweights were lower in the induced group, however, the number of babies born with birthweights less than the 3rd centile was significantly higher in the expectant group (30% vs 12%). A composite neonatal outcome combining death before discharge, Apgar Score, umbilical artery pH and admission to neonatal intensive care was used to assess fetal outcome. There was no significant difference in poor outcomes between the expectant group as compared to the induction group (6% vs 5%) and no difference in caesarean rates (45% both groups). The study concluded that expectant management for women is a equivocal options as long as fetal monitoringis used (here daily movement counts, twice weekly CTG and ultrasound were employed). A caution however is that the maternal age of this group was 27 and there was a tendency for older, more educated women not to participate. Customised growth charts were not used and therefore perhaps many of these participants would have been reclassified as normal, which may affect the generalisability of the results, particularly in the UK where these
have been widely adopted. Nevertheless, the high proportion of infant less than the 3rd centile in the expectant group does support that this remains a viable option for presumed FGR at term.

Further guidance is awaited from the results of the TRUFFLE study (Trial of umbilical and fetal flow in Europe) (Lees). This multicentre study is aimed at defining optimal timing of delivery between 26 and 32 weeks with women randomised to delivery based on cardiotocograph changes (primarily reduced short term variability) or on early of late changes in blood flow in the ductus venosus (the vessel carries oxygenated blood in the fetus before birth) i.e increased pulsatility index or loss of ‘a-wave’. This study will look at both short term and long term outcomes at 2 years and hopefully guide clinicians towards best practice.

The most recent meta-analysis on the use of fetal and umbilical Dopplers in high risk pregnancies has continued to support their use, however, high quality evidence is lacking and particularly in relation to FGR/SGA pregnancies (Alfirevic, Stampalija et al.). Eighteen completed studies involving just over 10,000 women were included. The use of Doppler ultrasound in high-risk pregnancy was associated a reduction in perinatal deaths (risk ratio (RR) 0.71, 95% confidence interval (CI) 0.52 to 0.98, numbers needed to treat = 203; 95% CI 103 to 4352). There were also fewer inductions of labour (average RR 0.89, 95% CI 0.80 to 0.99) and fewer caesarean sections (RR 0.90, 95% CI 0.84 to 0.97). No difference was found in operative vaginal births (RR 0.95, 95% CI 0.80 to 1.14 nor in Apgar scores less than seven at five minutes (RR 0.92, 95% CI 0.69). Only 7 studies focused on FGR specifically and the authors concluded that there was insufficient data in this sub group to say whether Doppler ultrasound has differential effect depending on the indication e.g. IUGR, hypertension or previous pregnancy loss.
1.1.7 Conclusion

Currently our methods for detecting the fetus at risk of FGR are not sufficient and may miss those at risk or lead to intervention in the small but normal fetus. An objective and more sensitive assessment of distinguishing the fetus with normal or abnormal growth would have enormous utility in guiding antenatal care, improving neonatal outcomes and focusing research. The placenta is of key importance in the pathophysiology of FGR, however in current practice, few tests relating to placental structure or function have been developed. New approaches such as 3D power ultrasound and angiography as well as MRI may allow non-invasive testing of these parameters. Three-dimensional power Doppler angiography (3-DPDA) facilitates the demonstration of blood vessels within an organ and provides a noninvasive method for the assessment of placental vasculature in humans in vivo (Campbell 2007). The power Doppler data within any three-dimensional (3-D) dataset can be processed to provide indices that represent the power Doppler signal, although the exact relationship to true blood flow is not clear, as for several MRI parameters in development. 3D Doppler indices such as the vascular index and vascular flow index have been shown to be altered in FGR (Pomorski, Zimmer et al.) and may correlate more closely than traditional 2D parameters (Luria, Barnea et al.). MRI however has some advantages over ultrasound; primarily the field of view is not limited by the size of the probe allowing whole placental imaging with ease. It is also not limited by reduced liquor volume, shadowing from ossification or obesity, which can prevent adequate assessment using ultrasound. However, MRI is costly and its use in pregnancy is much more limited which may hinder its development for use in FGR. Advanced ultrasound and MRI techniques are at a similar stage of development in relation to FGR; it seems likely that both may have a role to play but the exact nature of those roles is yet to be fully determined.
1.2 The Placenta in FGR

1.2.1 Placental phenotypes

Placental insufficiency is a major cause of FGR and understanding how the placenta differs in this condition is crucial in realising how tools such as MRI might be useful in identifying the disease.

The placenta represents the key interface between the mother and fetus and is the organ by which nutrient transfer and gaseous exchange occurs. Placental insufficiency is a major cause of FGR and increasing evidence supports that several aspects of placental structure and function are altered in the condition, including gross morphology, morphometric assessments of tissue structure at a microscopic level, blood flow in the uteroplacental circulations and molecular transport mechanisms for ions and nutrients. This constellation of specific abnormalities has been proposed as a representation of a placental phenotype of FGR (Sibley, Turner et al. 2005). In the mouse, a placental specific knockout of a single imprinted gene, encoding Insulin-like Growth Factor-2 (IGF-2), leads to a combination of placental alterations similar to those seen in human FGR (Constancia, Hemberger et al. 2002; Sibley, Coan et al. 2004). In this mutant mouse, placental weight is reduced prior to the onset of fetal growth restriction, supporting a causal relationship (Constancia, Hemberger et al. 2002). Although a single gene or even the IGF pathway alone is unlikely to be responsible for FGR as we see it in humans, a rigorously defined placental phenotype might help both in defining FGR and distinguishing it from other growth patterns, such as the constitutionally small baby.
1.2.2 Pathophysiology of placental insufficiency

Although FGR is rarely detected in the first half of pregnancy, abnormal placentation during this period is relevant to the pathophysiology of FGR. Understanding the pathophysiology of placental insufficiency is important in our appreciation of how we are most likely to detect FGR and at what gestation.

Placentation begins with implantation of the blastocyst beneath the uterine epithelium and subsequent differentiation into embryonic and extra-embryonic components. The placenta begins as cytotrophoblast, separated initially from maternal tissue by a shell of syncytiotrophoblast. Following implantation, cytotrophoblast begins to penetrate outside this shell in columns with cells breaking off to invade the uterine stroma, forming an extra-villous cytotrophoblast cell lineage (Benirschke 2000). (The remaining columns differentiate into villous cytotrophoblast, where proliferating cells fuse to form an overlying syncytiotrophoblast (Huppertz, Tews et al. 2001)). Extra-villous cytotrophoblast is thought to play a key role in forming plugs in the maternal spiral arteries preventing blood flow into the intervillous space, therefore providing a hypoxic drive for ongoing fetal angiogenesis within the developing placenta (Kaufmann, Black et al. 2003). This process is important in determining eventual placental size and shape; inadequate endovascular occlusion below the area destined to form the placenta could lead to excessive placental regression in the early second trimester, such that a previously central cord insertion becomes eccentric or even velamentous, often seen in FGR (discussed in next section).

At the end of the first trimester, the endovascular plugs disappear, maternal blood reaches the placental villi, and oxygenation of the whole placenta occurs. Extravillous trophoblast cells also surround the spiral arteries where they replace the muscularised vascular walls.
with a deposition, creating dilated channels conducting blood to the intervillous space (Lyall, Bulmer et al. 2001). The extra-villous trophoblast invades arteries in the inner myometrium to a depth of 3 to 5 mm. This is reduced to about 2 mm in pregnancies complicated by pre-eclampsia (Kadyrov, Schmitz et al. 2003), possibly due to the persistence of maternal leukocytes in the decidua secreting pro-apoptotic cytokines (Reister, Frank et al. 2001). Pregnancy therefore normally results in a low pressure high flow system into the placenta, however pregnancies complicated by FGR often have persistent high resistance, as evidenced by abnormal uterine artery Doppler waveforms (Cnossen, Morris et al. 2008). Although for some time this was largely thought to be secondary to the deficient invasion of the maternal vasculature, more recent evidence (from Power Doppler studies and post partum uterine vascular casts) has suggested that during the second trimester, blood flow to the intervillous space remains limited (Schaaps, Tsatsaris et al. 2005). The currently favoured explanation is that the interstitial extravillous trophoblast cells also secrete angiogenic and vasodilator signals to not only promote local blood flow to the uterus, but to mediate the increase in maternal cardiac output and blood volume and the decrease in blood pressure due to systemic vasodilatation (Hemberger, Nozaki et al. 2003). These changes in the microcirculations of the placenta, highlight its importance as a potential focus for detecting FGR. Currently ultrasound Dopplers only investigate the uterine and umbilical blood flows, related to ‘intra-placental flow’, but not a study of it directly. It is also apparent that placentation, at least in some cases, is abnormal from very early gestations, it therefore follows that prediction of FGR should be possible, if an appropriate test was available.

The placenta extracts a fixed proportion of the nutrient stream (70% glucose and 40% oxygen supplied to the uterus), therefore fetal supply is restricted to the surplus after placental needs have been met (Baschat 2004). Even mild dysfunction may restrict transfer to the fetus whilst placental nutrition is maintained. The fetus is able to respond to
such changes by adjustments in metabolism, endocrine axes and hematologic parameters as well as cardiovascular and behavioural responses. These adaptations and the restriction of fetal growth are the outcome of placental insufficiency and ultimately define the consequences of the disorder.

1.2.3 Gross placental morphology in FGR

Alterations in very basic morphological placental parameters are seen in association with FGR. When considering placental tests for this condition, these should not be disregarded. Although, due to a large amount of biological variation, the size or shape of the placenta alone is unlikely to be predictive of FGR, if considered as part of the placental FGR phenotype, they can be useful in conjunction with other parameters.

The relationship between placental weight and birthweight is complex. Teasdale reported that the weight of placenta from SGA infants was reduced when compared with controls, but changes in the placental weight to birthweight ratio were unclear (Teasdale 1984). The study concluded that the placenta is usually small because the infant is small, and its small size does not act as a contributory factor to fetal growth restriction. A further study found that placental weight to birthweight ratio was significantly associated with gestational age, female sex, Asian parentage, increasing maternal body mass index, increased maternal weight at booking, and lower socioeconomic status and concluded that the ratio was not an accurate marker of fetal growth, but a surrogate for other factors already known to influence health (Williams, Evans et al. 1997). Interestingly, the weight of the placenta in relation to birthweight was revisited in 15,047 appropriate for gestational age (AGA) and 1569 SGA infants (Heinonen, Taipale et al. 2001). The placental weight to birthweight ratio was significantly lower in SGA infants than in AGA infants with same birthweight.
SGA infants had smaller placentas than the controls, suggesting that placental weight is an influencing factor on fetal growth.

Placental weight is the most common way to characterize placental growth, but of note, weight is a summary of many dimensions of growth, measured by disc shape, the distance from the cord insertion site to the nearest disc margin and extent of branching of the villous and vascular nutrient exchange surface, reflected in the thickness of the chorionic disc. The normal placental disc shape is round oval. Abnormal lobation (bipartite or tripartite) of the placenta is associated with maternal smoking, maternal age over 35 and maternal diabetes mellitus (based on data from the National Collaborative Perinatal Project and analysis of placental morphology in placentas from 24,047 women (Salafia, Charles et al. 2006)). The umbilical cord insertion site marks the early confluence of principal chorionic vessels. Uniform placental growth about the cord insertion site will result in a centrally inserted cord, but if the placenta grows more in one direction than in others the cord insertion site may be eccentric, marginal, or (at the extreme) velamentous. Marginal and velamentous cord insertions are associated with fetal malformations (potentially implicating a primary fetal origin), but also with maternal diabetes mellitus and FGR (Naeye 1992).

Most of the placental growth in the third trimester is in increasing thickness, with progressive branching of the villous tree (Boyd 1970). Placental disc thickness is therefore an indirect measure of the development of the exchange surface of the placenta, essential to its successful support of fetal growth. However, there may be an optimal placental thickness; in normal placentas of normal thickness, subchorionic villi are already showing changes related to poor perfusion (Popek 1999). In thicker placentas, the increased depth of villous arborisation may therefore be less efficient. Abnormally thick placentas have been correlated with adverse pregnancy outcome (Raio, Ghezzi et al. 2004; Fisteag-
Kiprono, Neiger et al. 2006; Toal, Keating et al. 2008). Placentas from 60 women with abnormal uterine artery Doppler were examined at 19 – 23 weeks of gestation for placental shape abnormalities, described as a placental thickness >4cm or >50% of length. Those with abnormally thick placentas had higher odds of intrauterine fetal death (odds ratio, 4.5 95% CI, 1.3-15.6) and FGR (odds ratio 4.7, 95% CI 1.4 -15.1) (Toal, Keating et al. 2008). This highlights the importance of further research into the use of placental parameters as a tool for identifying the at risk fetus.

1.2.4 Placental blood flow in FGR

Uteroplacental vascular resistance has been extensively investigated as a predictor of FGR (see above). Prefumo et al. (Prefumo, Guven et al. 2004) investigated the relationship between the timing of disappearance of high-resistance uterine artery waveforms between the first and second trimester of pregnancy and birth weight in 662 pregnancies. A clear correlation between the longitudinal variation in uterine artery blood flow pattern and birth weight was observed, suggesting that the timing and extent of trophoblastic conversion of the maternal uteroplacental vessels is an important determinant of birthweight.

Vascularisation of the developing placental villi is seen in the second trimester. Fetal angiogenesis is crucial for successful placental development, although all the mechanisms are not completely understood. The pathology underlying the abnormal umbilical artery high resistance waveforms seen in FGR are complex and debates exist regarding the relative importance of the cord, stem artery and terminal villus pathology (Kingdom, Burrell et al. 1997). Umbilical arteries appear hypoplastic using morphometric analysis in FGR (Bruch, Sibony et al. 1997). Branching from these, stem artery density was initially
thought to be reduced in FGR (Giles, Trudinger et al. 1985), but since (using more rigorous stereological methods and immunohistochemistry) no major differences have been demonstrated (Jackson, Walsh et al. 1995; Macara, Kingdom et al. 1995). More distally, electron microscopy of placental villi and their vascular casts suggests a failure of formation of the terminal villi and an arrest of non-branching angiogenesis in FGR (Krebs, Macara et al. 1996). Thrombo-occlusive damage to the placenta, including central infarction, intervillous thrombosis and massive perivillous fibrin deposition are all seen in association with FGR, usually in the absence of maternal thrombophilia (Chaddha, Viero et al. 2004).

There is a growing body of evidence that the abnormal angiogenesis patterns seen in FGR, are influenced by angiogenic proteins such as vascular endothelial growth factors (VEGF) and angiogenic inhibitors (sFlt-1, soluble Fms-like tyrosine kinase) (Ahmed, Li et al. 1995; Wallner, Sengenberger et al. 2007). The exact roles of such factors is still undergoing research, however there are definite associations between maternal raised sFlt levels, abnormal uterine artery Doppler patterns and FGR (Schlembach, Wallner et al. 2007; Nevo, Many et al. 2008). If angiogenesis is abnormal in FGR, blood flow, particularly the microvasculature of the placenta, is also likely to be abnormal with effects on placental transfer.

It is likely that reduced blood flow contributes to fetal hypoxia in FGR (Itskovitz, LaGamma et al. 1983; Edelstone, Peticca et al. 1985), as oxygen is small and highly lipophillic, enabling it to diffuse readily across the placental barrier and causing its transfer to be flow–limited (Sibley and Boyd 1988). However, it is unlikely that blood flow reduction can sufficiently explain the reduced concentrations of various nutrients in the condition, such as glucose and amino acids, as the properties of the placental exchange
barrier primarily limits transfer of these hydrophilic molecules, rather than blood flow (Sideri M 1983; Sibley and Boyd 1988).

Currently, ultrasound imaging is not used to evaluate blood flow in the microcirculations of the placenta itself, only up- or downstream in the uterine or umbilical vessels. The development of techniques to study these microcirculations, such as MRI, are of key importance, given that, as discussed above, many of the changes seen in FGR are within the smaller vessels of the placenta itself.

1.2.5 Placental tissue morphology in FGR

Several aspects of placental tissue morphology are known to be altered in FGR. MRI offers a range of indices that relate to tissue structure, particularly relaxation times, discussed in the next section. Here, we discuss the alterations in tissue structure that may be foci for identifying this condition.

Substances passing from the maternal to fetal circulation, or vice versa, are faced with the placental barriers. The first, from a maternal start point, is the syncytiotrophoblast layer, which consists of the maternal facing microvillous plasma membrane (MVM), the trophoblast cytosol and the fetal facing basal plasma membrane (BM). The villous stroma is then reached, before crossing the fetal capillary endothelium to enter the fetal circulation. Human placental capillaries closely resemble other non-brain continuous capillaries, having wide paracellular clefts (Leach and Firth 1992), which allow relatively unrestricted transfer of molecules like glucose and amino acids across the capillary wall. Therefore it is the two polarized membranes of the syncytiotrophoblast that represent the main barrier for transplacental transfer (Sideri M 1983; Sibley and Boyd 1988).
Mayhew (Mayhew, Ohadike et al. 2003) quantified placental morphology using stereological techniques in pregnancies complicated by preeclampsia with and without FGR. FGR was associated with a placenta that had reduced volumes of all types of villi (stem, intermediate, terminal). The reduced elaboration of distal villi affected all compartments (trophoblast, stroma, capillaries) and was accompanied by smaller exchange surface areas and a thicker trophoblastic epithelium. It was concluded that FGR, but not preeclampsia, was associated with substantial changes in placental morphology of the placenta, including impoverished growth of villi and fetal vasculature. These results were reiterated in a study using visual image analysis to observe changes in the morphology (Daayana, Baker et al. 2004). Placental biopsies from nine normal pregnancies, five cases of pre-eclampsia, five cases of FGR, and five cases of pre-eclampsia with FGR were randomly sampled and the placental areas occupied by villi, syncytiotrophoblast, and syncytial cytoplasm were quantified. Only the FGR group showed a consistent reduction in villous area. Birth weight was positively correlated to total villous area occupied. Furthermore, increasingly positive relationships were recorded between both syncytiotrophoblast area and syncytiotrophoblast cytoplasm and birth weight, pointing to impoverished villus development in FGR. The changes seen in pre-eclampsia with FGR were more similar to those in the isolated pre-eclampsia group than those with isolated FGR, supporting that FGR and FGR seen in combination with pre-eclampsia have separate aetiologies. These FGR specific abnormalities are key targets to try to identify in utero, using techniques such as MRI.
1.2.6 Note on Stereological Placental Analysis

The nomenclature morphometry and stereology do not describe the same method. Morphometry is a two-dimensional quantitative method that uses a calliper or gauge, that may be part of a computer based image analysis system. Stereology is a test-system comprised of test points or lines orientated over a known frame area, which again may be computerised. Morphometry determines lengths, perimeters, areas whereas stereology estimates densities, such as volume density and surface area density by the application of specific formulae. The calculations are based on statistical principles (sample size, randomisation, tissue isotropy) and the aim of the sampling design is to obtain the maximum amount of quantitative structural information (for full details of stereological protocols for the placenta see Mayhew, 2006) (Mayhew 2006) and can require a significant amount of time and effort. Where very large data sets are analysed, the precision of individual measurement may be less important and a morphometric technique may be better suited in terms of cost and time. Very few studies have compared morphometry and stereology (Parsons, Marko et al. 1990; Rahman and Itakura 1996), none have compared techniques in the placenta (although Mayhew and Burton put forward a strong case for the latter(Mayhew and Burton 1988)). In studies contained within this thesis, a stereological technique was adopted after experimenting with the different methods. This was due to the small sample sizes achievable, the perhaps more robust methodology was preferred.

1.2.7 Placental transfer in FGR
During pregnancy, the placenta provides the major route by which all nutrients, ions, gases, water and other compounds are transferred to the fetus and by which waste products are removed. The placenta, therefore, is of fundamental importance to fetal health and growth. Although, for many years, the placenta was seen as a passive conduit to the fetus, its importance in regulation of placental transfer is increasingly acknowledged and several aspects of placental structure and function are altered in disease conditions. These are particularly relevant to disorders of fetal growth such as FGR.

Absolute fetal growth rate changes with gestation; it is very slow in the first trimester and from then on accelerates towards term. As material required for growth is supplied predominantly via the placenta, it follows that the transfer capacity of the placenta must also grow. Changes in transfer capacity cannot be explained by placental growth alone (at least on a macroscopic scale, as this is known to be highest in the first trimester and relatively slow towards term) and therefore other mechanisms must come into play. These increased demands could theoretically be met by one, alone or combined, of three changes: 1) alterations in blood flow to or within the placenta, 2) altered capacity of the placenta itself to facilitate transfer (determined by dimensions of the exchange barrier and availability/activity of specific transporter proteins in the plasma membrane) or 3) alterations in the driving forces across the placenta (the hydrostatic, osmotic and electrical gradients and supply of energy for active transport e.g. ATP).

As well as the well documented alterations in maternal and fetal blood flow in FGR (as evidenced by uterine and umbilical Doppler ultrasound measurements), there are changes in the structural variables of the exchange barrier and an ever growing wealth of literature describing a variety of changes in transporter expression and activity. In FGR, the surface area of the villi and fetal capillaries are significantly reduced (Mayhew, Manwani et al. 2007). Despite the degree of cross over between FGR and pre-eclampsia, these changes
are attributable to the former condition when seen in isolation, further evidence in support of differential disease processes for the conditions. (Daayana, Baker et al. 2004). The affect of FGR on the thickness of the placental exchange barrier is not clear cut. Stereological studies suggest a thickening of the trophoblastic component of the barrier although the overall thickness (comprising of trophoblast + stroma + capillaries) remains constant, suggesting a thinning of another component such as the stroma, with a possible compensatory shift of the capillaries to be in closer proximity with the trophoblast.

Whether the alterations in placental structure and function seen in FGR are cause or effect is a difficult issue to answer, but evidence from studies on a knockout mouse model, in which the placental-specific transcript of the insulin-like growth factor 2 gene (igf-2) was deleted, suggest the former. The altered placental phenotype in mutant mice pregnancies, similar to that seen in human FGR, was found to precede a decline in fetal growth. Undernutrition in the rat also leads to an altered placental phenotype prior to fetal growth decline. Knockout of the placental specific transcript of igf-2 in the mouse leads to a decrease in placental weight with reduced exchange surface area, an increase in the estimated harmonic mean thicknesses of the barrier and a strongly correlated decrease in permeability to diffusional markers, as well as fetuses which are 30% smaller than wild type (Sibley, Coan et al. 2004). Furthermore, placental growth restriction occurs at day 14 onwards (term is day 20), but fetal growth restriction does not occur until around day 18 or 19 (Constancia, Angiolini et al. 2005).

The reasons for this delay between placental growth restriction and that of the fetus may be due to a delayed reduction in the diffusional permeability of the mutant placentas to hydrophilic molecules, apparently arising from the altered placental morphology and changes in placental transporter activity or expression in the mutants. Various alterations in specific transporter mechanisms occur in association with human FGR. Impaired
amino acid transport generally is associated with impaired fetal growth and reduced fetal plasma levels of amino acids are seen in FGR. In FGR, the activity of the System A amino acid transporter in the placenta is markedly reduced and in the most severe cases of FGR, as determined by abnormal umbilical artery Doppler and fetal heart tracings, the most profound reductions in System A activity are seen (Glazier, Cetin et al. 1997).

The fact that some placental transporter activities (e.g. System A) go down in FGR whereas at least one, the Ca\(^{2+}\) ATPase increases is very interesting. The decreased in System A may be causative to FGR. The increase in Ca\(^{2+}\) ATPase may be adaptive to the FGR: placental supply adapting to specific demand signals from the fetus. In the igf-2 knockout mouse model, maternofetal transfer of [14C]methylaminoisobutyric acid (MeAIB, a specific non-metabolisable substrate of System A), measured in vivo, was increased by 50% per unit weight of placenta, in the knockout mice at day 16, but by day 19, [14C]MeAIB transfer was similar in knockout and wild-type pups. These data suggest that System A activity in the placenta can be upregulated to increase placental efficiency. However, this upregulation may not be sustainable, so that fetal growth restriction does finally ensue (Constancia, Hemberger et al. 2002). Furthermore, in the same model fetal calcium accumulation in the fetus of the knockout is reduced but transfer is then upregulated (with increased expression of calbindin-d9k) so that fetal calcium content is normal by term (Dilworth, Kusinski et al.). There is therefore good evidence of placental adaptation to fetal growth requirements in this model.

Further evidence for compensatory mechanisms comes from a study of System A transport in the MVM of human placentas from babies across the range of normal birthweights where activity was found to be highest in the smallest babies (Godfrey 1998). This is in contrast with the situation in frank FGR, where, as discussed, a downregulation of System A is seen.
Low birthweight infants are often found to be hypoglycaemic at birth and may have ongoing problems normalising glucose levels in the neonatal period. The reasons for this are not entirely clear. Fetal hypoglycaemia in FGR is unlikely to be due to altered placental GLUT 1 function, as protein expression and transport activity are unaltered. The impact of other members of the GLUT family has not been fully established, but it is important to note that not all transporter activities are altered in FGR.

Lipoprotein lipase is crucial in the process of fatty acid release to the fetus from lipoproteins and interestingly, its activity is found to be reduced in FGR, in keeping with the altered maternal/fetal lipid ratios also found in the condition.

$\text{Na}^+/\text{K}^+\text{ATPase}$ activity and expression is decreased in the MVM in FGR, which may result in a reduced driving force for a range of $\text{Na}^+$ dependent transport mechanisms. The activity and expression of the $\text{Na}^+/\text{H}^+$ exchanger (NHE), the primary pH regulating transporter in the syncytiotrophoblast, is reduced in association with FGR, which could, speculatively, contribute towards acidosis in these fetuses. Note that the activity of a lactate transporter on the BM is also reduced perhaps contributing further to the fetal acidosis

1.2.7.1 The placenta as a sensor

Our understanding of the control of placental transfer mechanisms remains incomplete but several lines of evidence support the view that the placenta is able to adapt to changes in its environment e.g. substrate levels in the maternal or fetal microcirculations, and adjust transfer accordingly, so that transport functions will be coordinated with maternal nutrient or ion availability and fetal demand. Therefore factors such as malnutrition, reduced blood
flow or hypoxemia may impact upon these regulatory mechanisms and as a consequence be up or down regulated. Examples of this include the reduction in the expression of System A amino acid transporter and increase glucose transporters in cultured term trophoblasts during hypoxia (Esterman, Greco et al. 1997; Nelson, Smith et al. 2003) and the effect of metabolic hormones such as insulin, IGF-1 and leptin, on nutrient transporters (Karl, Alpy et al. 1992; Ericsson, Hamark et al. 2005; Ericsson, Hamark et al. 2005). Disease states such as FGR and fetal overgrowth may in fact be due to a loss of placental ‘sensing’ and appropriate up or down regulation – as evidenced by the patterns seen in placental specific Igf2 knockout mice (Sibley, Coan et al. 2004).

Excitingly, another candidate ‘regulator’ of transport mechanisms has recently been postulated. mTOR (mammalian target of rapamycin) is a serine threonine kinase and represents an important nutrient sensing pathway in mammalian cells, controlling growth through regulation of translation and transcription in response to nutrient availability, in particular of branched chain amino acids, hypoxia and cellular energy status (Dennis, Jaeschke et al. 2001; Arsham, Howell et al. 2003; Jacinto and Hall 2003). mTOR is highly expressed in the cytosol of the syncytiotrophoblast and appears to have down regulated activity in FGR (Jansson and Powell 2006). Although much further research is required, such findings could eventually provide therapeutic targets for use in fetal conditions.

