An In-Vivo Structural MRI Investigation of Newborn Infants' Brains: Preterm Infants and Infants born with Intrauterine Growth Restriction

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Jessica Joanne Atkinson.

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Declaration

I certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university. I acknowledge that I have read and understood the University's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis. I certify that I have complied with the rules, requirements, procedures and policy of the University (as they may be from time to time).

The preterm infants were recruited as part of a larger randomised, controlled trial **(**Current Controlled Trials ISRCTN89493983; Regulatory body EUDRACT: 2005-003-09939). Ethical approval was granted by the North West Research Ethics Committee, and approved by the Research Governance Group at Liverpool Women's Hospital. The IUGR infants were also part of a larger research trial (reference number 09/H1011/2009) and ethical approval was approved by Oldham Local Research Ethics Committee.

Print Name:.....

Signature:……..

Date: ……..

Dedication

I would like to dedicate this thesis to my parents for their unconditional support and motivation. I would also like to dedicate this thesis to my brother (James Oliver Atkinson) for his additional support and continual belief in my ability. Finally I would like to dedicate this thesis to the families and infants who are the inspiration and motivation for this thesis, without their participation it would have not been possible.

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Table of Contents

Abstract

An In-Vivo Structural MRI Investigation of Newborn Infants' Brains: Preterm Infants and Infants born with Intrauterine Growth Restriction. Jessica Joanne Atkinson.

Introduction: It is well documented that preterm infants are less developed than term infants at term age and infants with IUGR are said to be of higher vulnerability. Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC) are sensitive to micro structural abnormalities and increases in anisotropy associated with premyelination are the earliest indications of the beginning processes of myelination. Furthermore reductions in brain volumes have been found in preterm infants compared to controls and IUGR infants. Thyroxine (T4) is necessary for normal growth and development of the central nervous system. Infants born preterm miss out on the maternal transfer of T4 that occurs during the third trimester and are born with an underdeveloped thyroid gland that is not yet producing sufficient amounts of T4.

Method: Sixty nine infants (51 preterm, 9 IUGR and 9 controls) were imaged on a 1.5 Telsa MRI Scanner. DTI analysis was performed using medical imaging software (DTIstudio). FA and ADC maps were used to draw regions of interest around the posterior limb of the internal capsule (PLIC), corpus callosum (CC), frontal lobes (FL) and occipital lobes (OL). The software Brain Voyager QX (version 1.9.10) was used for image realignment and demarcation of T2 Weighted images and the images were analysed using medical imaging software for structure- specific brain volume measurements (Easymeasure). Statistical analysis was conducted with repeated measures ANOVA using SPSS version 18.

Results: A significant interaction when investigating anterior vs. posterior structures and laterality of structures with treatment in the frontal lobes and posterior limb of the internal capsule was found suggesting a group difference between infants treated with levothyroxine and those receiving placebo. IUGR infants generally had lower FA and ADC than the control group. Generally lower structural volumes were found in the placebo and IUGR group.

Conclusion: Administration of levothyroxine affects the structures on different sides of the brain differently and raises structural volumes. Levothyroxine may be of particular benefit to infants with low levels of thyroxine in their blood (hypothyroxinemia) and male preterm infants. Preterm infants born with intrauterine growth restriction are of higher vulnerability than appropriate for gestational age preterm infants with lower FA, higher ADC and lower structural volumes. Further research is required to fully explore asymmetries in the preterm and IUGR brain and should look at administering levothyroxine to infants with low levels of thyroxine in their blood.

Publications

Atkinson, J.J., Parkes, L.M., Gamble, C., Ng, S.M., Turner, M., Weindling, A.M., Abernethy, L.J. & Sluming, V. (2011) 'Diffusion Tensor Imaging of the Internal Capsule and Corpus Callosum of Preterm Infants Born Under 28 Weeks Gestation', *Pediatr Res*, vol. 70, no. S5, pp. 149-149.

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S M Ng, M.A.T., C Gamble, M Didi, P Newland, S Victor, D Manning, P Settle, R Gupta, L Abernethy, L Parkes, J Atkinson & A M Weindling (2010) 'Longitudinal FT4 levels in the first 4 weeks of life are an independent factor affecting brain growth in extreme preterm babies born <28 weeks' gestation', *Endocrine Abstracts 2010*, vol. 24, no. OC1.1.