### 1.2.8 Conclusions

These accumulated observations enable us to describe a series of structural and functional alterations in the placenta that are specific to fetal conditions, even when accepting that groups included in studies may be heterogeneous. Further exploration of these abnormalities together could provide better information on the placental phenotype,
allowing more rigorous definitions of conditions such as FGR and fetal overgrowth and providing better biomarkers for use in clinical practice to identify and diagnose these conditions. Placental MRI is a potential candidate for this role.
1.3 Magnetic Resonance Imaging (MRI)

1.3.1 Basic Physics of MRI

An understanding of basic MRI physics is important when considering what MRI can tell us about tissue structure and function. As with all tests, it is subject to variation and error and is, to some extent, an approximation of the actual in vivo situation. MRI physics is a complex subject, suffice to say during MR the patient lies within a large, powerful magnet where the magnetic field is used to align the magnetization of the protons within the atomic nuclei in the body (most commonly hydrogen nuclei which are abundant in body tissues) and radio frequency fields are used to systematically alter the alignment of this magnetization. This causes the protons to produce a rotating magnetic field detectable by the scanner— and this information is recorded to construct an image of the scanned area of the body. A more in depth discussion of MRI physics is covered in the title Magnetic resonance imaging: Physical principles and sequence design (Haacke 1999). Bloch and Purcell were responsible for the original demonstration of nuclear magnetic resonance in 1945. They made their observations on either side of the United States within days of each other and subsequently shared the 1952 Nobel Prize for Physics (Bloch 1946; Purcell 1946).
Chapter 1 Figure 2. Basics of MRI. Figure showing a) spinning hydrogen protons with randomly orientated individual magnetic moments, b) alignment of most of magnetic moments within the magnetic field $B_0$ creating a net magnetisation (the
higher the field strength the greater the number of moments aligning), the magnetic moments also begin to precess in phase. In c) the precessing magnetic moments (and net magnetisation) are flipped into the transverse plane by the presence of a radiofrequency coil generating a magnetic field (B1) at 90 degrees at the Lamor frequency/resonant frequency of precession. If the radiofrequency pulse is then switched off the signal can be detected by a receiver coil. The signal then begins to decay by T1 and T2 relaxation as the protons return to their steady state.
1.3.2 Quantitative MRI indices

A major advantage of MRI is its ability to provide quantitative indices which relate to differences in tissue structure and function. These include indirect measurements in the form of physical properties such as MR relaxation times and magnetisation transfer, which are related to the bulk macromolecular and water content of the tissue of interest. MRI can also provide more direct measurements, without using injectable contrast, of blood flow and perfusion.

1.3.2.1 Relaxation time measurements

Most of the protons in tissues are found in water molecules with lesser amounts in organic molecules. In order to manipulate net magnetisation, a Radio Frequency (RF) pulse with a frequency that matches the Larmor frequency is applied. Protons spinning with the same frequency as the RF pulse will respond or resonate to that RF pulse. By sending an RF pulse with a certain strength (amplitude) and for a certain period of time it is possible to rotate the net magnetization into a perpendicular plane i.e. the net magnetization is ‘flipped’ by 90° (this angle is referred to as the flip angle and can vary between 0° and 180° in MR sequences). They protons also become ‘in phase’, so that the precession for all hydrogen protons becomes uniform. This process is called excitation and can be equated with the protons absorbing energy from the MR pulse. After the pulse is switched off, the relaxation process begins and can be divided into two parts: T1 and T2 relaxation. This is illustrated in Chapter 1 Figure 2.

T1 Relaxation: After the RF excitation pulse stops, the net magnetization will re-grow along the longitudinal direction, while emitting radio-frequency waves. T1 relaxation is
also known as Spin-Lattice relaxation, because the energy is released to the surrounding tissue (lattice). T1 relaxation relates to the protons flipped by 90° by the excitation pulse.

Not all protons are bound in the same way depending on the molecule they are in. Hydrogen in complex molecules such as fat are bound tightly, while those in water have a much looser bond. Therefore, the rate at which they release their energy is therefore different. T₁ is a time constant defined as the time it takes for the longitudinal magnetization to reach 63 % of the original magnetization.

**T2 Relaxation**: T2 relaxation occurs simultaneously, but is independent of T1. It relates to the protons relaxing from an *in-phase* situation to a total *out-of-phase* situation. Again, how the hydrogen proton is bound in its molecule is important. T₂ is defined as the time it takes for the spins to de-phase to 37% of the original value. The rate of de-phasing is different for each tissue. Fat tissue will de-phase quickly, while water will de-phase much more slowly.

The rate of T2 relaxation is quicker than T1 relaxation. T2 relaxation happens in tens of milliseconds, while T1 can take up to seconds. T2 relaxation is also called spin–spin relaxation because it describes interactions between protons in their immediate surroundings (molecules).

Thus, the contrast in the MR image largely relates to differences in the longitudinal (T₁) or the transverse (T₂) relaxation times of water protons present in the tissue. Cameron et al. studied the histology of 16 different tissue types in rats and correlated these with T₁ and T₂ relaxation times (Cameron, Ord et al. 1984). From the results they made the following generalizations;
1. Each normal tissue has characteristic and reasonably consistent $T_1$ and $T_2$ relaxation times.

2. Tissues with higher water content have longer $T_1$ relaxation times.

3. Tissue $T_2$ values are not as well correlated with water content as $T_1$ values.

4. Tissues with shorter $T_1$ values have higher calculated hydration fractions, greater amounts of rough endoplasmic reticulum, and a greater rate of protein synthetic activity.

5. Tissues with higher lipid content, associated with non membrane bound liquid droplets, tend to have longer $T_2$ values.

6. Tissues with greater all over surface area, whether in the form of cellular membranes or intracellular or extracellular fibrillar macromolecules, tend to have shorter $T_2$ values.

7. The differences in $T_1$ and $T_2$ values between tumour and normal tissues correlates with differences in the volume fraction of extracellular fluid and in the amounts of membrane and fibrillar surface area in the cells.

These last two points are of particular interest when studying the placenta, as a fall in $T_2$ and to a lesser extent $T_1$ were observed with advancing gestation, when increases in overall exchange barrier surface area would be expected (Gowland, Freeman et al. 1998). The clinical application of such indices has been studied in many human tissues and shown to be of use in the liver where it may help identify certain tumor histotypes, such as hepatocellular carcinoma and with careful analysis, may also help predict the degree of tumor differentiation (Bartolozzi, Cioni et al. 2001). Intracellular content of certain
substances such as glycogen, fat, melanin, iron and copper will also have a role in determining MR signal behavior and relaxation times have been investigated as a tool for studying iron load in the liver and brain (Thomsen 1996; Vymazal, Brooks et al. 1996).

Relaxation times can act as tissue biomarkers and are potentially of use in identifying the alterations in placental tissue seen in association with FGR.

1.3.2.2 Blood flow and perfusion measurements

In considering perfusion measurements, it is important to recall that there are a number of well established techniques for imaging blood flow including positron emission tomography, computed tomography and MRI contrast techniques. Particularly relevant to pregnancy however, are techniques which require no injected or inhaled contrast.

Intravoxel Incoherent Motion was initially developed by Le Bihan, in assessment of microscopic motions causing signal attenuation in diffusion MR imaging (Le Bihan, Breton et al. 1986; Le Bihan 1988). Attenuation of signal occurs when protons move fast enough within a magnetic field to become dephased (i.e. desynchronised in their inherent spins from other protons, giving a corresponding reduction in signal. IVIM measures a property of a tissue slightly different from classical perfusion as it is sensitive to molecular motion, however, in biological tissue this is predominantly constituted of the diffusion of water and movement of blood (Henkelman 1990).). Blood (or other fluids such as lymph), flowing in any direction will also contribute, rather than capillary flow in isolation. This is particularly of relevance when considering IVIM in the placenta, in which flow is slow and multi-directional. The concept introduced by Le Bihan is that water flowing in randomly oriented capillaries mimics a random pathed walk “pseudo-diffusion”. Diffusion itself relates to the random Brownian motion of water molecules, propelled by thermal energy, and is also in a random path. Both measurements are dependant on the b value.
(the b value is a factor of diffusion weighted sequences which summarises the influence of the gradients on the diffusion weighted images. The higher the value b, the stronger the diffusion weighting); pseudo-diffusion is also dependant on the velocity of the blood and the vascular architecture. However, the rate of signal attenuation resulting from pseudo-diffusion is greater than molecular diffusion in tissues, so its relative contribution to the MR signal becomes significant only at very low b values, allowing diffusion and perfusion effects to be separated (Le Bihan, Breton et al. 1988).

Arterial Spin Labeling (ASL) is a method of assessing blood flow, most extensively investigated in the brain (Detre, Leigh et al. 1992; Williams, Detre et al. 1992). ASL uses selective inversion as a method of tagging inflowing arterial blood. When the tagged blood reaches the tissue being imaged, it attenuates the signal as it has already undergone some relaxation. Subtraction of the tagged image from a control image gives a measure of the amount of tagged blood which flowed into the tissue. The quantity is closely related to the tissue perfusion. The sensitivity of the technique is improved at higher field strengths due to both intrinsically higher signal to noise ratio and the lengthening of blood T$_1$. However, it is particularly challenging to use ASL to study slow blood flow, as T$_1$ decay in the tagged blood may occur before reaching the tissue of interest, therefore preventing its detection (Chalela, Alsop et al. 2000). ASL has been investigated in a number of disease states including stroke (Chalela, Alsop et al. 2000), dementia (Johnson, Jahng et al. 2005), brain tumours (Weber, Thilmann et al. 2004) and epilepsy (Wolf, Alsop et al. 2001) although its role outside the CNS remains relatively unexplored. As ASL is able to tag blood in supplying vessels, differentiation of blood flowing into the placenta from either maternal or fetal directions may be possible and their relative contributions assessed.
1.3.4 Placental MRI

Fetal MRI is reviewed as a published article in Appendix I.

The placenta is of fundamental importance in the normal growth and development of the fetus and pregnancy. Specific alterations are seen in the placenta in association with FGR. Doppler assessments of uterine and umbilical blood flow are used in clinical practice and are indirect markers of placental function, examining blood flow up or downstream from the circulations within the placenta itself. MRI offers non invasive methods of examining blood flow and perfusion within the placenta and also provides quantitative indices, which relate to tissue structure.

1.3.4.1 Imaging the Placenta

The placenta is suited to MRI as it is relatively immobile and hence the problems created by fetal movement are not encountered to such an extent (Gowland 2005). It also lacks the air tissue interfaces found in other parts of the body which may cause image artefact and has a high moving blood volume, allowing the application of perfusion assessment techniques. Placental anatomy is well defined on MRI, with clear boundaries against the amniotic fluid and to a slightly lesser extent with the uterine wall. The volume of the placenta can be measured from a multi-slice set of images and has been investigated as a predictor of FGR (Baker, Johnson et al. 1995); generally lower placental volumes were seen on MRI in pregnancies complicated by this condition, but not falling outside the confidence limits set for the normal population (Baker, Johnson et al. 1995; Duncan, Sahota et al. 2001).
1.3.4.2 Placental structure

Histology has shown that the placenta matures and alters in structure with increasing gestation and quantitative MRI reflects these changes. $T_1$ and $T_2$ relaxation times, related to the structural and content of the placenta, are seen to fall with increasing gestation (Gowland, Freeman et al. 1998). The values are less than those seen for blood alone, suggesting changes in the villous tissues rather than just an increase in the vascular component are responsible for the drop. This could be due to either a change in volume or surface area, although studies looking at magnetisation transfer data suggest the latter. Magnetisation transfer can be used to quantify the ratio of bound protons to the total number of protons within a given sample. No change was seen in magnetization transfer ratios in the placenta at different gestations (Ong, Tyler et al. 2004), in particular no change in the bound fraction, which would be expected if macromolecular content had increased. This might suggests that the ratio of the villous tissue (non-vascular component) to total placental volume remains constant throughout gestation, but surface area changes (affecting relaxation times). Further correlation with placental morphometric assessments is, however, required.

Increased heterogeneities are often observed on MR images at later gestations and in complicated pregnancies (Gowland 2005). $T_1$ and to a lesser extent, $T_2$, fall in pregnancies complicated by FGR or pre-eclampsia (Gowland, Freeman et al. 1998). This may be due to the cumulative effects of small regions of infarction and fibrosis. Differences in magnetization transfer ratios were not seen in complicated pregnancies (Ong, Tyler et al. 2004) suggesting that even within the FGR placenta, where the total volume may be reduced, the ratio of non vascular: total volume (presuming a correspondence to bound protons : total protons) remains constant. The significance of these findings is not fully
clear, but may reflect an adaptive placental response, although the small numbers in these studies warrants further investigations.

1.3.4.3 Placental Function

As discussed, blood flow and perfusion can be depicted in several ways using MRI and with the exception of contrast agents, these methods can be applied to the placenta. The safety of contrast agents such as gadolinium remains controversial in pregnancy, as it is passed into the amniotic fluid and re-swallowed, the total fetal exposure is unknown (discussed in (Garcia-Bournissen, Shrim et al. 2006)). In the few women receiving contrast for medical indications (Marcos, Semelka et al. 1997), rapid enhancement of the placenta was demonstrated, initially following the lobular structure and becoming homogeneous with the uterus after around 45 seconds. Contrast imaging has been used to image placental blood flow in mice (Yeh 2006) and establishment of a safe contrast material would no doubt be advantageous for in utero assessment of perfusion. Using ultrasound, contrast micro-bubbles have been trialled as a means of qualitatively, although not yet quantitatively, assessing intervillous blood flow (Orden, Gudmundsson et al. 1999). Potentially these bubbles could also be detected on MRI images, an area which is yet to be explored.

In the placenta, ASL is challenging due to the slow and multi-directional blood flow, despite a high blood volume. FAIR (flow-sensitive alternating inversion recovery) ASL is a technique which can be used as an alternative, with an initial non selective inversion pulse acting on blood within the whole coil area which will in time, flow into the capillary network (Gowland, Francis et al. 1998). Using this technique, the average perfusion rate of the placenta was found to be around 176ml/100mg/min, more than would be predicted
based on knowledge of placental blood flow and weight (Francis, Duncan et al. 1998). This is most likely due to the multidirectional flow in the placenta and hence the measure is of moving blood within a tissue rather than classical perfusion through a tissue.

Mapping of these ‘perfusion’ values across the pixels in the placental image demonstrated that a significantly higher fraction of pixels had low perfusion values (<100ml/min/100g) in pregnancies complicated by FGR (as defined by IBR < 10th percentile) (Francis, Duncan et al. 1998). This study was performed at 0.5 T in 6 healthy pregnant volunteers and 9 women with pregnancies complicated by FGR. The numbers are small and it would be interesting to further investigate this method at increased field strengths where, as mentioned, inherent improvements would be expected.

The alternative technique is intra-voxel incoherent motion (IVIM). Initial studies in 11 women from 20 weeks gestation found the technique to be both sensitive and informative (Moore, Issa et al. 2000). The moving blood fraction (f%) within the placenta averaged at 26%, with a trend towards decreasing at later gestations. Interestingly, in a further study of nine women with normal pregnancies and six women with pre-eclampsia scanned within ten days of delivery, showed a reduction in f% using this technique at the basal plate (maternal fetal interface and region of the maternal spiral artery supply to the placenta) (Moore, Ong et al. 2008). The values at the basal plate appeared relatively constant for various gestations, although a complete dataset (at four different gestations) was only acquired in one individual due to exclusions. A similar pattern in FGR has not been observed, and changes in IVIM in this condition would be interesting to study, as well assessments at early gestations, to establish whether such findings predictive for pre-eclampsia or FGR.
1.3.4.4 Placental Magnetic Resonance Spectroscopy

Placental MR spectroscopy has been used as a research tool for over 20 years with the aim of investigating placental metabolism. Again, technical challenges have restricted the development of the technique and compared to other organs, very little data has been published with limited in vivo analysis. Problems due to movement in fetal MR spectroscopy are not as great in the placenta, however, the availability of expensive hardware and appropriate staff to maintain equipment remains a limitation. Additionally, different methodologies have prevented a cumulative analysis of the available data (McKelvey and Kay 2007). Despite this, differences have been observed between normal and FGR placentas. One such study of phosphorus spectroscopy examined placental tissue after delivery (by caesarean) from third trimester pregnancies complicated by FGR (Kay, Hawkins et al. 1992). Compared to normal pregnancies (in both groups delivered by caesarean and vaginally), mean concentrations of ATP (adenosine tri-phosphate), a molecule fundamental to energy metabolism and exchange, were raised. This is somewhat contradictory to other experiments performed by perfusing placentas in vitro, which suggested that periods of ischaemia would cause ATP to fall (Malek, Miller et al. 1995). It is possible that in FGR there is a compensatory mechanism underlying these effects, however, there are no in vitro studies of FGR placentas to evaluate this more conclusively.

1.3.4.5 Conclusions

The potential for MRI in imaging the fetus and placenta is yet to be fully realised. It is unlikely to supersede ultrasound entirely in the antenatal setting, partly due to its expense and probable lower patient acceptability, but it has a role in the assessment of several fetal anomalies. In the UK, referrals for MRI are increasing and have trebled at some centres...
over the last 5 years (Reeves 2008). The increased diagnosis of CNS and other
abnormalities will no doubt lead to increased terminations, but this should be balanced
against positive outcome of allowing parents to make a more informed decision.
1.3.5 Safety issues relating to Magnetic Resonance Imaging (in detail)

Maternal and fetal concerns regarding the safety of Magnetic Resonance Imaging (MRI) must be addressed when consenting a woman for an MRI scan in pregnancy. In non-pregnant subjects, MRI has been utilized in the clinical setting in more than 150 million persons, with very few serious injuries and no harmful effects demonstrated (Shellock and Crues 2004).

1.3.5.1 General safety concerns of MRI

There are a number of potential safety concerns during MR imaging including

1. Biological effects of the magnetic field.

The direct effect of magnetic fields on human DNA, genes, cells and tissues has been extensively investigated. No potential adverse effects have been clearly verified (WHO 2006). Additionally, there is no evidence for a cumulative effect of MR exposures on health, as demonstrated in health care workers and patients requiring repeat examinations (Kanal, Gillen et al. 1993).

2. Ferromagnetic attractive or ‘projectile’ effects.

Both internal implanted objects (e.g. aneurysm clip) and external (e.g. oxygen cylinder) can move within the magnetic field causing injury and fatalities have occurred (Chen 2001). Many of the injuries sustained during MRI have been secondary to this effect and demonstrate the importance of adhering to screening procedures and safety policies.
3. Potential effects of time varying magnetic field gradients.

During MRI, time varying magnetic field gradients may stimulate nerves or muscles by inducing electric fields in the patient (Shellock 2000). In MR, three orthogonal magnetic field gradients are switched on and off to select the region of diagnostic interest and to spatially encode the MR signals. As a general guide, the faster the imaging, the greater the rate of change of the gradient fields used and the resultant current density induced in the tissue. At extremely high exposure levels, beyond those in routine clinical use, cardiac stimulation may be a concern.

4. Effects of rapidly varying radiofrequency (RF) magnetic fields.

The majority of RF power transmitted for MRI is transformed into heat within the patients’ tissues. This effect is altered by many variables relating both to the MR procedure and individuals physiological response. Overall no excessive temperature elevations or damaging physiologic responses have been demonstrated at field strengths in clinical practice, although injuries have been sustained by excessive heating of conductive materials (e.g. electrodes, guidewires) (Kanal, Shellock et al. 1990).

5. Auditory Considerations.

Various forms of acoustic noise are produced in association with the operation of an MR system. The primary source is the gradient magnetic field activated during the MR procedure. In general, acoustic noise levels recorded for conventional MR procedures are below maximum permissible limits but has the potential to reach dangerous levels (Price, De Wilde et al. 2001).

6. Safety issues from superconductive systems involving large amounts of helium or nitrogen.
7. Psychological effects, such as claustrophobia and anxiety.

Additionally, there are safety considerations relating to the use of MR contrast agents.

1.3.5.2 Safety Concerns Specific to Pregnancy

MRI does not involve exposure to ionizing radiation and therefore does not carry the potential risks associated with X-Rays or Computerized Tomography (CT) imaging. The main imaging modality in pregnancy is ultrasound, which is generally considered safe, although previously concerns have been raised regarding heating effects in the fetus these have not been demonstrated to be harmful (Miller, Nyborg et al. 2002).

Thousands of women have now undergone MRI scanning in pregnancy, with no demonstration of adverse effects. However, the potential for harm in pregnancy may be greater and therefore all possible safety hazards should be considered again individually with respect to the fetus and the pregnant woman.

*Biological effects of Magnetic and Radiofrequency fields*

It is well known that rapidly dividing cells, as in the case of the developing embryo, are susceptible to the affects of a wide range of insults. Although no clear adverse effect have been demonstrated in human tissues (Rodegerdts, Gronewaller et al. 2000), data in the first trimester is limited and therefore MRI at this gestation is generally avoided. The potential effects of the magnetic field on cardiac function at later gestations have also been studied with no change in cardiotocograph parameters (Poutamo, Partanen et al. 1998).
In animal studies, there have been some conflicting results of the effects of in-utero exposure to MRI. A number of studies have shown no adverse effects including studies in mice with prolonged exposure to static fields (up to 624 days over 3 months) (Osbakken, Griffith et al. 1986), high strengths (6.7T) (Murakami, Torii et al. 1992) and pulsed magnetic fields (MRI associated) (McRobbie and Foster 1985). However, a few small studies in mice have suggested a link with intra-uterine growth restriction. Most of these studies are regarding MRI exposures much longer or at much higher strengths than those relating to clinical practice. Those most minimal regimes (still beyond those used in clinical practice) included exposure to 4.7 T for 8 hours (Carnes and Magin 1996) in which fetal weights were between 11 and 17% less than controls, and crown rump lengths were similarly reduced in animals exposed at 9 (time of spinal cord formation) days; no effect was found after exposure at 9 plus 12 days or 12 days (time of reproductive organ development) alone. In a follow up study by the same group at 4.0T (Magin, Lee et al. 2000), differences in CRL and birthweight were only observed in mice exposed to both MRI and ultrasound (ultrasound at day 9, MRI day 12). The sham (outside the magnet) MRI/ultrasound group also showed a downward trend, suggesting that environmental and handling conditions are a factor. (Interestingly, both sham and non-sham groups also showed post exposure weight loss in the mothers.) Another small study showed a reduced CRL compared to controls following exposure to MRI at 1.5T on day 7, although the raw data was not reported (Tyndall 1993). Another study demonstrated an effect on birthweight at 0.35 T after 16 hours of exposure (Heinrichs, Fong et al. 1988). The conflicting results are typical given the different imaging parameters, strains of mice and outcome measures used by the different groups. At high exposure times and field strengths caution is still required, although even within studies performed by the same group inconsistencies are found in the results.
This effect on growth has never been demonstrated in humans. A prospective study of 74 pregnancies exposed to MRI (EPI 0.5T) *in utero* showed no difference in individualized birth ratios when compared to matched controls (Myers, Duncan et al. 1998). Although infant birthweights were lower in the MRI group, this corresponded with a difference in gestational age at delivery (although no difference in premature delivery), which may have been a reflection of increased opportunity for medical intervention in this group.

*Heat*

Heat generated relating to the radiofrequency pulses is not thought to cause significant tissue heating outside the gravid uterus. Several studies have demonstrated only marginal increases in body temperature with MRI (i.e. <0.6°C) and even at very high levels of radio frequency energy exposure, a normal individual is well able to tolerate any changes (Shellock and Shields 2000). The fetus however is surrounded by fluid which is known to be poor at dissipating heat, demonstrated in other organs such as the lens of the eye (Shellock and Crues 1988). Additionally, the gravid uterus may fill the magnet bore, limiting the airflow around the patient and its cooling effect.

Direct *in utero* measurements of temperature increases would be difficult to measure in humans for ethical reasons, but have been measured in pregnant pigs undergoing half Fourier single-shot turbo spin-echo (HASTE). This technique is has the potential to deposit more heat than traditional imaging techniques, however no significant changes in temperature were observed in the fetal brain, abdomen, or amniotic fluid (Levine, Zuo et al. 2001). Additionally, the safety standards relating to radiofrequency fields include limits on the specific absorption rate (SAR). Researchers have used an electromagnetic solver to predict the SAR within the fetus in an anatomically realistic model of a pregnant patient.
and found that at 1.5T (at 64MHz) peak SARs were around 40-60% of those generated in the mother (Hand, Li et al. 2006).

In both clinical and research practice, both 0.5 Tesla and 1.5 Tesla magnets have been extensively used on thousands of women in pregnancy, though safety at 3 Tesla and beyond is much less established. As in adults and neonates, no harmful effects have been demonstrated from the associated magnetic and radiofrequency fields.

Auditory Considerations

The gradient magnetic field is the main source of acoustic noise associated with MRI procedures. Rapid alterations of currents within the gradient coils in the presence of a strong magnetic field give rise to significant forces (Lorentz) which act upon the coils, causing them to vibrate and impact against their mountings which in turn vibrate. This causes loud tapping, banging or chirping noises which will vary with the MRI imaging parameters. The noise can increase with the strength of the magnet and is at its highest with modalities such as echoplanar imaging (EPI), due to the extremely fast gradient gradient switching times and high gradient amplitude.

The problems associated with acoustic noise for adult patients vary from annoyance and difficulty communicating to temporary or potentially permanent hearing loss. Noise is defined in terms of frequency (Hz), intensity (dB) and duration and damage to hearing is dependant on these variables. The sound is affected by the source but also by the environment i.e. the proximity of surfaces that may reflect the sound, quantified by the sound pressure level (SPL). The ear is also not equally sensitive at all frequencies, peaking at around 4Hz, where the most damage can be done. Noise intensity can be weighted to reflect this in the dB (A) weighted, as opposed to linear, scale.
A temporary shift in hearing threshold was reported in 43% undergoing MRI (Brummett, Talbot et al. 1988) without hearing protection, however if the noise is sufficiently injurious, the result could be permanent hearing loss at specific frequencies. Permissible limits in the UK for occupational exposures are peak levels of 135dB and daily/weekly exposure limits of 85dB (HSE 2005). Safety standards for MRI recommend hearing protection is worn when noise levels exceed 85dB, offering a reduction when worn correctly of 10-30dB. Generally noise generated in MRI falls within recognized safety guidelines however EPI has been recorded as generating noise levels of up to 115 dBA (Shellock, Ziarati et al. 1998), a level which would be permissible for 15mins per day by US federal guidelines.

In neonates, numerous studies have suggested a link with prolonged noise exposure (e.g. in neonatal intensive care environments) and resulting cochlear damage (American Academy of Pediatrics 1997), however the effects of short term high noise levels has not been investigated. Preterm infants were also noted to have alterations in monitored vital signs during MRI procedures (Battin, Maalouf et al. 1998) however a detrimental effect on hearing nor any other adverse effect has never been confirmed.

In utero, development of fetal hearing organs is complete by 24 weeks and responses to vibroacoustic stimulation are elicited from this gestation onwards. The threshold for hearing appears to be around 40 dB at 28 weeks but falls near to adult levels by term (13.5 dB). Animal studies have suggested an increased sensitivity of the developing cochlea to noise induced damage (Lenoir and Pujol 1980; Pierson 1996), but this effect has not been demonstrated in humans. Noise exposure has been linked to congenital malformations, pre-term birth and decreased birth weight. In all studies the insult was prolonged noise exposure such as air craft or occupational noise, rather than the short exposures
experienced during MRI, although even here the evidence remains inconclusive (American Academy of Pediatrics 1997).

Attempts have been made to assess the sound levels reaching the fetus in utero. Studies in non-biological and sheep models have demonstrated a complex pattern of transmission of external noise. As expected, the sound is always significantly attenuated to some degree by air and by fluid. Sheep are a good model of human pregnancy as like in humans, the fetus begins to hear in pregnancy and the dimensions of the abdomen of human females and sheep are similar. At very low frequencies, less than 500Hz sound easily reaches the sheep fetal head, but above this sounds are attenuated by around 15-20dB (Gerhardt and Abrams 1996). At very high frequencies the sound pressure within some models would peak and trough, so that attenuation of sound was reduced (Lecanuet, Gautheron et al. 1998). In MRI however, a peak noise levels are found to be at the low to medium frequency region of the spectra, (a characteristic which is similar for different MR systems (Counter, Olofsson et al. 1997)) and are therefore likely to have the most significant attenuation.

In humans, sound transmission has been recorded in utero, after the rupture of membranes, which may lead to an underestimation of noise attenuation, as much of the amniotic fluid impeding the sound may have already been lost. One such study looked at sound transmitted from a vibroacoustic stimulator across frequencies of 55 – 220Hz to a hydrophone attached to the fetal ear under ultrasound guidance following artificial rupture of the membranes (Arulkumaran, Talbert et al. 1992). At a level of 107dB reaching the maternal abdomen, sounds recorded ranged between 65 to 96dB, an attenuation of approximately10 – 40dB. Again, the frequencies tested here are generally lower than those mostly generated by MRI noise, and therefore in MRI the noise is likely to be attenuated to a greater degree. Therefore a value of 10 – 40dB may be an underestimate both by the
presence of more amniotic fluid and by the low to medium sound frequencies generated. Interestingly background noise with no stimulation was up to 68dB, levels that might relate to normal conversation or a vacuum cleaner for adults.

Importantly, these levels however do not account for the impedance of fluid in the outer and middle ears. In sheep, an attempt was made to estimate this effect by measuring the electrical voltage generated (called the cochlear microphonic) in the inner ear in fetal sheep in utero and newborn lambs (Gerhardt, Otto et al. 1992). A much greater attenuation of sound was observed in utero; above 500Hz this was 35 – 45dB, significantly less sound than that reaching the fetal skull. This data would fit with a scientific model; in water, the vibration for a given sound pressure is about 1/4000 of that in air, equating to a difference in sound intensity of around 36dB. If the middle ear is also flooded, a situation which may be analogous to otitis media, a further reduction of around 20dB may occur. Therefore, although not directly measurable, there could be a reduction of a further 56dB on top of the 10 – 40dB predicted from the human experimental model (which may in itself be an underestimate of the situation in MRI with membranes intact).

Although it is difficult to quantify the exact sounds reaching the fetal cochlea, it is equally difficult to analyse what levels cause damage. In sheep the effects of steady state long duration exposures (120dB 16 hour broadband noise) (Gerhardt, Pierson et al. 1999) and impulse noises (20 rounds of 170dB peak -artillery level) (Pierson, Gerhardt et al. 1994) were studied. Changes were seen on histology suggestive that intense, long duration, low frequency sound exposure caused damage rather than high frequency impulse noise. Children with high frequency hearing loss tested aged 4 to 10 years of age were more likely to have been exposed consistently to 85-95dB noise, although one of the several weaknesses in this study was retrospective noise evaluation (Lalande, Hetu et al. 1986). Similar studies with impulse noise insults have not been performed.
Follow up studies of infants exposed to MRI in utero from 20 weeks have demonstrated no adverse effects on hearing. A series of 20 children from ‘high risk’ pregnancies exposed to EPI in utero had no abnormality by 3 years of age (Baker, Johnson et al. 1994). Although no clinical assessment was made as part of the study, the children were all deemed to have normal hearing by standard pathways of care for hearing assessment. A second series compared the results of a routine 8-month distraction test compared to matched controls. These infants had been exposed to MRI at four points during the pregnancy. Again normal results were found (Clements, Duncan et al. 2000). Of note, no other differences in neurological/developmental assessments were demonstrated. Both these studies used EPI, which as mentioned, produces some of the highest noise levels, although in neither study was this data recorded. Additionally both these studies were performed using 0.5 Tesla magnets. An increased risk might be predicted from a 1.5 Tesla magnet due to the increased field strength.

One study in the Netherlands followed up hearing in 35 children aged between 1 and 3 and 9 children between 8 and 9 who underwent MRI in a 1.5T system in the third trimester (Kok, de Vries et al. 2004). Data included health centre assessments at 3, 6, 9, 14, 24 and 36 months as well as a questionnaire regarding current health. Again, the noise levels were not measured but MR sequences included half-Fourier acquisition single shot turbo spin-echo (HASTE), which would be expected to generate high noise levels. The total examination times were between 40 and 70 minutes. No abnormalities in hearing function as a result of MR exposure were observed; 2 children failed the hearing screening at the health centre, one child was diagnosed as having cholesteatoma secondary to recurrent ear infections and the hearing of the second child was found to be normal on further assessment at the ear, nose and throat department.
Although numbers in these follow up studies are small, thousands of *in utero* MRI scans have now been performed and no effect on hearing has been seen. Difficulties arise in assessing the possibility for damage in that direct *in utero* measurements of sound reaching the cochlea cannot be measured ethically in humans and the threshold at which hearing may be affected in the fetus is not clear cut. All the evidence in non-biological, animal and human studies suggest there is a significant attenuation of noise reaching the fetus and that the size of this is probably larger than would be provided by ear protection worn by the adult. In industrial situations caution is advised; the American Conference of Governmental Industrial Hygienists recommended a threshold limit value that called for women to avoid steady state noise levels of $>115$dB and impulses $>155$dB peak (levels which are not exceeded in MRI, even in worst case scenarios). No such pregnancy specific recommendation exists in the UK, but sound levels are likely to be much below 135dB the recommended peak exposure level and for short durations. Efforts should be made to reduce noise where possible and researchers should continue to follow up those exposed to *in utero* MRI. However, women should feel confident that all the evidence suggests that the noise levels reaching the fetus are not of sufficient intensity or for long enough duration to cause damage and indeed an effect in humans has never been demonstrated.

*Maternal Considerations*

In the heavily pregnant woman, comfort within the MRI scanner may be an issue. Women may be scanned on their side or in a left lateral tilt position, with the aid of a support, to reduce vena cavit compression. There is an increased potential for anxiety or claustrophobia, as the abdomen may fill the magnet bore and some women may prefer to enter the scanner feet first. Very few groups have looked at the psychological impact of MRI in pregnancy. One small questionnaire in volunteers suggested it was generally well tolerated and that claustrophobia was uncommon.
Contrast agents

Gadolinium is not recommended for use in pregnancy. It is able to cross the placenta but it’s half life with the fetal circulation is not known, as it is excreted into the amniotic fluid and re-ingested and has demonstrated adverse effects in high doses in animal studies (Magnevist 2000). In 2006, a potential link with nephrogenic systemic fibrosis (a disorder with causes fibrosis of the skin and connective tissues in association with renal failure) has further prohibited it’s use in pregnancy, as the fetus may be more susceptible to developing this condition. Gadolinium has been used shortly prior to delivery for the assessment of placenta accreta (Tanaka, Sohda et al. 2001), when due to timing the effect of reabsorption is minimized.

1.4.5.3 Conclusions on Safety of MRI in Pregnancy

There is no evidence to suggest that MRI is unsafe in pregnancy provided the standard safety limits and protocols applicable to adults are adhered to. Importantly, no adverse affects have been demonstrated from MRI in pregnancy, including no association with FGR (a concern raised inconsistently in mice studies) and no effect on child hearing (it is likely that the amniotic fluid and flooding of the ear provides more than adequate protection). As technology advances and higher field strengths become available, it is right that both researchers and clinicians to continue to assess fetal outcomes. However, at low field strengths, safety of MRI in pregnancy is well established and women they should be reassured of this, as part of the counselling process.
1.4 Summary and Purpose of the Proposed Investigation

The review of the literature in the previous chapters highlights the importance of FGR as a serious complication of pregnancy with associated poor fetal outcomes. It also discusses the difficulties in diagnosing and predicting the condition. In clinical obstetrics, ultrasound biometry and Dopplers of the uterine and umbilical vessels are the main investigations available to diagnose and predict FGR. Despite rapid progress in the use of antenatal ultrasound, a sufficiently predictive test for the condition has yet to be developed. Additionally, it may not be always be possible to differentiate the small, but healthy fetus from one affected by FGR and ultrasound may miss the diagnosis, when the fetal growth is failing, but biometric measurements remain within normal limits. These difficulties in practice lead to large heterogeneous group of women (some with suspected FGR on the basis of biometric fetal measurements or amniotic fluid volumes and some with risk factors for FGR), all undergoing fetal surveillance and even facing unnecessary intervention, as the precise risks to the fetus are difficult to define and may only become clear at a late stage in the disease. Ultrasound Dopplers are an investigation of one aspect of placental function, that is blood flow in the uteroplacental circulations and they have been extremely successful in enabling us to stratify risk to the fetus in suspected FGR. However, as discussed in the review, other aspects of placental structure and function are altered in FGR, including morphology at the gross and microscopic level and blood flow within the placental microcirculations. Although it is not yet known how all such variables relate to fetal risk, development of tools to identify these altered placental features and the ‘placental FGR phenotype’ in utero, may allow more rigorous definitions of the condition, improve our ability to stratify disease and potentially even provide predictive tests for condition.
Specific aims of this project are;

1. To develop and use MRI techniques for examining placental structure and function in normal human pregnancies; including placental volumetry, placental MR relaxation times $T_1$ and $T_2$, and parameters relating to blood flow measured by Intra-Voxel Incoherent Motion (IVIM) and Arterial Spin Labelling (ASL) methods.

2. To carry out MRI examinations and perform measurements of the above placental indices in pregnancies complicated by Fetal Growth Restriction (FGR).

3. To correlate these data with stereological assessments of the placental morphology.

4. To compare data between groups affected by FGR and normal pregnancy, drawing conclusions as to whether MRI could be used as a clinical tool to distinguish these phenotypes.
1.5 Further notes on methodology

*MRI Relaxation times*

Relaxation times are MRI measurements that reflect the recovery of magnetisation following perturbation using a radiofrequency pulse in the scanner. The relaxation times are determined by the molecular environment of the hydrogen nuclei (the hydrogen nucleus is a single positively charged proton) within the tissue. In biological systems, water may be free in blood (or CSF) but is usually associated with large polysaccharides and proteins, which restrict proton movement. Relaxation occurs by two independent processes, referred to as longitudinal (spin-lattice) ($T_1$) and transverse (spin-spin) ($T_2$) relaxation, the rate of which depends upon the interaction between protons and their surrounding environment. The relaxation times $T_1$ and $T_2$ characterise these processes and may be measured non-invasively *in vivo*. Different body tissues have specific characteristic relaxation times, which reflect the number of protons that are ‘free’ i.e. in solution, ‘bound’ i.e. closely associated with macromolecules and those in an intermediate situation, often termed ‘structured’. $T_1$ and $T_2$ measurements were acquired with whole placental coverage, co-localised with the structural scans. The aim of achieving whole placental coverage was to try to allow cross-reference to the placenta when delivered i.e. a placenta lobule of interest on scan be matched to a lobule of interest following placental delivery. $T_1$ measurements were achieved using a 3D multiple flip angle fast field echo (FFE; spoiled gradient echo) technique with angles 2, 10 & 20 degrees with an acquisition time of 10 seconds for each volume (Fram, Herfkens et al. 1987). $T_2$ measurements, with the same geometry, were acquired with a double echo spin echo sequence using echo times of 6.3 ms and 200 ms on a slice by slice basis, repetition times 400ms each echo, taking 4 seconds per slice.
Routine methods of $T_1$ calculation e.g. involving partial saturation and inversion recovery were not selected because of the longer imaging time (in fetal and placental imaging, time is important to eliminate motion and furthermore, we hoped to study a range of parameters within a single MR examination). The method utilized was based on the variable nutation angle and gradient refocusing techniques as described by Fram et al (Fram, Herfkens et al. 1987). The authors found good correlations between this ‘variable flip angle’ technique and an alternative technique (partial saturation), but were able to achieve measurements in a fraction of the time. However, the variable nutation angle technique of $T_1$ calculation is not without limitation. While the $T_1$ values determined are highly correlated with those determined by partial saturation, the absolute values are different. This deviation was not felt to be important for our studies, as it is change in $T_1$ or patterns of change that were of interest, rather than the absolute values.

$T_2$ relaxation time method is a fairly routine and clinically practical spin echo sequence. The most common pulse sequence used in MR imaging is based on ‘spin echo’. It uses 90° radio frequency pulses to excite the magnetisation and one or more 180° pulses to refocus the spins to generate signal echoes named ‘spin echoes’. A 90° excitation pulse rotates the longitudinal magnetisation and the dephasing of the transverse magnetization starts. The following application of a 180° refocusing pulse generates signal echoes. The purpose of the 180° pulse is to rephase the spins, causing them to regain coherence and thereby to recover transverse magnetization, producing a spin echo. Double echo sequences include images with different weightings and / or echo times and obtain in phase and out of phase gradient echo images simultaneously without increasing the measurement time. This made it a good option for imaging the placenta/fetus where rapid scanning is a priority.
T₁ and T₂ values were calculated by fitting the relevant signal intensity relationships to the signal intensities recorded using the acquisitions outlined above using Matlab (The Mathworks). Relaxation times were recorded as median values from the distribution of fits obtained from each voxel across a selected region of interest (ROI). In methodological work up mean values were also calculated, but due to the skewed distribution of relaxation time values, with some very high values, the median value was selected as most relevant. These extreme values are felt to be most likely due to movement or image artefact.

As part of developing the analysis of relaxation times, different techniques for calculating the relaxation times were trialled. Initially, to calculate (n=17 normal pregnancies) T₁ and T₂, a region of interest was selected unsystematically from a homogeneous region of the placental image, unaffected by artifact, of sizes typically ranging between 250 and 800 pixels. For comparison of central and peripheral areas of the placenta, similar sized regions were selected in these areas (see Chapter 1Figure 3B), by visually assessing the site of the cord insertion and plotting the regions either close to this (centrally) or near the placental edge (peripherally). Where the cord insertion was not visible on the placental image, it was easily determined by studying the localising scans or fetal images (part of our normal scan protocol).
Chapter 1 Figure 3a and b. Placental images at 34 weeks. ROI shown are A) an unselected homogenous region and B) regions taken centrally (yellow) and peripherally (red) relative to the cord insertion seen (CI).

Another method used to select regions of interest was also tried, to improve repeatability and to be more in tune with the placental tissue sampling method. Using this method, 8 small regions across a placental region were selected as the ROI, each measuring 9mm x 9mm, giving a total of 648 pixels. The ROI was marked on a scan slice passing at a central depth through the placenta. The 8 regions were placed with 5 in a peripheral region and 3 in a central region, defined as above, to correspond with the methods used following delivery in placental tissue sampling. The mean signal over all 8 regions combined was then used to calculate $T_1$ and $T_2$. This is shown in Chapter 1 Figure 4.
For the third method, the whole placental image as the region of interest again on a mid-depth single slice. To avoid interference from artefact within the region (avoided by placement of the region in the previous method), the median signal values were used to generate $T_1$ and $T_2$. This is shown in Chapter 1 Figure 5.

Chapter 1 Figure 4. Placental image at 34 weeks. ROI taken by combining 8 points across the placenta, 3 central (yellow) and 5 peripheral (red).

Chapter 1 Figure 5. Placental image at 34 weeks. ROI taken by A) method 1; small homogenous region, B) method 2; whole placental region.
For all methods, ROIs were first selected on the plain structural image and then checked against the $T_1$ or $T_2$ sequence images and repositioned if overlying obvious artefact or not correct due to patient movement.

Repeatability was assessed for the 1st method by calculating the within subject coefficient of variation, for both a) taking repeat measurements by selecting different randomly selected homogenous regions of interest on identical scans (intra-observer error). 3 repeated measures were performed in 3 participants. Results are summarised in the table below.

<table>
<thead>
<tr>
<th>Participant</th>
<th>$T_1$ (ms)</th>
<th>$T_2$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Region 1</td>
<td>Region 2</td>
</tr>
<tr>
<td>21</td>
<td>1419</td>
<td>1190</td>
</tr>
<tr>
<td>22</td>
<td>1421</td>
<td>1068</td>
</tr>
<tr>
<td>25</td>
<td>1603</td>
<td>1388</td>
</tr>
</tbody>
</table>

Coefficient of Variation = 0.154

Coefficient of Variation = 0.240

Chapter 1 Table 1. Table showing 3 repeated measures in 3 participants and the relevant within subject coefficients of variation for different ROIs repeated on the same scan. (Method 1, small homogenous region.)
The second method of selecting the region of interest (8 point method) was also compared in terms of repeatability placental tissue sampling. Within subjects coefficients of variation are given below.

<table>
<thead>
<tr>
<th>Participant</th>
<th>( T_1 ) (ms) 8 point</th>
<th>( T_2 ) (ms) 8 point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Region 1</td>
<td>Region 2</td>
</tr>
<tr>
<td>21</td>
<td>1268</td>
<td>1321</td>
</tr>
<tr>
<td>22</td>
<td>1103</td>
<td>1218</td>
</tr>
<tr>
<td>25</td>
<td>1120</td>
<td>1148</td>
</tr>
<tr>
<td></td>
<td>Coefficient of Variation = 0.058</td>
<td>Coefficient of Variation = 0.073</td>
</tr>
</tbody>
</table>

Chapter 1 Table 2. Table showing 3 repeated measurements in 3 participants and the relevant within subject coefficient of variation for ROIs selected using the new 8 point technique. (Method 2, 8 point)

The 8 point ROI was subject to less variation when different regions are selected on the same scan i.e. less intra-observer error. This is probably related to the variation in relaxation times seen across the placenta, so that selecting one large ROI in one area only, is subject to greater variation than selecting 8 smaller points across the placenta. Although regions were repositioned if overlying obvious artefact, subtle artefact effects may also contribute to the variation seen in values seen in different areas. Taking a combined 8 point ROI would reduce the effect of such artefact, as only 1 or 2 small points may be involved, rather than the entire ROI. However, although these methods showed good intra observer variability, it was felt that perhaps they were not sampling the placenta maximally and another technique was trialled.

Prior to this central and peripheral regions of the placenta were compared (from values using the first method) to establish whether they should be assessed separately. The
Wilcoxon’s matched pairs test for non-parametric data was used. There were no significant differences between central and peripheral placental regions in terms of either T₁ (p=0.727) or T₂ (p=0.3379). There was also no clear pattern between paired (same placenta) central and peripheral values, see Chapter 1 Figure 6 and 7. On the basis of this it was decided the most appropriate method was the third method incorporating the whole placental region, as differences between central and peripheral areas were not felt to be significant and this sampled the placenta maximally.
Chapter 1 Figure 6. Scatter plot of T1 values taken from central and peripheral regions. A) Medians and interquartile ranges are plotted. B) Relationship between paired (same placenta) values are shown.
Chapter 1 Figure 7. Scatter plot of $T_2$ values taken from central and peripheral regions. A) Medians and interquartile ranges are plotted. B) Relationship between paired (same placenta values) are shown.
Repeatability was assessed for Methods 1 and 3 by calculating the within subject coefficient of variation by using identical regions on relaxation time scan sequences repeated 3 times during a single MRI examination. 3 repeated measures of relaxation times were calculated in 5 participants. Results are summarised in Chapter 1 Table 3 below.

<table>
<thead>
<tr>
<th>Method</th>
<th>$T_1$ coefficient of variation</th>
<th>$T_2$ coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 1. Small homogenous region</td>
<td>24.9%</td>
<td>31.4%</td>
</tr>
<tr>
<td>Method 3. Whole placental region</td>
<td>17.4%</td>
<td>17.2%</td>
</tr>
</tbody>
</table>

Chapter 1 Table 3. Table showing reporting coefficients of variation when using 3 repeated measures in 5 participants using the same ROI on repeat scans taken during the a single MRI examination.

On the basis of these findings, it was decided that method 3 was appropriate for determining relaxation time and therefore, it is this method used in the studies reported in this thesis. As well as showing good repeatability between scans, it also sampled a large placental area and is representative of the area we aimed to represent stereologically.
Measurements relating to blood flow

IVIM measures quasi random blood movement within a single imaging voxel and results in a bi-exponential signal attenuation in a standard pulsed gradient spin echo (PGSE) experiment. IVIM variables were measured using an interleaved EPI (Echo Planar Imaging) sequence (TE/TR 70ms/5000ms, matrix 128x128 with three slices –in plane resolution 3x3mm, 8mm slice thickness). Images were acquired with diffusion weighting \( b \) of 0, 1, 2, 5, 10, 15, 25, 50, 100, 200, 400 s/mm\(^2\). \( B \) is a MRI parameter which reflects the gradient on a diffusion weighted image. IVIM generates 3 indices: \( D \), \( D^* \) and \( f \). The effects of diffusion, quantified by the diffusion coefficient \( (D) \), and measured in units of mm\(^2\)/sec, are observed at high values of \( b \) and relate to smaller scale movement of water e.g. Brownian motion. The pseudo-diffusion coefficient \( (D^*) \) is associated with perfusion and is measured in units of mm\(^2\)/sec. Signal attenuation due to \( D^* \) is observed at lower values of \( b \), as it relates to larger scale movement. The value \( f \) measures the total volume of blood moving in the voxel compared to the total voxel volume and is quoted as a fraction or percentage \( f \). The complexity of blood movement within the placenta means that is necessary to determine the diffusion for the two separate compartments, \( D \) and \( D^* \). This was analysed by fitting the mean signal in a placental ROI to a bi-exponential decay model, to obtain individual values. Fitting was performed using MATLAB\textsuperscript{©} code. The ROI for calculating IVIM variables was selected as a large homogenous region in the middle each placental slice, unaffected by artefact. This region was drawn on the B0 image (the image acquired with only the static magnetic field applied, before the diffusion sequence is applied) using MRICro\textsuperscript{®} software.

The PGSE sequence employed in the studies has often used to measure \( f \), \( D \), and \( D^* \), predominantly in the brain but has been criticised for its low sensitivity (Turner, Le Bihan et al. 1990) . However, the expected volume of moving blood in the placenta is high,
reducing image signal-to-noise requirements and supporting that the placenta would be an ideal site in which to use this sequence.

In the development of the IVIM methods, it was important to establish whether there were any differences in indices moving across the placenta in a maternal to fetal direction as previous studies suggested an increase in flow, or at least in f, at the basal plate that was less significant in pre-eclampsia (Moore, Ong et al. 2008). A series of experiments were run to explore this, but no differences were found in normal (n=12) or FGR pregnancies (n=7). Attempts were also made to qualitatively examine images, although no clear patterns could be identified other than perhaps increased heterogeneity in FGR. Example images are shown in Appendix III.

Chapter 1 Figure 8. IVIM indices in planes moving across the placenta from a maternal to fetal direction, n=12 normal pregnancies.
Chapter 1 Figure 9. IVIM parameters $D$ and $D^*$ in planes moving across the placenta from a maternal to fetal direction, in FGR pregnancies $n=7$.

Chapter 1 Figure 10. IVIM parameters in planes moving across the placenta from a maternal to fetal direction, in normal pregnancy $n=2$. An different imaging protocol was designed with 7 slices across the placenta for this analysis.
FAIR ASL was used (Kim 1995; Kwong, Chesler et al. 1995). Using this technique two inversion recovery (IR) images are acquired by interleaving slice-selective inversion and non-selective inversion. During the inversion delay time after slice-selective inversion, fully magnetized blood spins move into the imaging slice and exchanges with tissue water. The signal enhancement (FAIR image) measured by the signal difference between two images is directly related to blood flow. The position of an imaging slice can be seen in Chapter 4 Figure 1a, overlaid on a structural image; typically this was a mid-depth single placental slice, running through the long axis of the placenta, giving disc shaped placental images. The sequence used is based on current methodology (Gardener and Francis 2010) with the adaptation that the half-fourier single-shot turbo spin echo (HASTE) acquisition was used following inversion. The scanning was carried out using a 1.5 T Philips Intera system (Philips Medical Systems, Best, NL) FAIR-HASTE ASL sequence parameters, TR/TE=3500/5.4ms; FOV=384x384mm; single slice voxel dimensions 3x3x8 mm$^3$. 7 inversion times were applied (300, 600, 900, 1200, 1500, 1800 and 2300 ms), each with 22 averages and a non-inversion acquisition to estimate initial magnetisation. The slice selective inversion was 20 mm outside the imaging slice and the non-selective inversion 400mm outside the imaging slice. Perfusion (f) was calculated for a region of interest (ROI) in the placenta that was of consistent position throughout scanning and within the placental borders; an example is shown in Chapter 4 Figure 1b. Individual images that were significantly affected by motion or artefact were excluded. Quantification of blood flow into physiologic units was carried out using the Buxton model (Buxton, Wong et al. 1998) applied to the mean signal differences observed in the ROI. This model is more general than alternative earlier models (Detre, Leigh et al. 1992; Williams, Detre et al. 1992) but reproduces these earlier models under more appropriate assumptions. Previous models assume single-compartment kinetics for water clearance and instantaneous
exchange of water between tissue and blood. In the Buxton model, these assumptions are relaxed and an analysis of error if these assumptions are not met is allowed. An example of signal differences with error bars using the ROI fit across time is shown in Chapter 4 Figure 1c. A voxel by voxel fit (perfusion calculated for each voxel and a mean of the all values calculated (a voxel being the 3D unit that make up the image / slice) was also performed as a secondary analysis.

FAIR ASL technique is unable to differentiate between the maternal and fetal circulations. However, given the similarity between perfusion estimates based on expected maternal blood flow to the placenta given above and placental f reported in this study, it would appear that by far the largest contributor to the placental perfusion is the maternal influx. Directionally dependent ASL techniques were explored as part of our preliminary work, but were disappointing. Attempts were made at magnetically labelling blood flowing into the placenta from the maternal aorta, but the time for the primed blood to reach the placenta appeared to be too long and very little signal change was detectable in the placenta. Similarly, attempts were made at priming blood in the region of the uterine arteries; again little signal change was observed, which was thought to be due to being unable to prime a sufficient volume of blood to observe a signal change.
Chapter 1 Figure 7 showing in A a directionally dependent technique (in this case STAR-ASL) where the inversion slab (yellow) and imaging slice through (red). The inversion slab is set to include the aorta and blood flowing in from this ‘tagged’ region can theoretically be detected by a signal change in the imaging slice. In B, FAIR-ASL shows the imaging slice (red). All blood beyond a set border of the imaging slice (white) would be ‘tagged’ and a change in signal sought within the imaging slice as blood flows in from both maternal and fetal directions.
Stereology

Morphometry and stereology are two techniques used commonly in the analysis of tissue morphology and have been used in the placenta (Daayana, Baker et al. 2004; Mayhew 2006). They differ in several aspects; morphometry typically uses random selection method of tissue biopsies whereas stereology uses a systematic random sampling method to ensure unbiased selection of tissue. Both randomly orientate tissue biopsies for slide preparation. Microscopic image analysis is then different; morphometry uses image analysis systems to manipulate field images (usually by pseudocolouration of the screen image) to generate whole areas per field (e.g. to give an indication of villous to non villous tissue) or drawing around surface manually to calculate surface area per field (e.g. to give an indication of capillary surface area). Stereology uses a grid lattice placed over the image to allow point or intersect counting to generate the same values.