Abbreviations

ADC= Apparent diffusion coefficient

AGA= Appropriate for gestational age

ANOVA= Analysis of variance

Ant= Anterior

Bo= External magnetic field

CC= Corpus callosum

CCA= Anterior corpus callosum

CCP= Posterior corpus callosum

CNS= Central nervous system

CP= Cerebral palsy

D= Diffusion coefficient

DLPFC= Dorsal lateral prefrontal cortex

DMPFC= Dorsal medial prefrontal cortex

DTI= Diffusion tensor imaging

DWI= Diffusion weighted image

FA= Fractional anisotropy

FID= Free induction decay

FL= Frontal lobe

FLL= Left frontal lobe

FLR= Right frontal lobe

FT= Fourier transform

FT4= Free thryoxine levels

GA= Gestational age

G= Gradient strength

GM= Grey matter

HIE= Hypoxic ischemic encephalopathy

IQ= Intelligence quotient

IUGR= Intrauterine growth restriction

LDLPFC= Left dorsal lateral prefrontal cortex

LDMPFC= Left dorsal medial prefrontal cortex

LHemisphere= Left hemisphere

LOLPFC= Left orbital lateral prefrontal cortex

LOMPFC= Left orbital medial prefrontal cortex

LPLIC= Left posterior limb of the internal capsule

MHz= Megahertz

MR= Magnetic resonance

MRI = Magnetic resonance imaging

NMV= Net magnetisation vector

OL= Occipital Lobe

OLL= Left occipital lobe

OLPFC= Orbital lateral prefrontal cortex

OLR= Right occipital lobe

OMPFC= Orbital medial prefrontal cortex

PD= Proton density

PFC= Prefrontal cortex

PLIC= Posterior limb of the internal capsule

Post= Posterior

PVL= Periventricular leukomalacia

RDLPFC= Right dorsal lateral prefrontal cortex

RDMPFC= Right dorsal medial prefrontal cortex

RF= Radio frequency

RHemisphere= Right hemisphere

ROLPFC= Right orbital lateral prefrontal cortex

ROMPFC= Right orbital medial prefrontal cortex

RPLIC= Right posterior limb of the internal capsule

S= Diffusion-sensitised

SD= Nondiffusion signal

SGA= Small for gestational age

SNR= Signal to noise ratio

STD= Standard

T= Distance between each slice

TIPIT = Thyroxine in Preterm Infants Trial

TE= Echo time

TR= Repetition time

TT3= Total triiodothyronine levels

TT4= Total thyroxine levels

T4= Thyroxine

V= Volume

VLBW= Very low birth weight

Wo= Larmour frequency

WM= White matter

X= Spatial location

Figures List

Tables List

CHAPTER 1

Introduction

With advances in neonatal care over the last few decades preterm infants and infants with intrauterine growth restriction are more likely to survive. Preterm infants and infants born with intrauterine growth restriction are known to have later developmental problems in comparison to infants born at term of appropriate for gestational age.

These infants are more likely to repeat a grade at school and have special educational needs and are less likely to continue into higher education. The differences seen between term, preterm and intrauterine growth restriction infants' development has largely been attributed to differences in brain development and maturation.

This thesis begins by exploring the literature on the development of the brain within the foetus on a cellular level. Next the implications of preterm birth and intrauterine growth restriction (IUGR) on brain development are reviewed.

Following this my thesis will go on to explore the anatomical brain using DTI and stereology on a sample of infants born preterm and a sample of infants with intrauterine growth restriction.

The results are discussed in the light of with previous research on DTI and Stereology in infants born preterm and infants born with IUGR. Conclusions are drawn and further research is suggested.

Chapter 2: Development of the Neonatal Brain

This chapter discusses previous literature on brain development beginning with formation of the neural tube, development within the fore brain, cellular proliferation and migration. The chapter then moves on to discuss formation of the mantle, development of the marginal zone and the neocortex. The cells produced within the ventricular layer, neurotransmitters and neuromodulators are also introduced. Next the discussion moves on to maturation of white matter and myelination which is of particular interest for the DTI measures of this thesis.

Next the chapter discusses how this brain development has been found to be different in infants born preterm in previous research and how brain development is linked to diffusion tensor imaging (DTI) and Stereology. Further differences within brain development are then explored including sex differences, asymmetry and motor difficulties.

Finally the role of thyroxine in brain development is considered and development in infants born with intrauterine growth restriction (IUGR) is discussed.

Chapter 3: Magnetic Resonance Imaging and Image Analysis.

This chapter introduces the reader to the physics behind a magnetic resonance imaging (MRI) machine including a brief introduction to MRI, magnetic moment, precessional frequency, larmour frequency and gradients. Moving on to discuss pulse sequence, image contrast, T1 and T2 weighting as well as spin echo sequences.