Stereology is concerned with extrapolating threedimensional (3D) structural quantities from simple counts or measurements made on 2D sectional images. Stereology is dependent upon random sampling, which is applied at all levels. Its virtue (and strength) resides in the fact that random sampling gives each part of the specimen, and every direction within it, an equal chance of being selected. Random sampling is, by definition, unbiased sampling. Stereology aims to be an unbiased numerical estimation of parameters, such as length, area, volume, that characterize the tissue. On the other hand, computer morphometry was developed to quantify the 3D morphology of structures seen in light microscopy, ranging in size from individual cells to larger anatomical entities. The researcher uses a set of criteria, e.g. colour when stained, to select the parts of the tissue that are to be analyzed. The goal is to obtain accurate representations of the anatomical structures. Computer microscopy's aim is accurate 3D mapping and quantification of biological tissue whether it be of individual cells, groups of cells, or entire histological
regions. Computer microscopy is not per se a statistically oriented methodology and was originally was not intended to perform sampling operations.

Taking into account the pros and cons of the different techniques, a robust stereological technique was decided upon for use in this study, as numbers of placentas were likely to be small. Initial work using morphometric techniques was however performed in a small number of women with normal pregnancies. These results are presented in Appendix 2.

The stereological technique in detail was, as for all the studies included in this thesis, from those reported by Mayhew (Mayhew, Ohadike et al. 2003; Mayhew, Manwani et al. 2007). Placentas were collected postnatally within 30 minutes of delivery time to prevent changes to tissue structure from dehydration and to try and replicate the in-vivo situation clearly. A random sampling method was devised that would allow more in-depth analysis of placental heterogeneity, matching regions of the placenta examined for morphology, with corresponding regions on the MR scan i.e. a mid depth MRI placental slice was matched with a mid depth analysis of placental morphology. This alters the reference volume for stereological calculations and therefore absolute values concerning the whole placenta are not reported here. A transparent grid with pre-numbered sampling windows at the intersections was placed with random orientation over the placenta. Taking the placental cord insertion as the central point, a circle was marked out with a diameter half that of the longest axis of the placenta. Within this circle was marked as ‘central’ and outside this circle was ‘peripheral’. Full-depth columns of tissue were dissected by superimposing the transparent grid over the placenta, bearing a systematic array of sampling windows (4cm apart). Using a random computer-generated number sequence, tissue samples were taken from the placenta until a minimum of 3 windows overlying the central region had been sampled. The ratio of central to peripheral windows sampled using
this method was generally 3:5. The resulting full depth tissue samples were then dissected into a top (fetal facing), middle and bottom (maternal facing) placental section (to allow analysis in relation to specific slices of the MR images) and fixed in 10% buffered formalin. Only middle sections are considered in this analysis, to correspond with the mid-depth level of the MRI scan plane. These samples were diced and embedded with random orientation in paraffin wax blocks and processed for histology.

Before imaging, cut sections (5 micrometres) were mounted on microscope slides, stained with haematoxylin and eosin (H&E), and observed under a Leitz (Dialux 22) microscope at a magnification of x250. Approximately 8 sections were investigated per placenta. For image analysis, a Q-Imaging (Fast 1394) digital camera acquired images from each sample. For each section, 5 fields were selected for imaging in a systematic fashion, moving around the section in system of partial turns of stage adjuster. In total, approximately 40 high-power fields of view were analysed per placenta. A typical field of view is shown in Chapter 1 Figure 11.

Images were acquired using Image-Pro Plus software (Version 6.3). Test point counting was employed to obtain volume densities. Intersection counting using a superimposed grid lattice was used to calculate surface area densities. Indices calculated included villous volume density, fetal capillary volume density, fibrin (intervillous fibrin deposition) volume density, villous surface area density, capillary surface area density. From this interfiles space (IVS) volume density (1 – villous volume density), total blood volume density (IVS + capillary volume density), ratio of fibrin: villous volume densities, ratio of capillary: villous volume densities and total surface area density (villous + capillary surface area densities) were calculated.
Chapter 1 Figure 11. Microscope images showing A) normal villous appearance with H&E stain and B) villous containing large amounts of fibrin staining bright pink.
Chapter 2

Magnetic Resonance Imaging Relaxation Time Measurements of the Placenta at 1.5 T
This chapter is the published article:


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2.1 Abstract

Placental insufficiency is a major cause of fetal growth restriction (FGR) and accumulating evidence indicates several aspects of placental morphology are altered in this condition. MRI provides quantitative indices that may be used in non-invasive assessment of the human placenta, such as relaxation time measurements, $T_1$ and $T_2$. We hypothesised that placental relaxation times relate to alterations in placental tissue morphology and hence may be useful in identifying the changes associated with FGR. We report on the first phase of testing this hypothesis, in a study of women in normal pregnancy.

**Aims:** To assess relaxation time measurements in the placenta in normal pregnancy and correlate these with gestational age and stereological analyses of placental morphology following delivery.

**Methods:** 30 women underwent MRI examination (1.5 T) between 20 and 41 weeks gestation. Placental $T_1$ and $T_2$ measurements were acquired from a mid-depth placental region, co-localised to a structural scan. Fixed, wax-embedded sections of these placentas collected at delivery were stained with haematoxylin/ eosin and subjected to stereological analysis.

**Results:** Placental $T_1$ and $T_2$ show a significant negative correlation with gestation, (Pearson correlation $p=0.01$, 0.03 respectively). 17 placentas were analysed stereologically. In the group as a whole there was no significant correlation between $T_1$ and $T_2$ and morphological features. However, in a subset of 7 pregnancies scanned within a week of delivery, a significant positive correlation was observed between the fibrin
volume density and the ratio of fibrin: villous volume densities and T2 (Spearman correlation p=0.02, 0.03 respectively).

**Discussion:** The correlations between placental T1 and T2 and gestation show that these variables are clearly influenced by changes in placental structure. Fibrin might be a key component but further work is needed to fully elucidate the major structural influences on placental T1 and T2.
2.2 Introduction

Fetal growth restriction (FGR) is a serious complication of pregnancy in which the fetus fails to reach its own genetically determined growth potential. It is common, affecting 3-10% of all first time births (Chiswick 1985) and is linked to a third of all antepartum deaths (CEMACH 2008). Placental insufficiency is a major cause of FGR and accumulating evidence indicates that several aspects of placental structure and function are specifically altered in this condition, including gross morphology (Toal, Keating et al. 2008), villous morphology at a microscopic level (Daayana, Baker et al. 2004), blood flow in the uteroplacental circulations and placental transport mechanisms for nutrients and ions (Sibley, Turner et al. 2005).

In clinical obstetrics, ultrasound biometry and Doppler ultrasound of the uterine and umbilical vessels are the main investigations available to diagnose and predict FGR. However, it may not always be possible to differentiate the small but healthy fetus from one affected by FGR. These difficulties particularly affect the large heterogeneous groups of women with suspected FGR (on the basis of biometric measurements, reduced amniotic fluid volumes, abnormal uterine artery Dopplers or risk factors for FGR), all undergoing intensive fetal surveillance or facing unnecessary intervention, as prognosis for the fetus is difficult to predict and a deterioration may only occur late in disease, if at all.

Doppler ultrasound of umbilical blood flow is an assessment of only one aspect of placental function (and does not directly measure blood flow in the placental microcirculations, the site of increased resistance (Mitra, Seshan et al. 2000; Baykal, Sargon et al. 2004; Mayhew, Wijesekara et al. 2004)), whilst several other aspects of placental structure and function are altered in FGR (Sibley, Turner et al. 2005). Various morphological changes occur in placental tissue in association with FGR. Gross placental
lesions are detected occasionally and are associated with poorer fetal outcomes and abnormal Doppler waveforms (Madazli, Somunkiran et al. 2003; Sebire and Sepulveda 2008). However, there are often more subtle alterations, such as impoverished development of the villi (Daayana, Baker et al. 2004) and changes in placental exchange barrier surface area and thickness (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004; Pallotto and Kilbride 2006; Mayhew, Manwani et al. 2007). Such subtle changes are not directly detectable using routine ultrasound, although altered vessel morphometry may in turn influence umbilical Doppler waveforms (Mitra, Seshan et al. 2000). The constellation of specific morphological and physiological abnormalities in the placenta has been proposed to represent a placental phenotype(s) of FGR (Sibley, Turner et al. 2005). The development of tools to identify a placental FGR phenotype in utero, may allow more rigorous definitions of the condition, improve our ability to stratify disease and potentially provide specific predictive tests.

Magnetic Resonance Imaging (MRI) provides a range of quantitative indices that may be used in non-invasive assessment of the human placenta; these remain relatively unexplored in this tissue and there is a need for further evaluation of their use as biomarkers of pregnancy disease. At the gross morphology level, MRI has been shown to detect changes in placental thickness and volume in FGR as compared to normal pregnancy (Damodaram, Story et al. 2010). However, other MRI markers may relate to fine tissue structure and function and therefore be good candidates for more detailed in utero placental assessment, with greater discrimination between placental phenotypes. The major source of image contrast in MRI comes from the variation in relaxation times between tissues. Relaxation times are MRI measurements that reflect the rate of hydrogen nucleus recovery following perturbation using a radiofrequency pulse in the scanner. The relaxation times are determined by the molecular environment of the hydrogen nuclei (the hydrogen nucleus is a single positively charged proton) within the tissue. In biological
systems, water may be free in blood (or CSF) but is usually associated with large polysaccharides and proteins, which restrict proton movement. Relaxation occurs by two independent processes, referred to as longitudinal (spin-lattice) \( (T_1) \) and transverse (spin-spin) \( (T_2) \) relaxation, the rate of which depends upon the interaction between protons and their surrounding environment. The relaxation times \( T_1 \) and \( T_2 \) characterise these processes and may be measured non-invasively \textit{in vivo}. Different body tissues have specific characteristic relaxation times, which reflect the number of protons that are ‘free’ i.e. in solution, ‘bound’ i.e. closely associated with macromolecules and those in an intermediate situation, often termed ‘structured’. Theoretically, \( T_1 \) will be shortest in tissues with mainly ‘structured’ protons, longer when ‘free’ and longest when ‘bound’. \( T_2 \) will be longest where protons are ‘free’ in solution and shortest where bound to macromolecules(Bottomley, Foster et al. 1984). These parameters have been studied in many clinical disorders but, although generalisations can be made regarding their histopathological correlates, it is not known specifically what changes in the placenta influence relaxation. In studies examining other tissue types, water content (related to vascular and intervillous volumes in the placenta) and membrane surface areas were found to be important influences on relaxation times(Cameron, Ord et al. 1984). As yet, no study investigating possible correlations between postnatal placental villous morphology and quantitative relaxation times has been conducted.

In this study we have examined relaxation time measurements in the placenta in normal pregnancy and evaluated evidence for correlations between these measurements and postnatal morphology of the placenta using stereological techniques. Based on the work described above in other tissues, indices relating to blood and non-blood volumes of the placenta were examined as potential structural correlates, as well as consideration of villous and vascular surface areas. The fibrin content of the placenta was also assessed; as a macromolecule, this may have an important influence on relaxation times in the placenta.
and is related to relaxation time values in other disease processes such as atherosclerosis and liver cirrhosis (Yu, Song et al. 2000; Bartolozzi, Cioni et al. 2001). In previous MRI studies at lower field strengths (0.5 T), a trend for shorter relaxation time measurements, $T_1$ and $T_2$, was observed in pregnancies complicated by pre-eclampsia and FGR (Gowland, Freeman et al. 1998). We hypothesised that the differences observed at 0.5 T in FGR placentas were related to altered placental morphology and therefore that $T_1$ and $T_2$ may be prognostic biomarkers of the abnormal placental structure associated with this condition, where there is high risk for adverse pregnancy outcome. To begin testing of this hypothesis, we investigated placental relaxation times in normal pregnancies at the increased field strength of 1.5 T, allowing improvements over the 0.5T measurements in signal-to-noise ratio and spatial resolution.

### 2.3 Methods

#### 2.3.1 Subjects

Ethical approval was obtained from the local NHS Research Ethics Committee and all women gave written informed consent. Women with normal pregnancies between 20 and 42 weeks gestation were recruited. Participants included had apparently uncomplicated pregnancies and subsequently delivered normal infants at term without adverse outcomes. Exclusion criteria were co-existing maternal systemic disease such as diabetes, renal disease or microvascular disease, multiple pregnancies, fetal anomalies and antenatal complications including pre-eclampsia, fetal growth restriction and pre-term delivery. Neonates with an individualised birthweight ratio (IBR) less than the 5th centile, a surrogate for FGR (Wilcox, Johnson et al. 1993; Sanderson, Wilcox et al. 1994), were excluded.
2.3.2 MRI

Women underwent a single MRI examination including acquisition protocols for placental structure and relaxation time measurements ($T_1$ and $T_2$). All scans were carried out on a 1.5 T Philips Achieva scanner using a 5 channel phased array cardiac coil positioned to optimise the signal from the placenta. The pregnant women were positioned supine, using a foam wedge to support a left-lateral tilt (preventing vena cava compression) and introduced feet first into the scanner, with their heads outside the bore to reduce potential for claustrophobia. Although no evidence has demonstrated any detrimental effects to the fetus as a result of MRI, in the interests of adopting a cautious approach, “SofTone” imaging magnetic field gradients were used, keeping gradient ramp speeds to below 50% of maximum to reduce noise exposure. Furthermore, the specific absorption rate (SAR) was restricted to 2 W/kg to minimise thermal effects. These restrictions represent hard limits on acquisition protocol optimisation, which aimed to achieve measurements within reasonable time limits, reducing issues of fetal motion and maximising patient comfort. All scanning sessions were limited to a maximum of 40 minutes, which was well tolerated by the women.

Women first underwent a series of 3 localising images, for orientation, followed by structural and relaxation time acquisitions, taking slices along the long axis of the placenta and through the shortest axis, giving disc shape placental images. This allowed whole placental coverage with the minimum number of slices. Structural scans were carried out using an implementation of the single shot fast spin echo sequence used routinely in fetal scanning; with a field of view of 384 x 384 cm$^2$, matrix size 128 x 128, 30 slices (interleaved), resolution 3x3x3 mm$^3$ and half scan (55%) to obtain the image in a single acquisition lasting 36 seconds. $T_1$ and $T_2$ measurements were acquired with whole placental coverage, co-localised with the structural scans. $T_1$ measurements were achieved
using a 3D multiple flip angle fast field echo (FFE; spoiled gradient echo) technique with angles 2, 10 & 20 degrees with an acquisition time of 10 seconds for each volume (Fram, Herfkens et al. 1987). T\textsubscript{2} measurements, with the same geometry, were acquired with a double echo spin echo sequence using echo times of 6.3 ms and 200 ms on a slice by slice basis, repetition times 400ms each echo, taking 4 seconds per slice.

T\textsubscript{1} and T\textsubscript{2} values were calculated by fitting the relevant signal intensity relationships to the signal intensities recorded using the acquisitions outlined above using Matlab (The Mathworks). Relaxation times were recorded as median values from the distribution of fits obtained from each voxel across a selected region of interest (ROI).

The ROI was drawn as the largest placental region possible ensuring that the outer borders were not crossed and typically encompassed between 500 – 1500 voxels. The ROI was marked on a scan slice passing at a mid-depth through the placenta and was always first selected on the structural image, (where the placental outline and cord insertion are most clearly seen) and then checked against the co-localised relaxation time scans and repositioned if affected by obvious artefact or patient movement. Chapter 2 Figure 1 shows a typical structural image through the placenta with the ROI selected.
Chapter 2 Figure 1. Structural scan through the placenta at 36 weeks showing placental ROI outline.
2.3.3 Stereology

All stereological analyses were made in placentas collected from women undergoing MRI using methods adopted from those reported by Mayhew (Mayhew, Ohadike et al. 2003; Mayhew, Manwani et al. 2007). Placental tissue samples were collected in 17 subjects delivered at term. Placentas were collected postnatally within 30 minutes of delivery time. A random sampling method was devised that would allow more in-depth analysis of placental heterogeneity, matching regions of the placenta examined for morphology, with corresponding regions on the MR scan i.e. a mid depth MRI placental slice was matched with a mid depth analysis of placental morphology. This alters the reference volume for stereological calculations and therefore absolute values concerning the whole placenta are not reported here. A transparent grid with pre-numbered sampling windows at the intersections was placed with random orientation over the placenta. Taking the placental cord insertion as the central point, a circle was marked out with a diameter half that of the longest axis of the placenta. Within this circle was marked as ‘central’ and outside this circle was ‘peripheral’. Full-depth columns of tissue were dissected by superimposing the transparent grid over the placenta, bearing a systematic array of sampling windows (4cm apart). Using a random computer-generated number sequence, tissue samples were taken from the placenta until a minimum of 3 windows overlying the central region had been sampled. The ratio of central to peripheral windows sampled using this method was generally 3:5. The resulting full depth tissue samples were then dissected into a top (fetal facing), middle and bottom (maternal facing) placental section (to allow analysis in relation to specific slices of the MR images) and fixed in 10% buffered formalin. Only middle sections are considered in this analysis, to correspond with the mid-depth level of the MRI scan plane. These samples were diced and embedded with random orientation in paraffin wax blocks and processed for histology.
Before imaging, cut sections (5 micrometres) were mounted on microscope slides, stained with haematoxylin and eosin (H&E), and observed under a Leitz (Dialux 22) microscope at a magnification of x250. Approximately 8 sections were investigated per placenta. For image analysis, a Q-Imaging (Fast 1394) digital camera acquired images from each sample. For each section, 5 fields were selected for imaging in a systematic fashion, moving around the section in system of partial turns of stage adjuster. In total, approximately 40 high-power fields of view were analysed per placenta.

Images were acquired using Image-Pro Plus software (Version 6.3). Test point counting was employed to obtain volume densities. Intersection counting using a superimposed grid lattice was used to calculate surface area densities. Indices calculated included villous volume density, fetal capillary volume density, fibrin (intervillous fibrin deposition) volume density, villous surface area density, capillary surface area density. From this interfiles space (IVS) volume density (1 – villous volume density), total blood volume density (IVS + capillary volume density), ratio of fibrin: villous volume densities, ratio of capillary: villous volume densities and total surface area density (villous + capillary surface area densities) were calculated.

2.3.4 Statistics

The normality of the distribution of the median relaxation time and stereological values across the subject group was tested using D’Agostino and Pearson omnibus normality test: $T_1$ and $T_2$ results were normally distributed and therefore analyses of their relationships with gestation were performed using parametric tests. Not all stereological variables were normally distributed, therefore non-parametric tests were used for all analyses involving stereological variables. Test of correlations were consistently two-tailed. Linear regression
analysis was performed to define linear trends in the data. Graphpad Prism (Version 4.01) was used for statistical analysis. No corrections were made for multiple statistical comparisons.

2.4 Results

2.4.1 Relationship between placental relaxation times and gestation

30 women underwent a single MRI examination at a range of gestations between 21.9 and 41.7 weeks (mean 32.9). Of the 30 women scanned, one scan was terminated before completing the entire T₁ sequence due to maternal claustrophobia, thus there were 29 remaining T₁ results.

Chapter 2 Figure 2 shows the relationship between relaxation times and gestational age at the time of scan. A significant negative correlation was seen between both T₁ and gestational age (Pearson’s p= 0.01) and T₂ and gestational age (Pearson’s p= 0.03). When expressed as a linear regression, this showed a fall in T₁ across gestation of 20.2 ms/week (95% confidence interval -35.1 to -5.2 ms/week, intercept = 1684ms, r²=0.22, p=0.01) and in T₂ of 2.4 ms/week (95% confidence interval -5.6 to -0.3 ms/week, interval intercept = 280 ms, r²=0.16, p=0.03). (Distribution of data across gestation; weeks 20-23+6, n=1, mean T₁ 1407ms, mean T₂ 217ms; weeks 24-27+6, n=7, mean T₁ 1170ms, mean T₂ 225ms; weeks 28-31+6, n=8, mean T₁ 1000ms, mean T₂ 195ms; weeks 32-35+6, n=2, mean T₁ 935ms, mean T₂ 198ms; weeks 36-39+6, n=10, mean T₁ 904ms, mean T₂ 194ms; weeks 40-41+6, n=2, mean T₁ 1017ms, T₂=163ms.)
Chapter 2 Figure 2. Graph showing relaxation times plotted against gestational age at time of scan. The linear regression lines for $T_1$ and $T_2$ are also shown.
2.4.2 Correlations between placental stereological analyses and relaxation times

From the 30 patients who were scanned, 17 placentas were analysed stereologically following delivery. Failure to analyse placentas was due to non-collection e.g. if the researcher was not informed of delivery, the woman delivered elsewhere or the researcher was not available. No significant correlations were observed between relaxation times measured at time of scan and placental stereological variables.

As placentas were collected following term deliveries (between 37 and 42 weeks, mean age of delivery 39.8 weeks), often some time after the MRI scan, and bearing in mind the change in $T_1$ and $T_2$ over gestation, three further analyses were performed. In the first, a subgroup population who all had scans within a week of delivery were analysed. In this subgroup (n=7, mean gestational age at scan 38.9 weeks, at delivery 39.1 weeks), significant correlations were observed between $T_2$ relaxation time and the fibrin volume density and fibrin: villous densities ratio (Spearman’s $p=0.02$ and $0.03$ respectively) (Chapter 2 Figure 3). In the second analysis, a subgroup of women who were scanned within 2 weeks of delivery (n=10, mean gestational age at scan 38.9 weeks, at delivery 39.5 weeks), were analysed: no significant correlations between relaxation times and stereological variables were observed. In the third analysis, the linear regression model for the relationship between gestational age and relaxation times (Chapter 2 Figure 2) was used to predict $T_1$ and $T_2$ values for each of the seventeen women (whose placentas had been analysed stereologically) for the exact day of their delivery. Using this method no significant correlations were found between $T_1$ and $T_2$ values and placental stereological variables.
Chapter 2 Figure 3. Graphs showing relationships between a) fibrin volume density and b) the fibrin:villous volume densities ratio and relaxation times in the subgroup population scanned within 1 week of delivery. Significant correlations were observed with $T_2$ (a) $p=0.02$ and b) $p=0.03$). Linear regression analysis demonstrated a linear trend (plotted line) between $T_2$ and fibrin volume density, $r^2=0.57$, $p=0.05$, and the ratio of fibrin: villous volume density, $r^2=0.59$, $p=0.04$. 
2.5 Discussion

The most important finding in this study is a clear correlation and linear relationship between placental $T_1$ and $T_2$ measurements at 1.5 T and gestational age. This relationship was previously demonstrated at 0.5 T (Gowland, Freeman et al. 1998), but given the differences in imaging and analysis methodologies, software and the effect of increased field strength on relaxation and particularly on $T_1$, the fact that this relationship persists in our study adds considerably to its validity. $T_1$ and $T_2$ relaxation times are shown to decrease by 20.2 ms and 2.4 ms per week of gestational 1.5 T respectively, giving a total fall of in $T_1$ of 400 ms and in $T_2$ of 50 ms between 20 and 40 weeks. The relationship supports the proposition that changes in placental tissue structure or composition that occur with advancing gestation significantly influence relaxation times. Of note, the gradient of decline in $T_1$ across gestation is twice that seen at 0.5 T (Gowland, Freeman et al. 1998), which may be attributable to the increase in field strength and might also support that the previously demonstrated differences in relaxation times in pregnancies complicated by FGR and pre-eclampsia (Gowland, Freeman et al. 1998), may be more readily observed at this field strength.

Our observations on gestational changes in placental $T_1$ and $T_2$ are similar to those seen at 0.5 T (Gowland, Freeman et al. 1998). However, our study at 1.5T shows a sharper decline in $T_1$ across gestation than was reported at 0.5 T. It is known that field strength strongly influences $T_1$ (Bottomley, Foster et al. 1984) and the trend for a steeper decline across gestation at 1.5 T than at 0.5 T (gradient 20.2 ms/week at 1.5 T and gradient 9.1ms/week at 0.5 T) suggests an enhanced ability to differentiate placentas with longer or shorter relaxation times at the higher field strength. This additional differentiability may be important, as shorter relaxation times have previously been associated with complicated pregnancies (Gowland, Freeman et al. 1998).
The origin of the observed change in relaxation times with advancing gestational age remains a matter for conjecture. In this study, relaxation time values did not correlate with villous volume densities or total blood volume densities, indicating that other changes in morphology or function, rather than just increases in solid or blood volumes, lead to the fall in $T_1$ and $T_2$ towards term. Previous studies of placental morphology support that villous densities do not change with gestational age, rather the total volume of the villi increase, owing to hyperplastic growth of the placenta (Mayhew, Wadrop et al. 1994). The total volume of the maternal vascular bed increases with gestational age (Mayhew, Jackson et al. 1993), but if this was the major influence on relaxation times then a rise rather than fall would be seen, as the $T_1$ and $T_2$ of oxygenated maternal blood \textit{in vivo} is around the upper end of the placental values (Barth and Moser 1997). That other changes in placental morphology may influence relaxation times, rather than the balance of vascular and non-vascular volumes, is supported by studies looking at MRI magnetisation transfer (an \textit{in-vivo} measure of bound protons to total protons) in the placenta which demonstrated no changes in these values or in non-vascular: total placental volume ratios assessed stereologically across gestation (Ong, Tyler et al. 2004). Other studies have also supported the view that the ratios of total volumes of blood and non-blood placental compartments remain fairly static across gestation (Boyd 1984) and if so, the fall in $T_1$ and $T_2$ observed across gestation must be due to other changes within placental tissue, rather than blood volume alone.

All placentas included in the analysis were delivered at term when large amounts of placental fibrin were likely to be present. Fibrinoid volume has previously been shown to
correlate positively with intervillous volume and villous surface area, but relative to intervillous volumes, increases significantly towards term (Mayhew and Barker 2001). Our data show a correlation between T_2 and placental fibrin deposition, but only when scan and delivery were within one week of each other (Chapter 2 Figure 3). As all analysis groups have similar mean gestational age at delivery, the correlations observed cannot be explained by the presence of more or less fibrin in placentas of any group and are more likely to reflect the importance of close timing of MRI and stereology. These data suggest that fibrin content might be one of the contributors to the fall in T_2 over gestation, although it is unlikely to be the sole determinant. Intuitively, a negative rather than positive correlation between fibrin and relaxation times would be expected as we might assume that the complex macromolecular structure of fibrin deposits would result in shorter relaxation times. Fibrin may, however, be a surrogate marker for a wider change in tissue quality that is influencing T_1 and T_2. Villous surface area may be a factor influencing relaxation times, (surface area of the villi expands with advancing gestation (Teasdale 1980; Boyd 1984; Teasdale and Jean-Jacques 1985)) but we did not find a significant correlation between these variables. It is likely that other factors not investigated here may also influence relaxation times. Potential candidates include reduced blood oxygenation (for example, deoxygenated blood generally has lower T_1 values (Silvennoinen, Kettunen et al. 2003)), variations in the fluid content of stromal channels in the villi (Castellucci and Kaufmann 1982) or changes in the tissue hydration fraction; found to be important in other tissues in animal studies (Cameron, Ord et al. 1984).

In this study, women were scanned deliberately at a range of gestations in order to assess the effect of gestational age on relaxation times. This caused difficulties in the analysis when relating MRI findings to stereology, as the stereological variables were always measured at delivery. There was a correlation between T_2 and fibrin content when only
those placentas delivered within one week of scan were considered, but not when all delivered within two weeks of scan were included, emphasising the importance of taking gestational changes into account. We attempted to do this by modelling the gestational changes in $T_1$ and $T_2$ and using the predicted values for day of delivery in correlations with stereological values. For several indices, a tighter relationship was seen using this approach but none reached significance at the 5% level. Although a significant linear relationship was observed between relaxation times and gestation, the broad confidence interval surrounding this slope may have hindered the linear regression model as an accurate predictor of $T_1$ and $T_2$ at delivery. In future studies we hope to scan more women closer to term in order to closely assess the links between MRI biomarkers and morphology, removing the influence of gestation. In practical terms this can be problematic, as delivery may be unpredictable, MR scanning may be prevented due to size of the gravid uterus or discomfort lying flat/still and women may also be less amenable to participation in research in the last few days before delivery.

Histological studies have demonstrated a range of differences in placentas from FGR pregnancies including evidence of tissue damage and repair, increased inter-villous fibrin deposition and reduced villous volume, surface area and capillary surface area (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004; Pallotto and Kilbride 2006; Mayhew, Manwani et al. 2007). Previous studies at 0.5 T demonstrated that in pregnancies complicated by pre-eclampsia and FGR, relaxation times were generally lower and frequently below the 90% confidence interval for the healthy population. From the stereological data presented in this current study it is unclear which morphological variables, if any, directly influence placental relaxation times and therefore whether a difference in relaxation times in FGR could be expected. However, the clear correlation with both $T_1$ and $T_2$ and gestation at scan supports the view that relaxation times may be important biomarkers of placental tissue change and therefore may be useful in detecting
pathological conditions such as FGR. Further work to test this hypothesis requires stereological measurements of the placenta together with measurement of relaxation times and other MRI variables closer to term. These structural measurements might also be combined with MRI assessment of placental blood flow such as those measured by Intra-Voxel Incoherent Motion and/or Arterial Spin Labelling.
Chapter 3

Potential Magnetic Resonance Imaging Biomarkers of the Placenta in Fetal Growth Restriction
This section is the article planned for submission for publication in the journal Obstetrics & Gynaecology:

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Potential Magnetic Resonance Imaging Biomarkers of the Placenta in Fetal Growth Restriction

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3.1 Abstract

MRI provides quantitative indices for use in the non-invasive assessment of the human placenta, such as measurements of placental shape and size, relaxation time measurements, $T_1$ and $T_2$, which relate to placental composition, $D$, $D^*$ (coefficients of diffusion and pseudo-diffusion) and $f\%$ (the fraction of moving blood per voxel) which relate to perfusion, measured using Intra-Voxel Incoherent Motion (IVIM). We hypothesised these indices could be assessed using MRI in utero and would be altered with advancing gestation and in FGR pregnancies, relating to changes in placental tissue morphology which occur in these states.

Methods: 53 (39 normal, 14 FGR) women underwent MRI examination between 20 and 41 weeks. Parameters of placental shape and size, placental relaxation times $T_1$ and $T_2$ and IVIM measurements $D$, $D^*$ and $f$ were acquired from a mid-depth placental region. Sections of these placentas collected at delivery were stained and subjected to stereological analysis of morphology.

Results: There was a significant positive correlation between placental volume, depth, the depth: volume ratio and gestation in normal pregnancy ($p=>0.0001, p=0.001$ and $0.02$ respectively) Placental volume was significantly lower in the FGR group (ANCOVA $p=0.0003$) and depth was significantly greater in FGR (ANCOVA $p=0.004$), by around 10mm. There was a highly significant difference in $log_{10}$ depth: volume between normal and FGR groups (ANCOVA $p=<0.000001$). In this analysis the correlation previously reported between $T_2$ and gestation in normal pregnancy was again significant ($p=0.002$) but not $T_1$. A significant difference was observed in $T_2$ between normal and FGR groups (ANCOVA $p=0.01$) but not $T_1$. Analysis of the IVIM variables $f$, $D$ and $D^*$ was possible in 26 participants; 16 normal and 10 FGR pregnancies. There was a significant negative
correlation between D and gestation in normal pregnancy (p=0.039) which was reduced in FGR pregnancies (ANCOVA p= 0.02). T₂ and D also correlated with stereological indices of placental morphology.

**Discussion:** These finding show that MRI placental biomarkers are altered with advancing gestation and in FGR. Further investigation is required to determine whether their use in FGR could be beneficial.
3.2 Introduction

Fetal growth restriction (FGR) is a serious complication of human pregnancy where the fetus fails to reach its genetically pre-determined growth potential. It is a common condition, affecting 5 -15 % of all pregnancies (depending on definition used) (Gardosi 2009) and is linked to a third of all antepartum deaths (CEMACH 2008). Growth restricted infants are at increased risk of neonatal morbidities, including intraventricular haemorrhage, respiratory distress syndrome and necrotizing enterocolitis (Gilbert and Danielsen 2003), often worsened by iatrogenic prematurity. In addition, serious neurological sequelae, such as cerebral palsy, are more common (Jarvis, Glinianaia et al. 2003) along with behavioral and emotional problems in childhood (Zubrick, Kurinczuk et al. 2000). Small size at birth also predisposes the infant to major diseases in adult life, such as diabetes, hypertension and cardiovascular disease (Hales, Barker et al. 1991; Leon, Lithell et al. 1998).

In clinical obstetrics, ultrasound biometry and Doppler assessment of the uterine, umbilical and fetal vessels is the mainstay of investigation. Nevertheless, these methods may still fail to differentiate a small but healthy fetus from one affected by FGR. Indeed, abnormal umbilical Dopplers may predict those at risk of adverse outcomes, particularly perinatal death (Madazli, Somunkiran et al. 2003; Sebire and Sepulveda 2008), but small for gestational age infants, with normal umbilical artery Dopplers, may still be at risk of adverse perinatal outcomes and subsequent neurodevelopmental complications (Figuera, Eixarch et al. 2007).

Placental insufficiency is a major cause of FGR and accumulating evidence supports that placental structure, function and blood flow are invariably altered in this condition. These potential pathologic features range from gross changes in placental size and structure
(Toal, Keating et al. 2008; (Damodaram, Story et al. 2010), changes in morphology at the microscopic level (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004), and attenuations in placental nutrient transport (Sibley, Turner et al. 2005). This constellation of abnormalities provides a placental phenotype for FGR (Sibley, Turner et al. 2005) and implies a more global assessment of placental characteristics would allow more rigorous definition of the condition, its clinical scarification and potentially its early prediction.

Magnetic resonance imaging (MRI) provides a range of quantitative indices that relate to tissue structure and function. It is therefore a realistic but relatively unexplored candidate for non-invasive in utero placental assessment. In this study we consider MR indices in 3 areas; those relating to macromolecular structure e.g. placental volume and depth, MR relaxation times, which relate to placental tissue composition, and indices measured by intravoxel incoherent motion (IVIM), which relate to blood movement. Recent studies have re-highlighted the importance of considering aspects of gross placental structure; both Toal et al. (Toal, Keating et al. 2008) using ultrasound and Damodaram et al. using MRI (Damodaram, Story et al. 2010) demonstrated a link between placental thickness and FGR. We hypothesised similarly, that placental dimensions would be altered in FGR and aimed to determine which parameters showed the greatest differences between groups and how these were placed in the context of alternative indices of structure and function.

Relaxation times $T_1$ and $T_2$ are MRI measurements that reflect the rate of recovery of magnetisation following perturbation using a radiofrequency pulse in the scanner.

Relaxation times $T_1$ and $T_2$ characterise the processes by which this recovery occurs and may be measured non-invasively in vivo. Different body tissues have specific characteristic relaxation times, which broadly reflect the number of protons that are ‘free’ i.e. in solution, ‘bound’ i.e. closely associated with macromolecules. In previous work we demonstrated that $T_1$ and $T_2$ measurements of the placenta were significantly affected by gestation and that macromolecules such as fibrin may influence relaxation times (Wright,
Following on from this, we hypothesised that $T_1$ and $T_2$ are altered in FGR, due to the abnormal placental morphology associated with this condition. IVIM techniques assess diffusion within a tissue by measuring the attenuation of signal when water protons in motion are travelling far enough within a magnetic field to become dephased (i.e. desynchronised in their inherent spins from other protons, giving a corresponding reduction in signal). These techniques are therefore sensitive to blood motion (Moore, Strachan et al. 2000; Moore, Ong et al. 2008). Blood (or other fluids such as lymph, flowing in any direction will contribute towards the attenuation, rather than capillary flow in isolation. Signal attenuation due to $D^*$, measured in units of mm$^2$/sec, is observed at lower values of b as it relates to larger scale movement and is the parameter most closely linked to perfusion. Diffusion, quantified by the diffusion coefficient (D) and also measured in units of mm$^2$/sec, is observed at higher values of b and are related to smaller scale movement of water. By performing this diffusion weighted MRI with at least two diffusion weightings, or b values, the differential signal attenuation at different b values can be used to calculate the diffusion coefficient (known as D or the apparent diffusion coefficient, ADC). In its simplest form, the slope of the line that describes the relationship between the logarithm of the signal intensity and the b value is D. If the calculation is repeated for each voxel of the DW-MRI, then D for every image voxel can be derived. Thus, voxels that show a steeper slope of signal attenuation with increasing b values will have higher D values (indicating higher water diffusivity) compared with voxels that show a gradual slope of signal attenuation. Cellular tissues (e.g., tumor tissues) in the body often return lower D values compared with native tissues, which facilitates their detection and characterization. The value of f measures the total volume of blood moving in the voxel compared to the total voxel volume and is quoted as a percentage. This is particularly of relevance when considering IVIM in the placenta, in which flow is slow and multi-directional. IVIM has been used to assess placental blood flow...
flow at 0.5 T (Moore, Strachan et al. 2000) and is of interest in FGR pregnancies as a means of assessing blood flow in the placental microcirculations.

Studies to date have touched on MRI as a potentially useful tool for *in utero* placental assessment, but there is a need for greater knowledge of what information can be provided and a better understanding of how these altered MRI indices relate to real differences in placental tissue structure and fetal outcomes. In the study reported here, we tested the overall hypothesis that analysis of indices of gross placental structure, placental relaxation times (relating to tissue composition) and placental IVIM (relating to blood flow) would be altered in FGR and relate to placental morphological characteristics assessed stereologically following delivery. Furthermore, we hypothesized that at field strengths of 1.5 T, improvements in signal-to-noise ratio and spatial resolution would improve placental visualisation and allow more accurate analysis compared to the majority of previous studies in this field which were conducted at 0.5 T.
3.3 Methods

3.3.1 Subjects

Ethical approval was obtained from the local NHS Research Ethics Committee and all women gave informed written consent. Women were recruited at a range of gestations between 20 and 42 weeks. Two groups of women were studied: those of normal pregnancy and those complicated by FGR. The FGR group were recruited on the basis of an ultrasound-estimated fetal weight <5th centile; this was confirmed after delivery with a customised birthweight centile <5 – a surrogate for FGR (Gardosi, Chang et al. 1992), calculated by Growth Centile Calculator (UK v5.15) (Gardosi J and Gestation Network). Neonates with birthweights between 5th and 10th customised centiles were excluded. Exclusion criteria for both groups included co-existing maternal systemic disease (diabetes, renal or microvascular disease), multiple pregnancies, the presence of fetal anomalies (antenatally or postnatally diagnosed), or the onset of pregnancy complications, e.g. pre-eclampsia, gestational diabetes or obstetric cholestasis. Women were classified as normal if they delivered at term (37-42 weeks gestation) without adverse maternal or neonatal outcomes. Some of the women in the normal pregnancy group were also included in our previous study investigation of placental relaxation times (Wright, Morris et al. 2011). In interpreting the FGR data, severity of disease was considered by birthweight. Umbilical Doppler assessments were not considered as they had not been performed as part of the study and timing in relation to scan was variable.
3.3.2 MRI

Pregnant women underwent a single MRI examination in the Manchester Wellcome Trust Clinical Research Facility. All scans were carried out on a 1.5T Philips Intera scanner using a 5 channel phased array cardiac coil, positioned to optimise the placental signal. The women were introduced feet first into the magnet, with their heads outside to reduce potential claustrophobia. They were positioned supine, using a foam wedge to support a left-lateral tilt, preventing inferior vena caval compression. Although safety of MRI in pregnancy has not been questioned, in adopting a cautious approach the specific absorption rate (SAR) was restricted to 2W/Kg to minimise thermal effects and “SofTone” gradients were used with gradient ramp speeds below 50% maximum to reduce noise exposure. These restrictions represent hard limits on sequence optimisation, aimed at achieving measurements within a reasonable time limit, with minimal fetal motion and patient discomfort. All scanning sessions were limited to 40 minutes maximum.

MRI examinations consisted of an initial series of 3 localising images. Localisers were obtained in sagital, coronal and axial orientations relative to the maternal abdomen. In all cases, the field of view was 375mm², with a resolution of 256x256 giving pixel size of 1.47mm. 15 slices were acquired, each 7mm thick with 1mm slice gap. A structural scan was acquired using a sequence running ‘coronally’ through the placenta (i.e. through the shortest axis and parallel to the width of the placenta, giving a disc shaped image). This sequence used an implementation of the single shot fast spin echo sequence used routinely in fetal scanning; with a field of view of 384x384cm², matrix size 128x128, 30 slices (interleaved), resolution 3x3x3 mm³ and half scan (55%), to obtain a single shot over 36 seconds. As far as possible, whole placental coverage was obtained with image slices in a ‘coronal’ plane to the placenta. From the utero-placental interface, placental volume was assessed manually by drawing a region of interest (ROI) on each placental image in
sequence using MRICro® (version 1.40) software (a computer package for quantitative MRI analysis). Chapter 3 Figure 1a shows a typical structural image with ROI selected. The volume of the placenta for each slice was calculated from the number of pixels in each ROI, multiplied by volume per pixel (in this case 3x3x3mm³). The total placental volume was then calculated by addition of the volumes of all placental ROIs. Volumes were measured by a single observer, blinded to patient identity, gestation and group.

Measurements of placental dimensions were performed using the Philips DICOM viewer version 2.6. For placental maximal depth, the cross sectional view of the placenta was selected from localizing scans (this was usually the axial image, but was dependent on placental orientation) and measurement taken from uteroplacental interface to placental surface, perpendicular to the uterine wall. Chapter 3 Figure 1b shows a typical axial image with a suggested maximal depth measurement. To obtain the width and length of each placenta, an image slice was selected from the placental volumetric scan (disc shaped image) which was taken at a mid-depth within the placenta. Width and length were measured between the borders of the uteroplacental interface, at right angles to each other. Each set of placental dimensions were measured by a single observer, blinded to patient identity, gestation and group.

T₁ and T₂ measurements were acquired with whole placental coverage, co-localised with the structural scans as described previously (Wright, Morris et al. 2011). T₁ measurements were achieved using a 3D multiple flip angle fast field echo technique (FFE; spoiled gradient echo) with angles of 2, 10 and 20 degrees. A 10 second acquisition was used for each volume. T₂ measurements, with the same geometry, were acquired with a double echo spin echo sequence using echo times of 6.3ms and 200ms on a slice-by-slice basis, repetition times 400ms each echo, taking 4 seconds per slice. T₁ and T₂ values were calculated using Matlab software (The Mathworks 2008a/2010a) by fitting the relevant
signal intensity relationships to signal intensities recorded using the acquisitions above. Relaxation times were recorded as median values across an ROI from the distribution of fits from each voxel. The placental ROI was drawn as the largest placental region possible, ensuring that the outer borders were not crossed, this typically encompassed 500–1500 voxels. The ROI was marked on a scan slice passing at a mid-depth through the placenta, and was always first selected on the structural image (where the placental outline and cord insertion are more clearly visible). These were then checked against the co-localised relaxation time scans and repositioned if affected by obvious artefact or patient movement. The measurements were again made by a single observer, blinded to identity, gestation and subject group.

IVIM measures quasi random blood movement within a single imaging voxel and results in a bi-exponential signal attenuation in a standard pulsed gradient spin echo (PGSE) experiment. IVIM variables were measured using an interleaved EPI (Echo Planar Imaging) sequence (TE/TR 70ms/5000ms, matrix 128x128 with three slices –in plane resolution 3x3mm, 8mm slice thickness). Images were acquired with diffusion weighting (b) of 0, 1, 2, 5, 10, 15, 25, 50, 100, 200, 400 s/mm². B is a MRI parameter which reflects the gradient on a diffusion weighted image. IVIM generates 3 indices: D, D* and f. The affects of diffusion, quantified by the diffusion coefficient (D), and measured in units of mm²/sec, are observed at high values of b and relate to smaller scale movement of water e.g. Brownian motion. The pseudo-diffusion coefficient (D*) is associated with perfusion and is measured in units of mm²/sec. Signal attenuation due to D* is observed at lower values of b, as it relates to larger scale movement. The value f measures the total volume of water moving within in the voxel compared to the total voxel volume and reflects the flowing blood volume, often quoted as a fraction f or percentage f. The complexity of blood movement within the placenta means that is necessary to determine the diffusion for the two separate compartments, D and D*. This was analysed by fitting the mean signal in
a placental ROI to a bi-exponential decay model, to obtain individual values. Fitting was performed using MATLAB© code. The ROI for calculating IVIM variables was selected as a large homogenous region in the middle each placental slice, unaffected by artefact. This region was drawn on the B0 image (the image acquired with only the static magnetic field applied, before the diffusion sequence is applied) using MRICro® software. All measurements were made by a single observer, blinded to identity, gestation and group. An example and typical ROI for a B0 image is shown in Chapter 3 Figure 2.
Chapter 3 Figure 1. a) Structural scan ‘coronally’ through the placenta at 36 weeks (at 1.5 T) showing placental outline; the border at the uterine interface is easy to define. Regions such as this were drawn manually on all images through the placenta using MRICro® and the volume on each slice added together to give a total placental volume. b) Typical localiser scan (axial plane) at 31 weeks (1.5 T), used to measure maximal placental depth (D).
Chapter 3 Figure 2. Example of IVIM B0 image, ROI (red), f, D and D* map across the placental ROI. The B0 image is the initial image acquired before the various MR gradients are applied to acquire the IVIM parameters. f is the fraction of moving water to non moving water per voxel, which reflects the flowing capillary blood volume; high values are white, low are black on the greyscale. D (the diffusion coefficient, reflecting the Brownian motion of water) and D* (the pseudo-diffusion coefficient, reflecting blood movement in a randomly orientated capillary network) are plotted with the highest values being white. Of note, these functional parameters appear in part to reflect the structural heterogeneity of the placenta.
3.3.3 Placental morphology

Stereology was performed on delivered placentas from women who had undergone an MRI scan. The methods used were adopted from Mayhew et al. (Mayhew, Ohadike et al. 2003; Mayhew, Manwani et al. 2007) as described by Wright, Morris et al. (Wright, Morris et al. 2011). Placentas were collected postnatally within 30 minutes of delivery. Systematic random sampling was performed by superimposing a transparent grid haphazardly over the placenta, bearing a systematic array of sampling windows (4cm apart). Using a random computer-generated number sequence, full-depth columns of tissue were dissected until 8 windows had been sampled. The resulting tissue was further subdivided equally into top (fetal facing), middle and bottom (maternal facing) placental sections, and fixed in 10% buffered formalin. These divisions reflected specific slices of MR images previously taken. On this occasion, only middle sections were considered, along with their associated mid-depth MR images. These samples were diced and embedded with random orientation in paraffin wax blocks, before being processed for histology. As this protocol alters the reference volume for stereological calculations, absolute values concerning the whole placenta could not be reported.

Before imaging, cut sections (5 micrometres) were mounted on microscope slides, stained with hematoxylin and eosin (H&E), and observed under a Leitz (Dialux 22) microscope at a magnification of x250. Approximately 8 sections were investigated per placenta. For image analysis, a Q-Imaging (Fast 1394) digital camera was used with Image-Pro Plus software (Version 6.3). For each section, 5 fields were selected in a systematic way, moving around the section in sequence of partial turns of the stage adjuster. In total, approximately 40 high-power fields of view were analysed per placenta. Test point counting was employed to obtain volume densities. Intersection counting, using a superimposed grid lattice, was also used to calculate surface area densities. Indices
calculated included: (i) villous volume ratio, (ii) intervillous volume ratio, (iii) fetal capillary volume ratio, (iv) fibrinoid volume ratio (intervillous fibrin-like deposition), (v) villous surface and (vi) capillary surface area densities. From these, total blood volume (intervillous + capillary volume ratios), ratio of fibrinoid: villous volume ratios, ratio of capillary: villous volume ratios, and total surface area densities (villous + capillary surface area densities) were calculated.

All placentas were assessed stereologically at delivery, but subset analysis was performed for placentas scanned within a week of delivery, in an attempt to negate the lag between MR and stereological variables (see Wright, Morris et al., 2011). Where this was not achieved (due to limited numbers), multivariate regression analyses was performed to account for gestational effects.

3.3.4 Statistics

The distribution of variables was assessed and data transformed where appropriate for parametric analysis. Two-tailed testing was employed for all correlations. Graphpad Prism (version 4.01) was for used statistical analysis and Graphpad InStat (version 3.10) for multiple regression analysis. IBM SPSS (version 20.0.0) was used for analysis of co-variance (ANCOVA) with homoscedasticity confirmed by Levene’s test. ROC curves were generated using Graphpad Prism (version 4.01).
3.4 Results

A total of 39 women with normal pregnancies and 14 with FGR were scanned. The characteristics of both groups are summarised in Chapter 3 Table 1. Of the 11 FGR pregnancies, 14 had birthweights below the 1st centile of the customised chart.

All 53 pregnancies were analysed when considering placental volumes and dimensions and relaxation times, bar one $T_1$ measurement in the normal group, in which the scan was incomplete. Due to the IVIM MR protocols being developed and introduced later in the study period, 26 pregnancies (16 of the normal group and 10 of the FGR group), were available for this analysis.
Chapter 3 Table 1. Summary of pregnancy and delivery characteristics for normal and FGR groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Group (n=39)</th>
<th>FGR Group (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± s.d)</td>
<td>33.5 ± 5.3</td>
<td>33.2 ± 3.8</td>
</tr>
<tr>
<td>Parity (%primparous)</td>
<td>43.6%</td>
<td>42.6%</td>
</tr>
<tr>
<td>Maternal BMI (mean ± s.d)</td>
<td>25.3 ± 4.4</td>
<td>24.0 ± 4.3</td>
</tr>
<tr>
<td>Gestation at delivery, wks (mean ± s.d)</td>
<td>39.7 ± 1.4</td>
<td>35.6 ± 3.7</td>
</tr>
<tr>
<td>Birthweight at delivery, g (mean ± s.d)</td>
<td>3446 ± 488</td>
<td>1781 ± 686</td>
</tr>
<tr>
<td>Birthweight centile (mean ± s.d)</td>
<td>42.5 ± 27.9</td>
<td>0.6 ± 1.2</td>
</tr>
</tbody>
</table>
3.4.1 Measurement of Placental Volume and Dimensions

There was no significant difference between the gestation at time of scan between normal and FGR groups for this comparison: mean±SE 33.5 ± 0.9 weeks and 33.2 ± 1.0 weeks respectively, unpaired t-test, although correction for gestation was made in the analysis when comparing groups by the use of ANCOVA.

Unsurprisingly, there was a significant positive correlation between placental volume in normal pregnancy and gestation (Pearson’s $r^2=0.67$, $p=>0.0001$), although this was not replicated in the smaller sized FGR group (Pearson’s $r^2=0.50$, $p=0.07$); the correlations appeared similar, albeit with greater variability in the FGR group. Placental volume was significantly lower in the FGR group when gestation was not considered (unpaired t-test $p=0.003$) and when gestation was corrected for (ANCOVA $F=14.879$, $p=0.0003$, partial $\eta^2=0.23$). The corrected means for volume were (mean±SE) 814.3± 35.7cm$^3$ and 545.7± 59.7cm$^3$ for the normal and FGR groups respectively. There was no significant interaction between group and gestation for this analysis.

There was a significant positive correlation between placental depth in normal pregnancy and gestation (Pearson’s $r^2=0.51$, $p=0.001$), this again was not replicated in the smaller FGR group (Pearson’s $r^2=0.27$, $p=0.34$), although the correlations appeared similar with greater variability in the FGR group. Placental depth was significantly greater in the FGR group (unpaired t-test $p=0.01$) and more so when gestation is corrected for (ANCOVA $F=8.874$, $p=0.004$, partial $\eta^2=0.15$). The corrected means for volume were (mean±SE) 39.6 ± 1.7mm and 49.4 ± 1.7mm for the normal and FGR groups respectively. There was no significant interaction between group and gestation for this analysis.
Depth: volume ratio was also considered as this had been highlighted as of interest in the study by Damodaram et al. (Damodaram, Story et al. 2010). There was a significant positive correlation between placental depth: volume ratio in normal pregnancy and gestation (Pearson’s $r^2=-0.37, p=0.02$), although this was not replicated in the FGR group (Pearson’s $r^2=-0.35, p=0.21$), again with similar correlations but greater variability seen in the smaller FGR group. Placental depth: volume was significantly greater in the FGR group when gestational effects are not considered (unpaired t-test $p<0.0001$). For ANCOVA analysis, log$_{10}$ transformation of the depth: volume ratio was used, as this demonstrated better homoscedasticity between normal and FGR groups (Levene’s test $p=0.185$) whilst maintaining a normal distribution. When gestation was considered as a co-variate, there was a highly significant difference in log$_{10}$ depth: volume between normal and FGR groups ($F=39.683$, $p=<0.000001$, partial $\eta^2=0.442$). The corrected means for the log$_{10}$ depth: volume ratio was (mean±SE) -1.299 ± 0.23 and -1.017± 0.38 for the normal and FGR groups respectively. There was no significant interaction between group and gestation for this analysis. The results for log$_{10}$ depth: volume is illustrated in Chapter 3 Figure 3. A further analysis of placental depth (width x length) was performed; there was no correlation between this parameter and gestation in either normal or FGR groups (p=0.52 and 0.52 respectively). As this variable was not normally distributed the log$_{10}$ transformation was used to compare groups which were significantly different (ANCOVA $F=0.132$, $p=0.0002$, partial $\eta^2=0.314$). (Corrected mean±SE = -2.47 ± 0.05 and -2.76 ±0.03 for normal and FGR groups respectively.) There was no interaction between group and gestation in this analysis (p=0.50) and variance was uniformly distributed (Levene’s test $p= 0.132$).
Chapter 3 Figure 3. Log placental Depth: Volume ratio in normal and FGR pregnancies plotted against gestational age. The most severe cases of FGR in terms of birthweight <2kg are highlighted in red.
3.4.2 Placental Relaxation Times

As noted measurement of placental relaxation times was possible for all 53 pregnancies examined excluding one participant in the normal group in whom the T₁ sequence was incomplete, therefore, as above, the groups were well matched in terms of gestation at scan. Relaxation time values are presented in Chapter 4 Figure 4. We previously reported a significant negative correlation between placental relaxation times and gestation in normal pregnancy (Wright, Morris et al. 2011). In this analysis the correlation between T₂ and gestation in normal pregnancy was again highly significant (Pearson’s r²=-0.47, p=0.002). For T₁, a trend to decrease with advancing gestation was observed, although this was not significant in this dataset (Pearson’s r²=-0.05, p=0.17). There was no correlation with either T₁ or T₂ and gestation in the smaller FGR group (Spearman’s p=0.15 and p=0.14 respectively); these data were analyzed using non-parametric tests due to the almost bimodal distribution of the data in the FGR groups. There was a significant difference in T₂ values between the normal and FGR pregnancy groups when compared directly (Mann-Whitney p=0.02) but no significant difference in T₁ (Mann-Whitney p=0.37). When gestation was corrected for using ANCOVA a significant difference was observed in T₂ between groups (ANCOVA F=7.045, p=0.01, partial η²=0.123, corrected mean±SE normal group 199.274±5.018, and FGR group 173.351±8.378). However, should be interpreted with caution, given an interaction seen between group and gestation in this analysis (p=0.03) and variance of T₂ may not be homogenous across groups (Levene’s test p=0.05). No significant difference was observed in T₁ between normal and FGR groups when considering gestation as a co-variate (ANCOVA F=1.626, p=0.208, partial η²=0.032, Levene’s test p=0.996, Interaction between group and gestation p=0.696). The difficulty in analysis of T₂ lies in the bimodal distribution seen in the FGR group, which cannot be normalized by standard transformations. One further analysis was
performed in an attempt to make sense of the two possible populations seen in the FGR group by splitting this group according to birth weights, either greater or less than 2kg. Although numbers are small (>2kg n=6, <2kg n=8), this split produced curves of 2 populations that appeared more normally distributed. Accounting for any gestational effect, the bigger >2kg babies were similar to the normal group in terms of the mean T$_2$ (ANCOVA F 0.244, p=0.624, partial $\eta^2=0.006$, >2kg mean ±SE 195.134±12.149) whereas the smaller <2kg babies, had significantly lower T$_2$ values as compared to the normal group (ANCOVA F12.620, p=0.001, partial $\eta^2=0.223$, <2kg mean±SE 159.310±10.368).