Next the chapter introduces diffusion weighted and diffusion tensor imaging, the latter which is to be used in this thesis. Within this fractional anisotropy (FA) and apparent diffusion coefficient (ADC) are discussed.

Finally the chapter introduces the image analysis method Stereology using cavalieri's principle and point counting.

Chapter 4: Methodology

This chapter outlines the method for data acquisition used in the studies. Firstly details of the preterm infant sample and secondly the intrauterine growth restriction sample are discussed, followed by information on the MRI sequences used and image analysis procedures for DTI and Stereology. Finally the statistical analysis is presented with the hypotheses.

Chapter 5: DTI Results and Thyroxine

In this chapter the DTI results for preterm and intrauterine growth restriction infants are presented. The results explored differences in DTI measures between structures of interest, between anterior and posterior structures, between left and right hemispheres and interaction with treatment of levothyroxine in the preterm infants. In the IUGR cohort differences between IUGR and a control group are explored. Further to initial analysis further analysis explored differences between total thyroxine (T4), free thyroxine (F4) and total triiodothyronine in placebo and thyroxine treated groups in the preterm cohort.

Chapter 6: Stereology Results

In this chapter the stereology results for preterm and intrauterine growth restriction infants are presented. The results explored differences in structural volumes between the structures of interest, between dorsal and orbital structures, between lateral and medial structures and between left and right hemispheres. Total hemisphere and prefrontal cortex volumes were also explored as was the interaction with treatment of levothyroxine in the preterm infants. In the IUGR cohort differences between IUGR and a control group were explored.

Chapter 7: Discussion

In this chapter the results are discussed and interpreted in line with the previous research and the hypotheses made. Firstly the results of DTI in the preterm cohort are discussed in line with previous research in normal development and DTI in preterm infants including research that also considers future neurodevelopment. Secondly the DTI results in intrauterine growth restriction are discussed. Thirdly and finally the stereology results in the preterm infants are discussed, followed by the stereology results in intrauterine growth restriction infants.

Chapter 8: Conclusions and future research.

Chapter 8 concludes the thesis with a brief final interpretation of the results in the light of previous research, and goes on to suggest possible future research ideas based on this thesis and the work that precedes it.

CHAPTER 2

Development of the Neonatal Brain

Aim of Chapter

This chapter aims to provide an overview of the literature on the development of the neonatal brain, brain development in preterm and IUGR infants and the role of thyroxine in brain development.

During foetal development the brain goes through organised structural changes (Huang 2010) which are demonstrated in figure 2.1. These changes begin early in uterine development where ectoderm is the predecessor for the central nervous system (CNS) (Richards, Plachez & Ren 2004). There are three basic processes in which ectoderm develop into the internal structures of the CNS: migration of neuroblasts, fusion of the neural plate into a neural tube and the division of the cells in the forebrain (prosencephalon) into separate regions including the cerebral hemispheres (Yakovlev 1959).

The regions of the central nervous system begin to form with initial clustering of the embryonic cells to form the fore-mid-and hind-brain. As the neural tube becomes fully formed, patterning (subdivision into distinct brain areas by gene expression) is seen in the rostro-caudal axis and along the dorsal-ventral axis (Lagercrantz 2002).

Brain Development in Embryo

Formation of the Neural Tube

Conversion of the neural plate into the neural tube takes place during the third and fourth week of gestation (Fenichel 1990). Formation of the neural tube involves changes in cell shape and cell- cell interactions of ecto-dermal cells. At this stage of development cellular

proliferation has a minor contribution. The ectoderm forms the neural plate in a process called neural induction. The neural plate then bends to form the neural groove. Neural folds are developed from the edges of the neural plate thickening (Lagercrantz 2002). On day 22 of gestation these neural folds begin to adhere so that the cells from the two neural folds merge (Schoenwolf 2009). This closes the neural groove and forms the neural tube (Lagercrantz 2002).

The neural tube will later differentiate to form the brain and spinal cord. Gene expression determines which regions of the neural tube will differentiate into the specific structures and cells of the nervous system. The development of the midline of the nervous system is also regulated by the same genetic patterning events and the formation of this midline is crucial for the later formation of the midline comissures such as the corpus callosum (Richards, Plachez & Ren 2004).

Development within the Forebrain

Initially the forebrain contains a single vesicle (the proscenphalon) although shortly after the proscenphalon has formed cellular proliferation and migration contributes to development either side of the single vesicle to form two hemispheres (Lagercrantz 2002). This occurs during the fifth week and the two vesicles that are formed are known as the telencephalon and diaencephalon (Schoenwolf 2009). Cellular projections include proliferation, migration and differentiation of cells into neurons and glia. These cellular projections work within the developing cortex to produce the six layers which make up the mature cortex (Richards, Plachez & Ren 2004).