We previously reported a correlation between placental T$_2$ and the placental fibrin content in normal pregnancy (Wright, Morris et al. 2011). This relationship persisted in this analysis; placentas delivered within a week of the scan (thereby reducing the lag between scan and assessment of placental morphology), showed a significant positive correlation between T$_2$ and the placental fibrin volume ratio assessed stereologically in normal pregnancy (n=10, Spearman’s $r^2=0.709$, p=0.027). No further correlations were observed between relaxation times and morphological variables in either normal (n=23) or FGR groups (n=5). Fewer FGR placentas were collected, as decision to delivery time was often short and the research team had not been made aware of the plan to deliver.
Chapter 3 Figure 4. Placental relaxation times, $T_1$ and $T_2$, in normal and FGR pregnancies plotted against gestational age. The most severe cases of FGR in terms of birthweight <2kg are highlighted in red.
3.4.3 IVIM Indices

As noted, analysis of the IVIM variables $f$, $D$ and $D^*$ was possible in 26 participants; 16 normal and 10 FGR pregnancies. The normal and FGR groups appeared well matched in terms of gestation at scan (mean±SE normal group 35.0 ±1.3, mean FGR group 32.3 ±1.3, unpaired t-test $p=0.193$), but analyses to account for gestational effects were also performed when comparing groups.

The relationship between $D$ and gestation in normal and FGR groups is shown in Chapter 3 Figure 5. There was a significant negative correlation between $D$ (the diffusion coefficient) and gestation in normal pregnancy (Pearson’s $r^2=-0.519$, $p=0.039$), which was not observed in FGR ($r^2=-0.307$, $p=0.389$), although there remained a trend for the values to fall towards term. There was also a significant difference between $D$ in FGR and normal pregnancy groups when compared directly (unpaired t-test $p=0.013$) and when gestation was considered as a co-variate (ANCOVA $F=12.502$, $p=0.02$, partial $\eta^2=0.343$). The adjusted mean±SE for $D$ were 2.00mm$^2$/mm$^3±0.117$ and 1.302mm$^2$/mm$^3±0.154$ for normal and FGR groups respectively. Of note, there was no interaction between gestation and group for this analysis ($p=0.99$) and variance in $D$ was uniform across groups (Levene’s test $p=0.41$).

There was no significant correlation between $f$ and $D^*$ and gestation in either group and no significant differences in these values between normal and FGR groups (ANCOVA $f$ $p=0.915$ and $p=0.984$ respectively).

IVIM indices were also correlated with placental morphological variables. In normal pregnancy $D$ was shown to correlate with placental villous volume ratio positively and intervillous space volume (and total blood volume ratio) negatively (n=11, Spearman’s
relationship persisted and was more significant when FGR pregnancies were also included in the analysis (total n=15 Spearman’s $r^2=0.554$, $p=0.032$ and $r^2=-0.525$, $p=0.0445$ respectively). When only placentas delivered within a week of the scan were considered (to negate the effects of the lag between scan and morphological analysis) the results failed to reach significance in this smaller group (n=7 normal pregnancy, n=9 all pregnancies). Multivariable regression analysis was also performed to allow analysis of the whole group whilst attempting to reduce the effect of varying gestation at the time of scan. In normal pregnancy this supported a relationship between D and the villous volume ratio when gestation at scan was accounted for (n=11, $r^2=0.559$, $p=0.038$). Furthermore, this relationship persisted and was more significant if FGR pregnancies were also included in the analysis (n=15, $r^2=0.461$, $p=0.025$). Correlations were also observed between $f$ and the intervillous volume ratio and total blood volume ratio in normal pregnancy (n=11, Spearman’s $r^2=-0.908$, $p=0.026$ and $r^2=-0.618$, $p=0.043$ respectively). No correlations were observed between $D^*$ and morphological variables in normal pregnancy.
Chapter 3 Figure 5. Placental D (the diffusion coefficient) in normal and FGR pregnancies plotted against gestational age. The most severe cases of FGR in terms of birthweight <2kg are highlighted in red.
3.4.4 Stereological Variables

Comparison of stereological variables between normal and FGR groups demonstrated a significantly reduced villous surface area density in FGR placentas (n=5) in comparison to the normal group (n=23) (unpaired t-test p=0.037), consistent with previous studies (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004). (Mean±SE Villous surface area normal 229.0±7.2 cm$^2$/cm$^3$ and FGR 192.9±11.2cm$^2$/cm$^3$).

3.4.5 Predictive Testing

The results for the above parameters are summarized in Chapter 3 Table 2. Although numbers were small, where differences between FGR and normal pregnancies were most statistically significant (see Chapter 3 Table 2), ROC (Receiver Operator Characteristic) curves were constructed, in an exploratory fashion, as a means of investigating and comparing the potential of these biomarkers in the assessment of FGR. As each parameter had been measured at a range of gestations, where gestation had a significant effect on the variable, results were expressed as a percentage of the predicted normal value for that gestational time point; the predicted normal values were estimated using the linear regression model for each parameter across gestation. The actual results were then expressed as a percentage of normal in relation to the predicted value for the same gestation and ROC curves constructed from these values. ROC curves for placental depth: volume ratio (and adjusted as a percentage of normal), T$_2$ relaxation time (and adjusted as a percentage of normal) and D (and adjusted as a percentage of normal) can be seen in Chapter 3 Figure 6.
Chapter 3 Table 2. Summary of placental measurements significantly different between normal and FGR groups (correlations with gestation were all significant, p<0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of Participants</th>
<th>Correlation with gestation</th>
<th>ANCOVA Normal vs FGR</th>
<th>Adjusted mean Normal group</th>
<th>Adjusted mean FGR group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>FGR</td>
<td>($r^2$)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Placental Volume</td>
<td>39</td>
<td>14</td>
<td>0.67</td>
<td>0.0003</td>
<td>814.3cm$^3$</td>
</tr>
<tr>
<td>Placental Depth</td>
<td>39</td>
<td>14</td>
<td>0.51</td>
<td>0.0040</td>
<td>39.6mm</td>
</tr>
<tr>
<td>Placental Depth: Volume</td>
<td>39</td>
<td>14</td>
<td>-0.37</td>
<td>&gt;0.001</td>
<td>-1.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(log10 values)</td>
<td></td>
<td>(log10 values)</td>
</tr>
<tr>
<td>Relaxation time $T_2$</td>
<td>39</td>
<td>14</td>
<td>-0.47</td>
<td>0.01</td>
<td>199.3ms</td>
</tr>
<tr>
<td>D (diffusion coefficient)</td>
<td>16</td>
<td>10</td>
<td>-0.52</td>
<td>0.02</td>
<td>2.0mm$^2$/mm$^3$</td>
</tr>
</tbody>
</table>
Chapter 3 Figure 6. ROC curves of potential MRI biomarkers for FGR. The area under the curves were as follows: a) Depth: Volume Ratio, 0.8790, p=<0.0001; b) Depth: Volume Ratio % of predicted normal, 0.8974, p=>0.0001; c) T2, 0.7097, p=0.020; d) T2% of predicted normal, 0.6813, p=0.045; e) D, 0.7625, p=0.027, f) D% of predicted normal, 0.8529, p=0.002.
3.5 Discussion

These data represent the first study combining structural MR analysis with quantitative MR indices of both placental composition and function, based on proton behavior within the tissue, in both normal and FGR pregnancies. The most important finding is that differences can be detected in the FGR placenta relating to gross structure, microstructure and water movement; identifying a placental phenotype in FGR that has reduced volume but is thickened, has low T_2 relaxation times and low values of D, the diffusion coefficient.

At the gross morphology level, MRI has previously been shown to detect changes in placental thickness and volume in FGR as compared to normal pregnancy (Damodaram, Story et al. 2010). The potential importance of MR analysis in FGR is further seen in a study demonstrating that placental pathologies (e.g. infarction) detected by MRI correlate with histological findings and pregnancies at risk for adverse outcome and fetal death, independently of umbilical artery Doppler status (Linduska, Dekan et al. 2009). Data from our study is consistent with previous publications (Toal, Keating et al. 2008; Damodaram, Story et al. 2010), showing significant differences between normal and FGR pregnancies in terms of placental volume, depth and particularly the placental depth: volume ratio. This simple parameter was in fact the most convincing as a potential biomarker of FGR when considering the partial η^2 and ROC curves. Although the small sample sizes must be considered in the interpretation of this analysis, the data suggest that around 44% of the difference between log_{10} depth: volume is accounted for by the difference between normal and FGR groups. Although the ROC curves were generated only in an exploratory manor given the small numbers in the study, if this parameter was used as a test for FGR, a depth: volume ratio >96% of the predicted value, would offer 67% sensitivity and 93%
specificity for the condition. A novel marker, placental depth: (width x length) ratio was also considered in this analysis. Although the results were not as convincing as the depth: volume ratio, this parameter may be more applicable in real case analysis, as measurement of placental volume performed manually slice-by-slice is relatively laborious, whereas width x length is measured easily. In these data, where women were scanned at a whole range of gestations, this parameter was not as predictive of FGR as the depth: volume ratio.

The relaxation times are determined by the molecular environment of the hydrogen nuclei (the hydrogen nucleus is a single positively charged proton) within the tissue. In biological systems, water may be free in blood (or CSF) but is usually associated with large polysaccharides and proteins, which restrict proton movement. Relaxation occurs by two independent processes, referred to as longitudinal (spin-lattice) (T1) and transverse (spin-spin) (T2) relaxation, the rate of which depends upon the interaction between protons and their surrounding environment and those in an intermediate situation, often termed ‘structured’. Theoretically, T1 will be shortest in tissues with mainly ‘structured’ protons, longer when ‘free’ and longest when ‘bound’. T2 will be longest where protons are ‘free’ in solution and shortest where bound to macromolecules (Bottomley, Foster et al. 1984). In previous MRI studies at lower field strengths (0.5 T), a trend for shorter relaxation time measurements, T1 and T2, was observed in pregnancies complicated by pre-eclampsia and FGR (Gowland, Freeman et al. 1998) although the numbers were small. In our previous study (Wright, Morris et al. 2011), we also demonstrated a significant fall in both T1 and T2 with gestation and a correlation between T2 and placental fibrin content. The current study is consistent with those data, although the relationship between T1 and gestation was not seen, the relationship with T2 and gestation was observed and it is also in T2 values that a significant reduction was seen in FGR. This would support the view that it is T2 rather than T1 that is reflecting the morphological changes occurring in the placenta with
both changing gestation and disease processes. However, although generalisations can be made regarding histopathological correlates of relaxation times (Cameron, Ord et al. 1984), it is not known specifically what changes in the placenta influence relaxation. In our previous study of women with normal pregnancies correlations were observed between the fibrin content of the placenta and placental T₂ (Wright, Morris et al. 2011), although we concluded that this was unlikely to be the sole determinant. We hypothesised, by studying placentas from both normal and FGR pregnancies, that the wider variation in morphology might allow improved analysis of the relationship between morphological variables and MR relaxation times. However, the relationship between fibrin and T₂ was only observed when normal pregnancies were considered and no new morphological influences on relaxation times were identified. The total volume of the maternal vascular bed increases with gestational age (Mayhew, Jackson et al. 1993), but if this was the major influence on relaxation times then a rise rather than fall would be seen, as the T₁ and T₂ of oxygenated maternal blood in vivo is around the upper end of the placental values (Barth and Moser 1997). It is likely that other factors not investigated here may also influence relaxation times. Potential candidates include reduced blood oxygenation (for example, deoxygenated blood generally has lower T₁ values (Silvennoinen, Kettunen et al. 2003)), variations in the fluid content of stromal channels in the villi (Castellucci and Kaufmann 1982) or changes in the tissue hydration fraction, found to be important in other tissues in animal studies (Cameron, Ord et al. 1984). This failure to characterise the key placental component influencing T₂, should not obscure our important observation that shorter T₂ relaxation times are seen in FGR and furthermore this may also be influenced by the severity of FGR, as demonstrated by separating the FGR group by birthweight. In larger analyses, it would be interesting to further assess this relationship by examining FGR groups stratified in terms of severity by e.g. Doppler measurements of blood flow in the fetal or umbilical vessels.
Currently, ultrasound imaging is not used to evaluate blood flow in the microcirculations of the placenta itself, only up- or downstream in the uterine or umbilical vessels. The development of techniques to study these placental microcirculations, such as MRI, are of key importance, given that many of the changes seen in FGR are within the smaller vessels of the placenta itself (Mitra, Seshan et al. 2000; Mayhew, Ohadike et al. 2003). Electron microscopy of placental villi and their vascular casts suggests a failure of formation of the terminal villi and an arrest of non-branching angiogenesis in FGR (Baykal, Sargon et al. 2004). It is likely that reduced blood flow contributes to fetal hypoxia in FGR as oxygen is small and highly lipophillic, enabling it to diffuse readily across the placental barrier and causing its transfer to be flow–limited (Sibley and Boyd 1988). In previous studies of IVIM parameters at 0.5T in the placenta, the moving blood fraction ($f$) within the placenta averaged at 26%, with a trend towards decreasing at later gestations and a relationship between D and gestation was not observed (Moore, Issa et al. 2000). Also in previous studies of FGR, no difference was seen in D between normal and FGR pregnancies (n=13 normal, 7 FGR) (Moore, Strachan et al. 2000). This may have been due to small numbers, a lower magnetic field strength used for scanning and the definition of FGR (a reduced growth velocity on sequential ultrasound scans) being less specific for FGR. Also, pre-eclamptic patients or other FGR aetiologies were not excluded. This study had identified altered patterns of $f$ between maternal and fetal sides of the placenta; this pattern was not observed in our data when similar regional analyses were performed (data not shown). Our study demonstrated a fall in D (the diffusion coefficient) across gestation and a significant reduction in values in FGR. This is in agreement with a recent study considering diffusion weighted imaging (DWI) in the placenta and studying the apparent diffusion coefficient (ADC) (Bonel, Stolz et al. 2010). This parameter is related to D, but relates to the properties of diffusion occurring within a particular voxel. The Bonel et al. study, which evaluated the DWI in normal and FGR
(<10th centile birthweight) pregnancies, concluded that placental dysfunction was associated with restricted diffusion and reduced ADC. Our data support this conclusion.

Various morphological changes occur in placental tissue in association with FGR. Gross placental lesions are detected occasionally and are associated with poorer fetal outcomes and abnormal Doppler waveforms (Sebire and Sepulveda 2008). However, there are often more subtle alterations, such as impoverished development of the villi (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004; Pallotto and Kilbride 2006; Mayhew, Manwani et al. 2007) and changes in placental exchange barrier surface area and thickness (Mitra, Seshan et al. 2000; Baykal, Sargon et al. 2004; Mayhew, Wijesekara et al. 2004). The correlations between D and villous tissue volume are perhaps consistent with morphological studies; if more tissue is present, this may represent the placenta that is undergoing rapid growth and expected levels of small scale movement of water molecules would be high (giving high D values), as is the case in tumour tissues (Sakuma, Tamagawa et al. 1989). Therefore if villous volume is reduced in FGR and there is little growth and development of the villi (as illustrated in previous morphological studies (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004)), D would also be reduced. Another explanation would be that if the villous tissue is more restrictive to diffusion, even in the absence of reduced villous volume (as found in this study, alongside reduced villous surface area which might also influence diffusion), D would be reduced in FGR. Further examination of the characteristics of diffusion parameters within the placenta is warranted; potentially these biomarkers might provide information on the functioning on the placental microcirculations, which have remained difficult to assess in vivo. Although exploratory, the ROC curve for D adjusted as a percentage of the predicted normal was promising with an area under the curve of greater than 0.8.
In conclusion, this study illustrates that MRI biomarkers show potential in identifying the FGR placenta. Given the small numbers of studies in the literature, optimisation of scan protocols and scan interpretation still requires much work, as well as analysis of what information can be gained from MRI, in addition to that provided by ultrasound, by both qualitative and quantitative analysis. Although our study focused on an initial exploration of differences between normal and FGR groups as a whole, there are hints that the severity of FGR may be detected by MRI techniques and these may be useful in stratifying disease and rigorously defining phenotypes. Although at present ultrasound remains the optimal tool for the evaluation of the placenta in FGR, there is accumulating evidence to support the role of MR in assessment of this disease. Abnormalities in fetal brain development in growth restriction have also been demonstrated using MR techniques such as diffusion tensor analysis and proton Magnetic Resonance Spectroscopy (Charles-Edwards, Jan et al. 2010). We suggest that as units continue to increase their volume of MR imaging for a variety of fetal conditions, placental assessment should be included, as it may provide additional information on disease severity. Animal studies have recently studied the use of treatments including melatonin and growth hormone in animal models of FGR (de Boo, Eremia et al. 2008; Richter, Hansell et al. 2009), with evidence of potential improvement in outcome following treatment. As the origins of FGR lie in placental dysfunction, MRI could potentially also provide a means of analysis of interventions designed to improve placental function.
Chapter 4

Arterial Spin Labelling in the Assessment of Placental Perfusion at 1.5 T
This chapter is the article planned for submission for publication in the journal ‘Placenta’ and adapted for thesis format:

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Arterial Spin Labelling in the Assessment of Placental Perfusion at 1.5 T.

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4.1 Abstract

Blood flow to the placenta can be assessed by Doppler ultrasound in the uterine and umbilical arteries. However, there is currently no technique available to measure perfusion within the placenta itself. Here we present in vivo measurements of perfusion in the human placenta using a Magnetic Resonance Imaging (MRI) perfusion imaging technique; Flow Sensitive Alternating Inversion Recovery (FAIR) Arterial Spin Labelling (ASL). ASL is a technique which uses endogenous water excited by the MR pulse as a tracer and offers a means of measuring placental perfusion without the use of contrast agents. We carried out ASL placental perfusion measurements in 9 women during the third trimester using a FAIR-HASTE ASL technique at 1.5 T and correlated these findings with measures of placental morphology assessed stereologically at term. Mean perfusion ± S.D. was 111±28 ml/min/100g. A significant negative correlation was seen between perfusion and gestation (p=0.026) with perfusion falling by 6.3ml/min/100g per week. No significant correlations with placental morphology were observed. A trend for reduced perfusion in small for gestational age pregnancies was observed (n=4); a reduction of 10ml/min/100g was seen compared to average sized infants when gestational differences were corrected for. Good inter- and intra-observer variability was demonstrated. We conclude that this FAIR ASL technique is suitable for measurement of perfusion in the placenta at 1.5 T and offers potential as a technique for use in complicated pregnancies.
4.2 Introduction

Fetal growth restriction (FGR) is a serious complication of pregnancy in which the fetus fails to reach its own genetically determined growth potential. Placental insufficiency is a major cause of FGR and accumulating evidence indicates that several aspects of placental structure and function are specifically altered in this condition, including blood flow in the uteroplacental and fetoplacental circulations, as evidenced by over 200 studies into the use of Doppler ultrasound in this condition. In clinical obstetrics, measurement of blood velocity by Doppler ultrasound in the uterine and umbilical vessels is the main imaging technique used to assess blood flow in predicting or diagnosing FGR, but does not directly measure blood flow in the placental microcirculations; the maternal intervillous space and fetal placental vessels and capillaries.

It is well established that the spiral arteries of the uteroplacental circulations are transformed during early pregnancy, by invasion of extravillous trophoblast, into wide bore, low resistance vessels (Brosens, Robertson et al. 1967; De Wolf, De Wolf-Peeters et al. 1980). Failure of this invasion and consequent vessel transformation is associated with FGR, and probably underlies the abnormal uterine artery Doppler measurements seen in this condition (Brosens, Pijnenborg et al.). The pathology underlying the abnormal umbilical artery high resistance waveforms seen in FGR is complex and debate exists regarding the relative importance of cord, stem artery and terminal villous pathology (Krebs, Macara et al. 1996) and indeed of humoral regulation of vessel resistance (Mills, Wareing et al. 2005). However, electron microscopy of placental villi and their vascular casts suggests a failure of formation of the terminal villi and an arrest of non-branching angiogenesis in FGR, supporting the importance of the placental microcirculations in this condition (Lee and Yeh 1986; Krebs, Macara et al. 1996; Baykal, Sargon et al. 2004).
Better understanding of blood flow in the microcirculations of the placenta, maternal intervillous space and fetal villous vessels, either side of the exchange barrier, is important as this will ultimately determine transfer rates of both oxygen, in the maternofetal direction, and of carbon dioxide in the fetomaternal direction (Sibley and Boyd 1988).

Abnormalities in flow and therefore transfer of these highly diffusible gases will directly contribute to FGR. Therefore, the development of tools to identify abnormal blood flow in these microcirculations may allow more rigorous definition of the condition, improve our ability to stratify disease and potentially provide specific predictive tests.

An increasing body of work suggests that Magnetic Resonance Imaging (MRI) may be a very useful way of assessing placental structure and function in vivo (Damodaram, Story et al. 2010; Wright, Morris et al. 2011). The purpose of the study reported here was to determine the usefulness of one MRI technique, Arterial Spin Labelling (ASL), to measure placental perfusion in normal pregnancy. ASL perfusion imaging has gained wide acceptance for its value in clinical applications during recent years (Chen, Chiu et al.). Its capability for non-invasive (without the use of radio-isotopes) and absolute perfusion quantification makes it attractive for many clinical applications, including placental analysis. Magnetically labelled endogenous blood water can be used as a tracer for perfusion MRI. Classically, an inversion pulse is used to tag inflowing spins at a level proximal to the imaging slab, and, following a transit delay to allow these tagged spins to enter the imaging plane and exchange with tissue, control and label images are obtained. The pair-wise subtraction of many of these 2 images yields maps of brain tissue perfusion, expressed in units of ml/100 g/min. ASL essentially measures the a signal difference within the tissue of interest caused by the inflow of equilibrium magnetization from MR ‘labelled’ areas outside the tissue of interest and is therefore directly related to blood flow into the tissue (Detre, Leigh et al. 1992; Williams, Detre et al. 1992). This may be achieved in the placenta using a technique called FAIR ASL (Flow-sensitive Alternating
Inversion Recovery) Two previous studies, using MRI Echo-Planar Imaging (EPI) at 0.5 T (Tesla), explored FAIR ASL in the placenta in small numbers of women (Duncan, Gowland et al. 1998; Gowland, Francis et al. 1998). Both studies included pregnancies at a wide range of gestations between 20 and 40 weeks and huge variability in the data was observed (perfusion varying between around 50 and 350 ml/100g/min), which bore no relation to gestation. EPI was one of the first MRI sequences used in applications like diffusion, perfusion, and functional MRI, but is extremely sensitive to image artefacts and distortions, which may have in part been responsible for the high variability seen in these earlier studies. In the current study, the perfusion measurements were performed using a FAIR ASL with the adaptation that the half-fourier single-shot turbo spin echo (HASTE) used in image acquisition was used following inversion (EPI not used) (Kim 1995; Kwong, Chesler et al. 1995). In this particular scheme, an inversion recovery sequence is performed twice: one with and one without slice selective gradient to label the arterial spins. The perfusion-weighted signal then comes from the difference in signal intensity due to the absence of inverted arterial spins in the slice-selective inversion recovery sequence. For placental imaging, all regions outside the placenta are selected and labelled so inflow of blood (from any direction) from these labelled regions into the non-labelled placental slice is detectable by the altered signal. Directionally dependant ASL techniques (which would enable flow from the maternal and fetal circulations into the placenta to be determined separately) were explored in preliminary work but found to be ineffective in the placenta (see Discussion). Incorporated into this study, the FAIR ASL sequence has been optimized for future measurements to give maximum signal to noise ratio in the fitted value of the perfusion rate. The higher Tesla used in this study (1.5 T as opposed to 0.5 T in previous studies), gives intrinsically higher Signal to Noise Ratio (SNR) and also lengthens blood T1 which should enhance the ASL technique and allow more robust measurements of perfusion than seen in earlier studies(Wang, Alsop et al.
2002). Furthermore, the use of background noise suppression by the application of multiple inversion pulses (Dixon, Sardashti et al. 1991; Garcia, Duhamel et al. 2005) has become more attractive to improve ASL robustness and SNR; here 22 averages were used as opposed to 2 in previous studies. We hypothesized that these techniques would enable enhanced measurement of placental blood flow at 1.5 T in normal pregnancy and that measured perfusion would correlate with differences in placental vasculature assessed stereologically following delivery.
4.3 Methods

4.3.1. Subjects

Ethical approval was obtained from the local NHS Research Ethics Committee and all women gave written informed consent. Women with normal pregnancies in the third trimester (between 28 and 42 weeks gestation) were recruited from antenatal attendances at St Mary’s Hospital, Manchester. Perfusion measurements were performed on 9 women with normal healthy pregnancies, i.e. women with no significant past medical history, who had no known complications of pregnancy and who subsequently delivered an infant at term (37-42 weeks) with no adverse immediate adverse outcomes. Birth weight centiles were calculated using the Growth Centile Calculator for the UK v 5.16 (Gardosi J and Gestation Network; Gardosi, Chang et al. 1992).

4.3.2 MRI

FAIR ASL was used (Kim 1995; Kwong, Chesler et al. 1995). Using this technique two inversion recovery (IR) images are acquired by interleaving slice-selective inversion and non-selective inversion. During the inversion delay time after slice-selective inversion, fully magnetized blood spins move into the imaging slice and exchanges with tissue water. The signal enhancement (FAIR image) measured by the signal difference between two images is directly related to blood flow. The position of an imaging slice can be seen in Chapter 4 Figure 1a, overlaid on a structural image; typically this was a mid-depth single placental slice, running through the long axis of the placenta, giving disc shaped placental images. The sequence used is based on current methodology (Gardener and Francis 2010) with the adaptation that the half-fourier single-shot turbo spin echo (HASTE) acquisition was used following inversion. The scanning was carried out using a 1.5 T Philips Intera
system (Philips Medical Systems, Best, NL) FAIR-HASTE ASL sequence parameters, TR/TE=3500/5.4ms; FOV=384x384mm; single slice voxel dimensions 3x3x8 mm³. 7 inversion times were applied (300, 600, 900, 1200, 1500, 1800 and 2300 ms), each with 22 averages and a non-inversion acquisition to estimate initial magnetisation. The slice selective inversion was 20 mm outside the imaging slice and the non-selective inversion 400mm outside the imaging slice. Perfusion (f) was calculated for a region of interest (ROI) in the placenta that was of consistent position throughout scanning and within the placental borders; an example is shown in Chapter 4 Figure 1b. Individual images that were significantly affected by motion or artefact were excluded.

Quantification of blood flow into physiologic units was carried out using the Buxton model (Buxton, Wong et al. 1998) applied to the mean signal differences observed in the ROI. This model is more general than alternative earlier models (Detre, Leigh et al. 1992; Williams, Detre et al. 1992) but reproduces these earlier models under more appropriate assumptions. Previous models assume single-compartment kinetics for water clearance and instantaneous exchange of water between tissue and blood. In the Buxton model, these assumptions are relaxed and an analysis of error if these assumptions are not met is allowed. An example of signal differences with error bars using the ROI fit across time is shown in Chapter 4 Figure 1c. A voxel by voxel fit (perfusion calculated for each voxel and a mean of the all values calculated (a voxel being the 3D unit that make up the image / slice) was also performed as a secondary analysis.
Chapter 4 Figure 1a) Imaging slice position on sagital localizing scan, 1b) Sample ROI on placental image, 1c) Graph showing measured signal difference within the ROI across time (ms) (ROI fit); error
**4.3.3 Placental analysis**

All stereological analyses were made in placentas collected from women undergoing MRI using methods previously reported by Wright et al. (Wright, Morris et al. 2011), adapted from those reported by Mayhew (Mayhew, Ohadike et al. 2003; Mayhew, Manwani et al. 2007). Placental tissue samples were collected in 5 subjects delivered at term. Placentas were collected postnatally within 30 minutes of delivery time. A random sampling method was devised (Wright, Morris et al 2011) that would allow more in-depth analysis of placental heterogeneity, matching regions of the placenta examined with MR with morphology, so that a mid-depth MRI placental slice was matched with a mid depth analysis of placental morphology. This alters the reference volume for stereological calculations and therefore absolute values concerning the whole placenta are not reported here. A transparent grid with pre-numbered sampling windows at the intersections was placed with random orientation over the placenta and a random-number generated sequence used to select samples. The tissue was fixed in 10% buffered formalin. Only middle sections are considered in this analysis, to correspond with the mid-depth level of the MRI scan plane. These samples were diced and embedded with random orientation in paraffin wax blocks and processed for histology. Before imaging, cut sections (5 micrometres) were mounted on microscope slides, stained with haematoxylin and eosin (H&E), and observed under a Leitz (Dialux 22) microscope at a magnification of x250. Approximately 8 sections were investigated per placenta. For image analysis, a Q-Imaging (Fast 1394) digital camera acquired images from each sample. For each section, 5 fields were selected for imaging in a systematic fashion, moving around the section in system of partial turns of stage adjuster. In total, approximately 40 high-power fields of view were analysed per placenta.
Images were acquired using Image-Pro Plus software (Version 6.3). Test point counting was employed to obtain volume densities. Intersection counting using a superimposed grid lattice was used to calculate surface area densities. Indices calculated included villous volume density, fetal capillary volume density, fibrin (intervillous fibrin deposition) volume density, villous surface area density, capillary surface area density. From this interfiles space (IVS) volume density (1 – villous volume density) and total blood volume density (IVS + capillary volume density) were calculated.

4.4 Results

The perfusion values are shown in Chapter 4 Table 1 for each of the 9 subjects. The mean gestational age at time of scan was 33.4 weeks (range 29.3 – 36.9 weeks). Mean gestation at delivery (and time of placental analysis) was 40.2 weeks. In this study group the mean perfusion rates were found to be 111 - 127 ml/min/100g depending on the method of analysis used and the observer. Columns A to C refer to the ROI analysis method; the observer selects a placental region of interest (see Methods) and the mean signal from that region is used to calculate perfusion. Column A refers to the initial analysis using this technique, Column B to a second analysis performed by the same observer i.e. redrawing the placental region on the same images, whereas in Column C, the regions of interest were drawn by a second observer, thereby allowing analysis of inter-observer error using this technique. Column D refers to an alternative method to calculate perfusion in which the signal from each voxel in the MR image is used to calculate a perfusion value and the mean of all voxel values taken as the final perfusion value.
Chapter 4 Table 1. Perfusion values (f) in ml/min/100g calculated from the mean signal in a region of placenta for using A) ROI method and B) ROI method using regions drawn by the same observer on different occasions C) ROI drawn by a second observer and D) using a voxel by voxel fit (f calculated from the signal in each voxel and a mean of all values calculated).

<table>
<thead>
<tr>
<th>Subject</th>
<th>A (ROI fit Observer 1)</th>
<th>B (ROI fit repeat Observer 1)</th>
<th>C (ROI fit Observer 2)</th>
<th>D (Voxel by Voxel fit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85.0</td>
<td>93.7</td>
<td>88.7</td>
<td>108.8</td>
</tr>
<tr>
<td>2</td>
<td>114.8</td>
<td>131.9</td>
<td>124.5</td>
<td>120.3</td>
</tr>
<tr>
<td>3</td>
<td>73.1</td>
<td>71.2</td>
<td>84.3</td>
<td>71.0</td>
</tr>
<tr>
<td>4</td>
<td>102.4</td>
<td>102.4</td>
<td>85.5</td>
<td>100.3</td>
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<tr>
<td>5</td>
<td>113.8</td>
<td>114.7</td>
<td>118.4</td>
<td>162.7</td>
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<td>6</td>
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<td>123.1</td>
<td>114.8</td>
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<td>7</td>
<td>156.6</td>
<td>157.5</td>
<td>172.9</td>
<td>212.3</td>
</tr>
<tr>
<td>8</td>
<td>131.3</td>
<td>122.6</td>
<td>111.4</td>
<td>142.5</td>
</tr>
<tr>
<td>9</td>
<td>85.2</td>
<td>89.9</td>
<td>88.9</td>
<td>110.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td><strong>111 ± 28</strong></td>
<td><strong>113 ± 27</strong></td>
<td><strong>111 ± 29</strong></td>
<td><strong>127 ± 41</strong></td>
</tr>
</tbody>
</table>
Chapter 4 Figure 2 shows the variation in placental perfusion rate as pregnancy advances; there was a significant negative correlation with gestational age when using the ROI technique (Pearson’s r=-0.73, p=0.03, r=-0.69, p=0.04) respectively). Linear regression modelling showed that f decreased by 6.3ml/min/100g each week so that at 28 weeks the expected perfusion would be 145ml/min/100g and at term (40 weeks) this would have fallen to 70ml/min/100g. There was no significant correlation with gestation when the voxel to voxel fit analysis (Column D in Table 1) was used (Chapter 4 Figure 2).

4.4.1 Variability

The intra-observer and inter-observer correlation coefficients for the ROI fit were r=0.96, p=<0.001 and r=0.87, p=0.0012 respectively, based on 2 readings for each subject taken by a single observer (Columns A and B of Table 1) and a second independent observer (Columns A and C of Table 1). The correlation coefficient between ROI fit values and voxel by voxel fit values (Column A and D of Table 1) was 0.80, p=0.005; there was no significant difference between any group (Compared group to group by Paired t-test).
Chapter 4 Figure 2. Variation in perfusion, $f$, with gestation using the ROI fit (black) and voxel by voxel fit (red). ROI fit by a single observer showed a significant negative correlation with gestation (Pearson’s $p=0.026$). Using the voxel by voxel fit, no significant correlation was observed. Linear regression models are plotted for the observed values.
4.4.2 Comparison of data from pregnancies with Appropriate for Gestational Age and Small for Gestational Age babies

Although women at recruitment were not known to have any antenatal problems, 4 of the 9 women subsequently delivered infants that were less than the 10th customized birthweight centile, often used as a surrogate definition of small for gestational age (SGA); 2 of these were below the 5th centile, often used as a surrogate definition of fetal growth restriction (FGR) and 5 had normal birthweight centiles between the 10th and 90th centiles, often referred to appropriate for gestational age (AGA) (Gardosi J and Gestation Network). Direct comparison of f between AGA (10th centile and above) and SGA (less than the 10th centile) groups showed no significant difference between groups (unpaired t-test), although there was a trend for lower values in the SGA group; mean ± S.D. 115 ± 29 ml/min/100g versus 103 ± 30 ml/min/100g, AGA versus SGA respectively. When gestation is considered as a covariate, there was again no significant difference between groups (ANCOVA F=0.478, p=0.515, partial $\eta^2=0.074$). However, the corrected means ± S.E for f were 116 ± 9 ml/min/100g and 106 ± 11 ml/min/100g for the normal and SGA groups respectively. There was no significant interaction between group and gestation for this analysis. There was no significant correlation between f or f predicted for the day of delivery (using the linear regression model in Chapter 4 Figure 2) and the individualised birthweight centiles.

4.4.3 Correlations with placental stereology

5 placentas were analysed stereologically. The remaining placentas were not collected due to difficulties with placental collection at time of delivery e.g. the researcher was not informed of delivery or was not available to perform placental analysis. There were no
correlations between f and placental villous volume, capillary volume, fibrin volume, intervillous (blood filled) space volume or total blood volume (capillary volume + intervillous space volume). There was also no correlation with villous surface area or capillary surface area in this sample. As there was some lag between time of MRI and time of placental analysis (which occurred following delivery), the linear regression model for the ROI method (shown in Chapter 4 Figure 2) was used to estimate f for the day of delivery as a means of minimizing the effect of the change in gestation. There remained no significant correlations with stereological variables and f predicted for the date of delivery, when this step was taken.
4.5 Discussion

This study has shown it is possible to apply FAIR ASL to the measurement of perfusion rates in the placenta at 1.5 T. The observed perfusion value of 111ml/100g/min are in keeping with estimates of placental blood flow; at term the maternal blood supply to the placenta is estimated to be 600 ml/min (Browne and Veall 1953) and taking the mass of the placenta to be 550g (Boyd 1970), this would give an approximate expected classical placental perfusion rate of about 110 ml/100 g/min. Our data also show reasonable agreement with the previous studies at 0.5 T. Duncan et al. measured perfusion in 20 normal pregnancies (Duncan, Gowland et al. 1998), reporting mean placental perfusion (f) to be 209ml/100g/min (S.D. not given) between 20 and 40 weeks (the mean gestational age at time of scan is not given but from plotted graphical data it was around 31 weeks). The same group using the same technique also report on f in 15 normal pregnancies (Gowland, Francis et al. 1998), finding mean perfusion (f) ± S.D. to be 176 ± 24 ml/min/100g (at a mean gestational age of 31 weeks), slightly higher than our observed value; mean perfusion (f) ± S.D. 111 ± 28 (at a mean gestation of 33.4 weeks). This may in part be due to the range of gestations studied; we observed a significant negative correlation between f and gestation which supports that perfusion falls towards term; although this linear relationship was not demonstrated in previous studies, it would explain the lower mean value of f as in this study all women were scanned in the third trimester. The difference in observed values, may have also have been secondary to noise in the sample which was minimised in this study with the removal of scans affected by movement, increased field strengths and high number of averages acquired at each time point (22 compared to 2 in the previous studies). There is also much less variability in the data at 1.5 T; using the ROI technique the range of values is over 100ml/min/100g as opposed to 300ml/min/100g range at 0.5 T (Gowland, Francis et al. 1998).
The fall in f towards late gestation is supported by evidence from ultrasound Doppler studies, which demonstrated that in the umbilical vein (which carries blood from the placenta to the fetus), blood flow decreased linearly from 139 ml/min/100g to 65ml/min/100g between 27 and 40 weeks (Lingman and Marsal 1986). This shows remarkable similarity with our data, which demonstrated a fall in perfusion equating to 152ml/min/100g to 70ml/min/100g over the same time period. The higher values we observed are likely to reflect that using FAIR ASL is a measure of both maternal and fetal blood flow in combination, as opposed to that of the umbilical vein which is downstream in the maternal to fetal circulation. Furthermore, in a normal capillary network, where the blood flow is unidirectional, ASL will measure classical perfusion. However, in the placenta, where blood is flowing in vasculature latticed in a variety of directions (Boyd and Hamilton 1967), ASL might overestimate perfusion, and might be more reasonably said to be measuring blood movement. Nonetheless, blood movement may be the relevant measure in the placenta, as it is this movement that ensures that the maternal blood circulates adequately, so values of f may be functionally more useful in this regard.

It is reassuring that our data show good intra- and inter-variability between observations and, furthermore, that the voxel-by-voxel technique demonstrated similar results to the ROI mean signal method. The similarity of these two methods supports the view that the majority of voxels are not severely affected by noise, although the slightly higher values are likely to reflect the presence of some noise (the FAIR ASL technique is sensitive to in-plane motion), hence the ROI method was chosen in preference for analysis.

This study was designed to test the feasibility of ASL measurements on the placenta and was not powered to consider differences between AGA and SGA or FGR groups. However, as the birthweight centiles in the sample by chance included a number of SGA and FGR infants, this relationship between perfusion and birthweight was considered. It is...
therefore of interest that a trend for reduced perfusion in the SGA group was observed and this certainly requires further investigation using the developed ASL techniques. Previous studies have shown similar trends; Francis et al. showed a reduction in the proportion of the placenta image with $f < 100\text{ml/min/100g}$ using FAIR ASL (with EPI) in 9 SGA versus 6 normal pregnancies (Francis, Duncan et al. 1998). The results were confounded by a difference in mean gestational age at time of scan between groups; 36.5 weeks in the normal group and 33.9 in the SGA group. However, our finding, that $f$ decreases as pregnancy advances, would suggest that the difference between SGA and normal pregnancies studied by Francis et al. might be underestimated. Duncan et al. (Duncan, Gowland et al. 1998) who also found a trend for reduced overall mean placental $f$ in SGA pregnancies. Clearly the scene is now set for a larger study of the utility of placental ASL measurements in complicated pregnancies.

Our study did not demonstrate any correlations between placental stereological variables and perfusion. This analysis was limited by both the small sample size and delay between scan and delivery, although attempts to correct for the latter were made using the linear regression model. This relationship would be worth continuing in a larger study, including stereological analysis in a true FGR group who have undergone ASL.

Another limitation of the study reported here is that the FAIR ASL technique is unable to differentiate between the maternal and fetal circulations. However, given the similarity between perfusion estimates based on expected maternal blood flow to the placenta given above and placental $f$ reported in this study, it would appear that by far the largest contributor to the placental perfusion is the maternal influx. Directionally dependent ASL techniques were explored as part of our preliminary work, but were disappointing. Attempts were made at magnetically labelling blood flowing into the placenta from the maternal aorta, but the time for the primed blood to reach the placenta appeared to be too
long and very little signal change was detectable in the placenta. Similarly, attempts were made at priming blood in the region of the uterine arteries; again little signal change was observed, which was thought to be due to being unable to prime a sufficient volume of blood to give a detectable change in $T_1$.

There is a growing body of evidence that supports the view that investigation of a range of potential pathologic features of the placenta may be important in understanding FGR; ranging from gross changes in placental size and structure (Toal, Keating et al. 2008, (Damodaram, Story et al. 2010), changes in morphology at the microscopic level (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004), and attenuations in placental nutrient transport (Sibley, Turner et al. 2005). This constellation of abnormalities provides a placental phenotype for FGR (Sibley, Turner et al. 2005). Previous research in this field by our group has demonstrated a range of alterations in placental structure and function detectable by advanced MRI techniques across gestation (Wright, Morris et al. 2011). In FGR, we have demonstrated an increased placental depth: volume ratio, shorter placental relaxation times and a reduction in $D$, the diffusion coefficient (Wright et al, unpublished data). These findings support that a more global assessment of placental characteristics using MRI may be worthwhile and potentially allow more rigorous definition of FGR, its clinical scarification and possibly its early prediction.

In summary this paper presents in vivo measurements of perfusion in the human placenta using ASL. Our work details an optimised sequence for ASL placental perfusion scanning. The data suggest that the implementation of the FAIR-HASTE ASL technique can be used successfully in the placenta and may, in the future, be applied to the investigation of microcirculatory flows in normal and FGR pregnancy.
Chapter 5

Overall Conclusions and Future Work
The studies reported in this thesis demonstrate that quantitative MRI techniques are feasible in the placenta at 1.5 T and provide important new information on the structure and function of the placenta \textit{in vivo}. We focused on the relationships between potential MRI biomarkers and gestation and the differences observed between normal and FGR pregnancies. We have identified a placental phenotype in FGR pregnancy that is characterised by (i) a reduction in placental volume and increased depth, (ii) altered structural composition (as evidenced by alterations in MR relaxation times), (iii) altered diffusion characteristics and (iv) preliminary ASL data suggest that perfusion measured by this technique is reduced. Furthermore, there are hints in the data that those with more severe FGR also have a more marked deviation from the normal AGA phenotype.

Data from our study is consistent with previous publications showing an increase in placental volume with advancing gestation (Duncan 2001; Damodaram, Story et al. 2010). MRI is the only tool available to accurately study placental volume \textit{in vivo} as, beyond the first trimester, 3D ultrasound is limited by the size of the field of view (Metzenbauer, Hafner et al. 2001). We also observed a reduction in placental volume in FGR, consistent with several histopathological studies (Boyd and Scott 1985; Biswas and Ghosh 2008). The change in volume with gestation is likely to be a reflection of fetal growth, whereas in growth restricted pregnancies, the aetiology is less clear and may be secondary to increased placental apoptosis rather than a reflection of smaller fetal size \textit{per se} (Kaufmann, Black et al. 2003). In addition to a reduced size, FGR placentas also had an increased depth, giving a thickened, globular placental appearance. This appearance has been previously demonstrated in association with FGR using ultrasound (Fisteag-Kiprono, Neiger et al. 2006; Toal, Keating et al. 2008) and MRI (Damodaram, Story et al. 2010) and it appears that these simple gross morphological assessments may be more relevant in the assessment of FGR than previously realised. Particularly when combined with accurate volumetry as a depth: volume ratio, they may also be indicative of poor fetal
outcomes (Damodaram, Story et al. 2010). Although the ROC curves generated for this simple parameter in our studies were merely exploratory, as a test for FGR, a depth: volume ratio >96% of the predicted value, would offer 67% sensitivity and 93% specificity for the condition; a biomarker that perhaps might be useful, in combination with other factors. Other macroscopic morphology (e.g. infarction) detected by MRI have also been previously demonstrated to correlate with histological findings and pregnancies at risk for adverse outcome and fetal death, independently of umbilical artery Doppler status (Linduska, Dekan et al. 2009). Our data support a growing body of evidence for the use of structural MRI in evaluating the placenta in FGR pregnancies, not currently routine practice, as it may provide additional evidence on disease severity and outcome.

The studies reported in Chapter 2 demonstrated a correlation and linear relationship between placental $T_1$ and, more convincingly, $T_2$ measurements at 1.5 T and gestational age, consistent with previous work at 0.5 T (Gowland, Freeman et al. 1998). At 1.5 T $T_1$ and $T_2$ relaxation times were shown to decrease by 20.2 ms and 2.4 ms per week of advancing gestational age respectively, giving a total fall of in $T_1$ of 400 ms and in $T_2$ of 50 ms between 20 and 40 weeks. The relationship supports the proposition that changes in placental tissue structure or composition that occur with advancing gestation significantly influence relaxation times. The exact nature of this change, however, remains a matter for conjecture. In this study, relaxation time values did not correlate with villous volume densities or total blood volume densities, indicating that other changes in morphology or function, rather than just increases in solid or blood volumes, lead to the fall in $T_1$ and $T_2$ towards term. The total volume of the maternal vascular bed increases with gestational age (Mayhew, Jackson et al. 1993), but if this was the major influence on relaxation times then a rise rather than fall would be seen, as the $T_1$ and $T_2$ of oxygenated maternal blood in vivo is around the upper end of the placental values (Barth and Moser 1997). That other changes in placental morphology may influence relaxation times, rather than the balance
of vascular and non-vascular volumes, is supported by MRI magnetisation transfer (an in-vivo measure of bound protons to total protons) in the placenta which demonstrated no changes in these values across gestation (Ong, Tyler et al. 2004) and other studies of placental morphology demonstrating that ratios of total volumes of blood and non-blood placental compartments also remain fairly static (Boyd 1984). Our data showed a correlation between $T_2$ and placental fibrin deposition, suggesting that fibrin content might be one of the contributors to the fall in $T_2$ over gestation, although it is unlikely to be the sole determinant. Fibrin may, however, be a surrogate marker for a wider change in tissue quality that is influencing $T_1$ and $T_2$. No other individual stereological variable analysed correlated with relaxation times, although several trends were seen, supporting a hypothesis that a combination of factors or more generalised change in tissue quality is key in determining relaxation times, fitting with data from initial studies correlating tissue types and relaxation times (Cameron, Ord et al. 1984). Other potential influences include reduced blood oxygenation (for example, deoxygenated blood generally has lower $T_1$ values (Silvennoinen, Kettunen et al. 2003)) and variations in the fluid content of stromal channels in the villi (Castellucci and Kaufmann 1982).

In previous MRI studies at lower field strengths (0.5 T), a trend for shorter relaxation time measurements, $T_1$ and $T_2$, was observed in pregnancies complicated by pre-eclampsia and FGR (Gowland, Freeman et al. 1998) although the numbers were small. In the study here, significantly shorter $T_2$ relaxation times were seen in FGR and furthermore this may also be influenced by the severity of FGR, as demonstrated by separating the FGR group by birthweight (Chapter 2). In larger analyses, it would be interesting to further assess this relationship by examining FGR groups stratified in terms of severity e.g. by Doppler measurements of blood flow in the fetal or umbilical vessels. Our data support the view that it is $T_2$ rather than $T_1$ that is reflecting the morphological changes occurring in the
placenta with both advancing gestation and disease processes, as it is in this parameter that the differences across gestation and in FGR are most clearly observed.

Currently, ultrasound imaging is not used to evaluate blood flow in the microcirculations of the placenta itself, only to or from the placenta in the uterine or umbilical vessels. The development of techniques to study these placental microcirculations, such as MRI, are of key importance, given that many of the changes seen in FGR are within the smaller vessels of the placenta (Mitra, Seshan et al. 2000; Mayhew, Ohadike et al. 2003; Mills, Wareing et al. 2005). Electron microscopy of placental villi and their vascular casts suggests a failure of formation of the terminal villi and an arrest of non-branching angiogenesis in FGR, damaging the development of these microcirculations (Baykal, Sargon et al. 2004). It is likely that reduced blood flow within these microcirculations contributes to fetal hypoxia in FGR as oxygen is small and highly lipophilic, enabling it to diffuse readily across the placental barrier and causing its transfer to be flow–limited (Sibley and Boyd 1988). We studied blood flow by two techniques; IVIM and ASL.

In previous studies of IVIM parameters at 0.5T in the placenta, the moving blood fraction \(f\) within the placenta averaged 26%. There was a trend for \(f\) to fall in later gestations, but a relationship between the diffusion coefficient (D) and gestation was not observed (Moore, Issa et al. 2000). We observed a fall in D across gestation and more importantly, a significant reduction in D in FGR pregnancies. In previous studies of FGR, no difference was seen in D between normal and FGR pregnancies (n=13 normal, 7 FGR) (Moore, Strachan et al. 2000), however, a reduction in D in FGR was seen in our data at 1.5 T, which may be due to methodological changes or more specific inclusion criteria for FGR. This would be in agreement with evidence from diffusion weighted imaging (DWI) (Bonel, Stolz et al. 2010). We also observed a correlation between D and villous volume, consistent with the morphological changes that occur in FGR. If less villous tissue is
present as seen in FGR (Mayhew, Ohadike et al. 2003; Daayana, Baker et al. 2004), less Brownian motion or small scale movement of water molecules would be expected and D would also be reduced. Another explanation would be that if the villous tissue is more restrictive to diffusion, by changes in the exchange barrier, tissue macromolecules or hydration fraction, even in the absence of reduced villous volume (as in this study), D would be reduced in FGR. Further examination of the characteristics of diffusion parameters within the placenta is warranted; potentially these biomarkers might provide important information on the functioning of the placental microcirculations, not previously possible in vivo.

We demonstrated that FAIR ASL can be applied to the placenta in the measurement of perfusion at 1.5 T. Our data showed reasonable agreement with the previous studies at 0.5 T; (Duncan, Gowland et al. 1998; Gowland, Francis et al. 1998), finding mean perfusion (f) ± S.D. to be 111 ± 28 ml/min/100g (at a mean gestation of 33.4 weeks). We observed a significant negative correlation between f and gestation which supports that perfusion falls towards term. This relationship was not demonstrated in previous MRI studies, but is in keeping with evidence from Doppler ultrasound (Lingman and Marsal 1986). Certainly variability in the data was reduced at 1.5 T compared to 0.5 T, likely due to both inherent improvements from increased field strength and advances in scan protocols. Using our ROI technique all values lay within approx 100ml/min/100g of each at 1.5T, as opposed to around 300ml/min/100g for 0.5 T.

As the ASL study group by chance included a number of SGA and FGR infants, a relationship between perfusion and birthweight was considered. It is of interest that a trend for reduced perfusion in the SGA group and more so in the FGR group was observed and this certainly requires further investigation, using this now developed ASL technique. Previous studies have shown similar trends; Francis et al. showed a reduction in the
proportion of the placenta image with f < 100ml/min/100g using FAIR ASL (with EPI) in 6 normal and 9 SGA pregnancies (Francis, Duncan et al. 1998). This difference was also reported by Duncan et al. (Duncan, Gowland et al. 1998) who also found a trend for reduced overall mean placental f in SGA pregnancies. The observed finding in our study supports a growing body of small studies demonstrating that ASL can be used to assess placental perfusion and that reduced perfusion, so measured, is associated with FGR. Importantly, we were able to correct for any gestational effect in our study and this trend remained present. It would certainly be worthwhile continuing this analysis in a larger more rigorously defined group of women.

In conclusion, the studies in this thesis illustrate that MRI biomarkers show great potential in identifying the FGR placenta. Given the small numbers of studies in the literature, optimisation of scan protocols and scan interpretation still requires much work, as well as analysis of what information can be gained from MRI, in addition to that provided by ultrasound. Although our study focused on an initial exploration of differences between normal and FGR groups as a whole, there are hints that the severity of FGR may be detected by MRI techniques and these may be useful in stratifying disease and rigorously defining phenotypes. Although at present ultrasound remains the optimal tool for the evaluation of the placenta in FGR, there is accumulating evidence to support the role of MR in assessment of this disease. Abnormalities in fetal brain development in growth restriction have also been demonstrated using MR techniques such as diffusion tensor analysis (Tolsa, Zimore et al. 2004; Lodygensky, Seghier et al. 2008) and proton Magnetic Resonance Spectroscopy (Charles-Edwards, Jan et al. 2010). We would advocate that MR imaging is used more frequently in FGR, as it may provide additional information on disease severity. Most studies in this field are limited by the fairly small numbers involved and there is a case that, given the evidence that MR is safe in pregnancy, more widespread use might allow us to realise its potential. 3D ultrasound of the fetus has been widely
adopted in developed countries as it is acceptable to women, who often value the images provided, which has enabled its development. Similarly, it may be appropriate in some women to offer MRI, ideally in a setting which combines clinical and research elements, such as the ‘Placenta Clinics’, initially commenced by Kingdom’s group in Toronto and adopted in several centres including Manchester. These manage the clinical needs of pregnancies with FGR, or at high risk of FGR, but also collate data from ultrasound and biochemical blood tests. This approach allows large numbers of women to be accessed for recruitment and is likely to be important in the development of investigations and management of FGR, in which poor outcomes measures, such as intrauterine death of the fetus (stillbirth), are rare (Toal, Chan et al. 2007).

The main limitations of the study are in the small numbers studied. MRI is an expensive test and funding was limited, however the main constraint on participants scanned was time not money. At the outset of the body of the work no scan protocols were fully developed and therefore development of the protocols ran alongside recruitment and data collection. This was a huge hinderance in terms of time as many scans did not yield the data expected and there was substantial fine tuning along the way which could affect validity of the results. A study of this design ideally needs all scan protocols to be in their final form before recruitment begins, however, in reality, it is difficult to secure funding for development work without a clear clinical link in todays climate. If running a similar study in the future, it would be ideal to run all indices in every woman, allowing comparison between parameters within the same placenta e.g due to subadequate development of the scans many women who had relaxation time measurements did not have IVIM measurements. Again, numbers of placentas collected were low, this was due to collection problems when working independently on a project without assistance, requiring essentially to be on call 24 hours given the unpredictable nature of delivery.
In terms of validation of these techniques there is still much to be done. Various tests were run by me in the course of my PhD such as inter and intraobserver agreement and experimenting with different ways of analysing the data, however, a full and robust assessment of the physics of the scan protocols felt beyond my realm.

Another big weakness in the study is not being able to compare to other parameters such as uterine artery Dopplers. This would have greatly assisted in assessment of whether the MRI findings bore relation to measures of placental function in clinical practice. If doing the study again, all women would have an ultrasound placental assessment and MR assessment on the same day. It may be feasible in the future to run such a study from the base of the ‘Placenta Clinic’, incorporating several imaging and biochemical markers of placental function.

Currently, there are no treatments available for FGR specifically and management revolves around timing of delivery, hence the importance of imaging techniques that can predict fetal outcome. Recently animal studies have assessed the use of potential hormonal agents including melatonin and growth hormone in animal models of FGR (de Boo, Eremia et al. 2008; Richter, Hansell et al. 2009), with evidence of potential improvements in outcome. Similarly, Sildenafil (which regulates blood vessel diameter in some vascular beds) (Stanley, Andersson et al. 2012) and Tempol (an antioxidant) (Stanley, Andersson et al. 2012) have been demonstrated to improve pup growth in mouse models of FGR, also improving blood flow in the uteroplacental circulations assessed by Doppler ultrasound. As the origins of FGR lie in placental dysfunction, MRI could potentially assist in the assessment of such interventions.
References


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Appendix 1

**MRI of the fetus**

This section is the published review article (adapted to thesis format):


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Abstract

The role of MRI in fetal imaging is expanding. The depth of structural information provided by MRI places it as more than just a useful adjunct to ultrasound, as several structures are more clearly visualised and many of the limitations of ultrasound are avoided. Currently, MRI is most frequently utilised in the fetal central nervous system and is valuable in ventriculomegaly, agenesis of the corpus callosum and posterior fossa abnormalities. Outside this, MRI remains primarily a research tool, with increasing interest in thoracic abnormalities and scope for development in other niche areas. MRI is able to accurately determine fetal organ volumes and weight, although whether such measurements could play a role in conditions such as fetal growth restriction, has yet to be fully established. Techniques such as diffusion weighted imaging, magnetic resonance spectroscopy and functional imaging are also being remodelled for use in the fetus, improving our knowledge of in utero metabolism and development.
Introduction

Over the past 50 years, the use of imaging in the assessment of fetal wellbeing has increased dramatically, with ultrasound at the forefront of this development. Now, alongside major advances in technology and expertise, magnetic resonance imaging (MRI) is playing an increasing role. Initial attempts at MRI in pregnancy were limited by fetal movement, despite attempts at immobilisation with maternal benzodiazepines or pancuronium bromide via cordocentesis (Weinreb, Lowe et al. 1985; Williamson, Weiner et al. 1989). Fortunately, the development of ultra fast imaging techniques, has negated the need for such intervention. Sequences such as the Half-Fourier spin turbo (HASTE) and echo planar imaging (EPI) can obtain single images in less than 1 second and allow a complete examination in around 20-30 minutes. Example images for the fetus are shown in Appendix 1 Figures 1a and 1b.

Conventional MRI is used successfully in pregnancy for the diagnosis of adnexal masses and appendicitis, and may be considered in evaluating abnormalities such as placenta accreta (Kier, McCarthy et al. 1990; Maldjian, Adam et al. 1999; Eyvazzadeh, Pedrosa et al. 2004). Pertinent to this review, MRI is increasingly utilized in the assessment of fetal disorders and we aim to review the current indications, discuss more recent advances and highlight how MRI may play an increasing role in this field.
Appendix 1 Figure 1a. Example of fetal MRI in normal pregnancy at 36 weeks gestation cephalic presentation.
Appendix 1 Figure 1b. Example of fetal MRI in normal pregnancy at 36 weeks gestation breech presentation.
Fetal Anomalies

Referrals for in utero MRI have risen, predominantly to confirm or further characterise abnormalities seen on ultrasound. Unlike ultrasound, the images are unaffected by reduced liquor volume (often found in association with the anomaly) and shadowing of more posterior structures or by ossification, is avoided. Due to the wealth of structural information it provides, MRI is increasingly used when planning in utero or early neonatal surgery. Additionally, paediatric specialists may preferentially request MRI, as its use in neonates may found a greater familiarity with the technique.

Central nervous system

Large prospective studies are still required to assess the additional value of MRI for fetal central nervous system (CNS) anomalies, but initial comparisons are promising. Additional clinically important findings were found in around 50% of subjects (Levine, Barnes et al. 2003; Twickler, Magee et al. 2003; Whitby, Paley et al. 2004), with changes in diagnosis in 30% and management in 10-15%. Changes in management included termination or continuation of the pregnancy (particularly prior to 24 weeks gestation), direction of perinatal care and direction of mode or location of delivery (Levine, Barnes et al. 2003). Although most CNS abnormalities requiring MRI are an indication for delivery in a tertiary referral centre, for a small number of patients it may allow delivery in a community hospital e.g. confirmation of a normal spinal cord on MRI following detection of a spinal stenosis on ultrasound. Currently, there is no evidence to support that MRI alters timing of delivery. MRI has also been used to assess CNS damage after the demise of a monochorionic twin or following interventional procedures, as parenchymal damage can be visualised earlier, although the full extent may not be apparent until two weeks after the event (Levine 2006).
MRI is more reliable in detecting the cause and prognostically important associated abnormalities of ventriculomegaly (Benacerraf, Shipp et al. 2007), diagnosing agenesis of the corpus callosum and may prove particularly useful in viewing cortical malformations (d'Ercole, Girard et al. 1998; Glenn and Barkovich 2006). Areas not easily accessed by ultrasound, such as the posterior fossa, are better visualised, although a number of false positives are reported here (Poutamo, Vanninen et al. 1999). MRI is sensitive for intracranial haemorrhages and has been used in disorders such as alloimmune thrombocytopenia (Dale and Coleman 2002). The human brain develops throughout pregnancy, with major developmental events occurring in the latter half of gestation, including neuronal proliferation and migration. It should be emphasised therefore that a normal MRI or ultrasound scan performed routinely between 19 and 22 weeks does not exclude abnormalities of such processes. Although such abnormalities are rare, diagnosis in the index or subsequent pregnancy may be well beyond 24 weeks, presenting difficulties for parents faced with the option of a late termination and indeed for the counselling clinician, as the functional outcomes of such anomalies are often not clearly defined.

Ultrasound is an effective method of identifying myelomeningocele and hindbrain malformations in most cases, however evaluation of neural tube defects can also be performed with MRI. There are conflicting reports of the diagnostic advantages of MRI over ultrasound (Mangels, Tulipan et al. 2000; Aaronson, Hernanz-Schulman et al. 2003; Griffiths, Widjaja et al. 2006), but MRI should certainly be performed if there is doubt regarding the diagnosis. In a study of 50 cases of suspected spinal cord abnormality, fetal MR findings differed from ultrasound in 10 cases, MRI correctly diagnosing normality or reclassifying the abnormality to one that was less severe, such as lipomyelomeningocele (Griffiths, Widjaja et al. 2006). MRI can also be useful in assessing the degree of hindbrain herniation in cases of Chiari II malformation, as well as identifying other
associated CNS anomalies. A multi-centre study is currently underway in the USA to evaluate the role of prenatal repair of myelomeningoceles and if fetal surgery is shown to improve outcomes, fetal MRI may become part of routine assessment in affected pregnancies. MR imaging of the spine is also helpful in cases of vertebral anomalies. In particular it may detect underlying spinal cord abnormalities and could be of use in assessing other anomalies, for example gut artresia in VATER syndrome (von Koch, Glenn et al. 2005).

Thorax

In a study of 74 fetuses referred for thoracic abnormalities on ultrasound, MRI demonstrated additional findings in 38% and changed management in 8% (Levine, Barnewolt et al. 2003). MRI can help differentiate thoracic masses and is useful both diagnostically and prognostically in congenital diaphragmatic hernia (CDH). The involvement of the liver in the hernia is more easily identified on MRI; this is important, as this feature carries a higher mortality and is grounds for in utero surgery in some centres.

Currently, MRI does not accurately visualise the beating fetal heart, but the development of cine image techniques such as 2D-FIESTA (Fast Imaging Employing Steady-State Acquisition) may lead to the introduction of such techniques in the future (Shen, Guo et al. 2007).
Abdomen and Pelvis

The role of MRI in the abdomen is less clear, as most pathologies are easily seen on ultrasound and ultrasound has the added value of visualising peristalsis. One potential use of MRI is to determine the level of bowel obstruction, as meconium is bright and detectable in the rectum by 20 weeks gestation (Zizka, Elias et al. 2006). MRI can also be complementary in genitourinary abnormalities, particularly when there is coexisting oligohydraminos and can be useful in differentiating cystic renal disease (Hormann, Brugger et al. 2006). Additionally, it is superior in the diagnosis of sacrococcygeal teratomas, often incompletely visualized by ultrasound, due to shading by the iliac bones (Kirkinen, Partanen et al. 1997).

Other anomalies

Fetal cleft lip and palate is an area in which MRI shows promise but there is little published data. Whereas MRI and US are equivocal in detecting cleft lip anomalies, MRI is particularly useful in identifying of extension a cleft into the posterior soft palate, often obscured on ultrasound by shadowing from the surrounding bony structures and overlying fetal tongue (Ghi, Tani et al. 2003). MRI provides an accurate diagnosis allowing parents to be counselled appropriately regarding speech and hearing difficulties and also provides an opportunity to assess for associated CNS anomalies. Images highlighting the change in appearance of the fetal brain across gestation are shown in Chapter 1 Figures 4a, b and c.

Fetal and Neonatal Surgery

A rapidly expanding area for MRI is in evaluating the fetus prior to in utero surgery or ex utero intrapartum treatment (EXIT) procedures, increasingly performed for potential neonatal airway obstruction secondary to cervical teratoma or lymphatic malformation. In
uterine MRI may also be useful if complex surgery is likely to be required in the early neonatal period for example in cases of conjoined twin
Appendix 1 Figure 2. Fetal MRI in normal pregnancy at a) 22 weeks and b) 32 weeks and c) 36 weeks, showing changes in appearances of brain sulcation and improved definition of spinal and internal organ anatomy at later gestations.
The Role of Fetal MRI – Organ Volumetry

Knowledge about fetal growth and organ development has greatly improved over the past 50 years, largely contributed to by imaging techniques. MRI is able to accurately predict both organ volumes and fetal weight (Baker, Johnson et al. 1995; Duncan, Issa et al. 2005), of interest in various fetal disorders. In fetal growth restriction (FGR - often defined for research purposes as an individualized birthweight ratio of less than the 5th or 10th percentile for gestational age), ultrasound remains the best predictor of fetal weight (Duncan, Issa et al. 2005), but whether this is the case in macrosomia, has yet to be established. Many such pregnancies are in obese women, where ultrasonic image quality is affected, although the diameter of the magnet bore would prohibit examinations in the very obese.

MRI volumetry has confirmed liver volume to be decreased in FGR, although this was not highly predictive of outcome (Duncan, Issa et al. 2005). Interestingly, MRI may also assess physiological processes; changes in fetal liver signal intensity are seen alongside changes in erythropoiesis activity (Duncan, Baker et al. 1997).

Inadequate maturation of the lungs limits the chance of surviving a premature delivery. MRI can accurately determine lung volume, even when small and in CDH can assess the ipsilateral lung, often not seen on ultrasound (Busing, Kilian et al. 2008). There is much speculation about the use of MR lung volumetry in classifying the prognosis of CDH and several ratios have been explored including lung: head volume and actual: expected lung volume. As yet, no sufficiently predictive test based on these measurements has been established, suggesting that pulmonary hypoplasia is not the only factor determining survival in CDH (Betremieux, Gaillot et al. 2004; Gorincour, Bouvenot et al. 2005; Bonfils, Emeriaud et al. 2006). Interestingly, a rise in MR signal intensity is seen as
alveolar fluid is deposited and the lungs mature, but so far it is unclear whether such observations could be used prognostically. MR spectroscopy and diffusion weighted imaging present alternative strategies for assessing lung maturity (see below).

The phenomenon of ‘head sparing’ is classically reported in FGR, where head circumference is preserved despite failing growth, presumably due to redirected blood flow to the brain. Interestingly, MR images show a reduction in brain volume in FGR, although the head circumference remains spared (Duncan, Issa et al. 2005), which may be important in our understanding of this condition.

The Role of Fetal MRI – Advanced Imaging Techniques

Beyond an anatomical survey, MRI can provide a wealth of information on more functional aspects. Several techniques used in children and adults are being developed for the fetus, under the constraints of ethical considerations and minimising scan time.

Diffusion weighted imaging

Diffusion weighted imaging (DWI) assesses the Brownian motion of water molecules and can be evaluated by measuring the apparent diffusion coefficient (ADC). It has been used after birth in detecting hypoxic-ischaemic damage and white matter disorders. DWI patterns have been characterised in the normal fetal brain (Righini, Bianchini et al. 2003), but unlike in neonates, a chronic rather than acute pattern of ischaemic change is seen, with a likely increase in the ADC. As yet, the prognosis of white matter abnormalities with a raised ADC is unknown, although they correlate strongly with fetopathologic findings of cerebral oedema with astrogliosis (Guimiot, Garel et al. 2008). If earlier lesions are found to have improved outcomes, the technique could play an important role in the prevention of disorders such as cerebral palsy.
Other uses include assessing fetal renal function, with abnormalities demonstrated in the presence of huge dilatations and nephropathies (Savelli, Di Maurizio et al. 2007). Furthermore, the bright signal can be useful in locating the kidney, in cases of suspected agenesis or ectopic kidney. DWI may also have a role in assessing lung maturation, as changes are seen with increasing gestational age (Moore, Strachan et al. 2001), mainly reflecting an increase in pulmonary vascularisation.

*MR Spectroscopy*

MR spectroscopy can be used to estimate the concentration of various molecules within a tissue of interest and continues to be developed. In the fetal brain, several markers of development and ischaemia have been targeted, whilst in the lung, phospholipid components of surfactant were the focus. Unfortunately, the long scan times required (allowing fetal movement) have hindered the development of this technique and although some findings are of interest, the clinical applications are yet to be fully realised.

*Functional MRI*

Functional MRI (fMRI) gives information on pre- and post-stimulus blood flow to various parts of the brain. When an external stimulus causes increased neuronal activity, blood flows to that site, which fMRI detects by estimating the amount of oxyhaemoglobin present. Using this technique in the fetus, both temporal (auditory) and frontal lobe (visual) activation has been demonstrated (Hykin, Moore et al. 1999; Fulford, Vadeyar et al. 2003). Although still requiring development, it may be possible to use such methods diagnostically or as markers of fetal health.
Placental MRI

The placenta is of fundamental importance to the normal growth and development of the fetus and is suited to MRI as it is relatively immobile. The volume of the placenta can be easily measured and is generally low in pregnancies complicated by FGR, although not falling outside the confidence limits for the normal population (Baker, Johnson et al. 1995). The placenta matures with increasing gestation and MRI reflects this, with $T_1$ and $T_2$ relaxation times falling with increasing gestation, suggesting a change in the structural make-up (Gowland, Freeman et al. 1998). Relaxation times may also be altered in pregnancies complicated by FGR or pre-eclampsia, perhaps due to multiple areas of infarction and fibrosis.

Placental perfusion can be depicted in several ways using MRI, with the exception of contrast agents, such as gadolinium, which remain controversial in pregnancy. Arterial Spin Labelling (ASL) tags arterial blood upstream with a radiofrequency pulse and detects it as it flows into the main field. Mapping ASL values across the placental image demonstrates a higher number of pixels with low perfusion values in pregnancies complicated by FGR (Francis, Duncan et al. 1998). Intravoxel incoherent motion (IVIM) measures the reduction in signal when protons flow fast enough within the field to be dephased. Using this technique, the moving blood fraction in the placenta is around 26% (Moore, Issa et al. 2000), with a reduction at the maternal-fetal interface in pregnancies complicated by pre-eclampsia (Moore, Ong et al. 2008).

MR spectroscopy is less affected by fetal movement and has been attempted by several groups, with various methodologies employed. In vivo data is limited, but some interesting results have been obtained in post delivery placentas, such as higher
concentrations of adenosine triphosphate (ATP) in FGR pregnancies (Kay, Hawkins et al. 1992).

**Safety of MRI in Pregnancy**

A full review of MRI safety is beyond the scope of this article, but importantly, no adverse affects have been demonstrated from MRI in pregnancy, including no association with FGR (a concern raised inconsistently in mice studies) and no effect on child hearing (it is likely that the amniotic fluid and flooding of the ear provides more than adequate protection) (Arulkumaran, Talbert et al. 1992; Baker, Johnson et al. 1994; Carnes and Magin 1996; Clements, Duncan et al. 2000; Magin, Lee et al. 2000; Kok, de Vries et al. 2004). The contraindications to MRI (such as cardiac pacemakers or other ferromagnetic objects) remain the same as in the adult and guidelines have been set by the International Electrotechnical Commission (IEC) on the amount of heat (termed specific absorption rate) that can be generated (International Electrotechnical Comission (IEC) 2002). Also, as data is limited, MRI should be avoided in the first trimester. The magnetic field strength used in obstetric imaging is restricted in the UK to below 2.5 Tesla by the Medical Devices Agency (Medical Devices Agency 2002). As technology advances and higher field strengths become available, it is right that both researchers and clinicians to continue to assess fetal outcomes. However, at low field strengths, safety of MRI in pregnancy is well established and women they should be reassured of this, as part of the counselling process.
The Role of Fetal MRI - Conclusions

As our understanding of MRI improves, limitations become apparent, as fetal MRI and its interpretation are not yet available in many centres. The inevitable effect of improved diagnostic capabilities, is an increase in terminations, balanced against the positive outcome of more informed decision making for parents. In the long-term it is unlikely that MRI will replace ultrasound in the antenatal setting and outside the CNS it remains primarily a research tool. However, it has already adopted several niche roles and the potential for development is vast. This, alongside advances in other imaging techniques such as 3D ultrasound, makes for an exciting time ahead in fetal assessment.

END OF PUBLICATION
Appendix II

Morphometric Placental Analysis and correlations with MRI indices

In FGR, it is known that aspects of placental structure are altered, primarily a reduction in villous volume and exchange barrier surface area are observed. It is also known that relaxation times are a reflection of the molecular complexity to which the hydrogen protons are bound and hence differ dependant upon the components of the tissue and on other factors, such as the presence of membrane structures. In previous studies at 0.5 T (Gowland et al, 1998), a fall was seen in relaxation times with advancing gestation and in complicated pregnancies. The origin of these changes is, however, unknown and comparisons with placental tissue morphology and relaxation times have not been reported. We hypothesized that placental relaxation times are correlated with parameters of placental tissue morphology and in this first phase tested the correlation with morphometric analysis of the villous volume and the presence of fibrin.

Morphometric analysis

Similarly to stereologic investigations, good morphometric measurements require the unbiased selection of tissue, i.e., all parts and orientations of each placenta must be given equal opportunity for selection. To achieve this, I adopted a multistage random sampling method in which tissue selection was randomized for location and orientation, adopted from Mayhew (Mayhew et al, 2003). As we wished to compare central and peripheral regions of the placenta, adaptations to Mathews’ techniques were made, detailed below.

7.2 Aims
1. To carry out initial morphometric placental analyses of villous and fibrin content.
2. To correlate these morphometric analyses with placental relaxation times.
3. To compare central and peripheral placental regions in terms of morphometry

**Methods**

Placentas were collected postnatally within 30 minutes of delivery time. Taking the placental cord insertion as the central point, a circle was marked out on a transparent sampling grid with a diameter half that of the longest axis of the placenta. Within this circle was marked as ‘central’ and outside this circle was ‘peripheral’. Full-depth columns of tissue were dissected by superimposing the transparent grid over the placenta, bearing a systematic array of sampling windows (4cm apart). Using a random computer generated number sequence, 3 tissue samples were taken from windows overlying the central region and 5 from the peripheral region. The resulting full depth tissue samples were then dissected into a top (fetal facing), middle and bottom (maternal facing) placental section and fixed in 10% buffered formalin. Only middle sections are considered in this analysis to date. These samples were diced and each sample was embedded haphazardly in paraffin wax blocks and processed for histology. Before imaging, cut sections (5 micrometres) were mounted on microscope slides, stained with hematoxylin and eosin (H&E), and observed under a Litz (Dialux 22) microscope at a magnification of x250. 8 sections were investigated per placenta. For image analysis, a Q-Imaging (Fast 1394) digital camera acquired images from each sample. In total, 40 high-power fields of view were analysed per placenta. Images were acquired using Image-Pro Plus software (Version 6.3). Once acquired, images were pseudocolored and a threshold applied. This manipulation provided a boundary between the objects measured, i.e. those with pixel intensities falling within the thresholding range, and those to be excluded: those falling outside the desired color
intensity. Repeated adjustments of the threshold allowed specific areas of the image to be defined and the area occupied by each compartment calculated. This measurement was finally represented a percentage area of the entire field. All manipulations were conducted in accordance with the manufacturer's instructions.

A)

B)

FIBRIN
Appendix II Figure 1. Microscope images showing A) normal villous appearance with H&E stain and B) villous containing large amounts of fibrin staining bright pink.
Statistical Analysis

To generate $T_1$ and $T_2$ values, the 8 point ROI method was selected as this corresponded best with the 8 point placental tissue sampling techniques. Relaxation times were calculated from the mean signal over the entire 8 point region of interest. Similarly, the morphometric values were taken as the means from all 8 placental samples i.e. from 40 microscope fields.

Initial correlations were made using Spearman’s rank correlation. Wilcoxon’s matched pairs test (non parametric) was used to assess whether differences were present between central and peripheral placental regions in terms of morphometry.

Results

At the time of write-up, 7 placentas were delivered and had been processed for analysis.

Correlations between placental morphometric analyses and relaxation times

Villous area is reported as villous area per field; this is the mean value for all 40 fields investigated across one placenta. Similarly fibrin area per field is the mean value for all 40 fields.
Appendix II Figure 2. Graph showing relaxation times in relation to the villous area per field.

Appendix II Figure 3. Graph showing relaxation times in relation to the fibrin area per field.
Appendix II Figure 4. Graph showing relaxation times in relation to the ratio of fibrin: villous area.

A trend for relaxation times to increase as the villous area increased was observed. A significant correlation was present between $T_2$ and villous area (Spearman’s rank correlation $p=0.048$) but not $T_1$ ($p=0.302$). There were no significant correlations between relaxation times and fibrin area or the ratio of fibrin area to villous area, although trends for a fall in relaxation times as fibrin content increased were observed.

Comparing central and peripheral placental regions in terms of morphometry

Fields from samples taken from central or peripheral areas were grouped together (central = 15 fields, peripheral = 25 fields) and the mean calculated for each group. There were no significant differences between the groups (Wilcoxon’s matched pairs test) in terms of villous area or fibrin area per field ($p=0.375$, $p=0.297$ respectively). Similarly there were no patterns to the relationship between morphometric values taken from paired (same placenta) central and peripheral areas, see Figures 17 and 18 B.
Areas are expressed here as percentage area per field, to highlight that the differences between the groups are small, 3-5%. The median villous area per field is 65-70% and fibrin 3-7% - these values are similar to morphometric values obtained in previous studies (Boyd et al. 1977, Daayana et al. 2004).
Appendix II Figure 5. Scatter plot showing villous area per field (here expressed as percentage area per field) in central peripheral regions. A) Medians and interquartile ranges are shown. B) Relationship between paired values (same placenta) are shown.
Appendix II Figure 6. Scatter plot showing area fibrin per field (here expressed as percentages) in central peripheral regions. A) Medians and interquartile ranges are shown. B) Relationship between paired (same placenta) values are shown.

Correlations between morphometric measurements and relaxation times were poor when both parameters are subdivided into the central and peripheral regions, Spearman’s rank correlations summarized in the table below.
### Appendix II Table 1. Table showing correlations between morphometric measurements and relaxation times in central and peripheral regions.

<table>
<thead>
<tr>
<th>Spearman’s rank correlation</th>
<th>$T_1$</th>
<th>$T_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>central</td>
<td>peripheral</td>
</tr>
<tr>
<td>Villous area central</td>
<td>P=0.91</td>
<td></td>
</tr>
<tr>
<td>Villous area peripheral</td>
<td></td>
<td>P=1</td>
</tr>
<tr>
<td>Fibrin area central</td>
<td>P= 0.75</td>
<td></td>
</tr>
<tr>
<td>Fibrin area peripheral</td>
<td></td>
<td>P=1</td>
</tr>
</tbody>
</table>

### Discussion

There are no previously published reports of how relaxation times correlate with morphological or histological placental tissue parameters. The trends observed are promising, although the small numbers studied so far prevent any firm conclusions being drawn. Additionally, in these initial analyses, the effect of varying gestational age at the time of scan has not been accounted for and will be considered using multi-level modelling of these data, later in the study. However, the increase in $T_1$ and $T_2$ alongside increased villous area is of particular interest, as FGR pregnancies are known to have reduced villous area (Daayana et al. 2004). These data suggest that placental tissue with a low villous area would have a corresponding low $T_1$ and $T_2$ value. This fits well previous studies showing that in complicated pregnancies, placental relaxation times generally lie below the 95% confidence interval for the population (Gowland et al, 1998).
No differences were observed between central and peripheral regions of the placenta in either of the morphometric parameters, suggesting that the placenta is relatively homogenous from region to region in normal pregnancy. This finding may be linked to the mixed/early gestations at which women were scanned, as although central and peripheral regions have not been compared morphometrically in the past, at term placentas often have multiple area of infarction, necrosis and repair which can be seen macroscopically. It would be expected that the greatest variation in morphometric parameters would therefore be seen at later gestations (Boyd et al. 1977) and to draw out these differences, women at gestations of 36 weeks and above will be targeted for the remainder of the study.

The origin of the rise in relaxation times with increasing villous area remains a matter for conjecture. Again these data suggest that the observed changes are not due to an increase in blood volume; as the villous area increases, the intervillous space filled with maternal blood will decrease and less maternal blood will be present. As previously mentioned, the relaxation times for maternal blood are at the high end of the values seen here, so if less blood is present a corresponding fall not rise in $T_1$ and $T_2$ would be expected. Of course, the effect of the volume of blood in the fetal capillaries has not been considered in the morphometric analysis so far and will be investigated later in the study. The fall in relaxation times with increased villous area may relate to surface area of the villi. If the motion of the protons on the surface area of the villi is restricted, producing a fast relaxing compartment in fast exchange with the bulk water protons, then a relative increase in surface area could explain the observed changes in relaxation times. At low villous volumes, the relative surface area may be still be high (due to branching of stem villi out into small terminal villi) and this could explain the short relaxation times. Further morphometric analysis of such parameters is, however, required. The trend for a fall in relaxation times as the fibrin content increases is more easily explained, as complex
molecular structures such as fibrin restrict the movement of hydrogen protons and, similarly to adipose tissue, have long $T_1$ and $T_2$ relaxation times. The small increases in fibrin seen may not entirely explain the corresponding increases in relaxation times, but may in fact be a surrogate marker for a wider change in tissue structure or repair.
Appendix II Figure 1. Examples of IVIM images (B0) with corresponding maps of indices measured (F, D and D*) across the placental region of interests in 3 subjects with normal pregnancies.
Appendix II Figure 2. Examples of IVIM images (B0) with corresponding maps of indices measured (F, D and D*) across the placental region of interests in 3 subjects with FGR pregnancies.