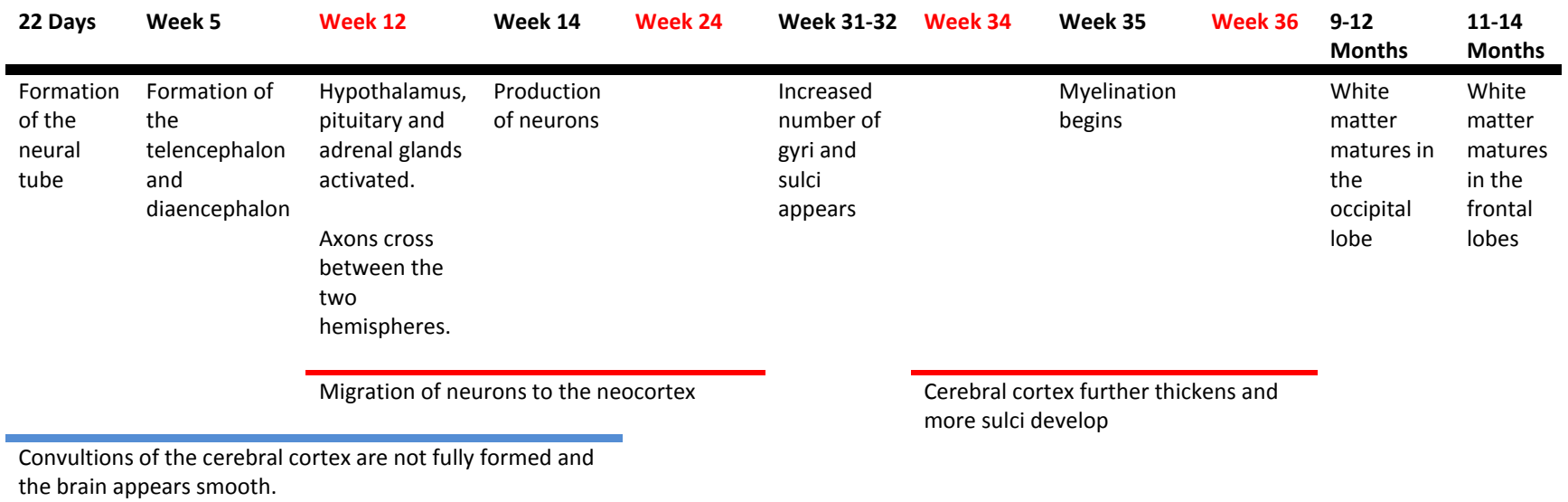


Figure 2.1 Timeline of events in Neonatal Brain development

Cellular Proliferation

Cellular proliferation occurs in the ventricular zone (Richards, Plachez & Ren 2004; Schoenwolf 2009). The closed neural tube contains proliferating cells which are involved in self-renewal and production of neurons and glial cells (Lagercrantz 2002). The first cells to be produced in the ventricular layer are known as post mitotic neurons, these neurons migrate to form the next layer known as the mantle (Schoenwolf 2009). Proliferative events including the production of neurons occur between 8 and 16 weeks (Volpe 1987). Neurons migrate to the neocortex between 12 and 24 weeks (Sandrine Passemard et al. 2010).

Migration

Migration is a complex step which occurs early in embryonic and foetal development; in humans migration of neurons to the neocortex occurs between the third and fifth month of gestation (Lagercrantz 2002; Volpe 2008). The cell layer of the neural tube is initially a single layer and converted to a multilayer structure via migration. Stem cells are confined to the inner layer known as the ventricular layer and the newly formed cells migrate to separate layers of the cortical plate and spinal cord. During migration neurons are guided by the specialised cells known as radial glial cells (Lagercrantz 2002).

Formation of the Mantle

As cellular proliferation of stem cells is sustained in the developing CNS, there are more and more newly formed cells which migrate out of the ventricular layer forming a second layer around the neural tube. This second layer becomes thicker as more cells are added to it and is known as the mantle or intermediate zone (Lagercrantz 2002).

Development of the Marginal Zone

In the intermediate zone there are postmitotic neurons which establish a pattern of axonal connections (Lagercrantz 2002). Other nerve fibres involved in connecting neurons in the CNS include dendrites and synapses which with axons form the

complicated neuronal circuits which transmit nerve pulses (Tang 2003). Eventually many of these axons will be coated in myelin sheaths by the glial cells known as oligodendrocytes (Lagercrantz 2002; Schoenwolf 2009). The process in which axons are coated in myelin sheaths is known as myelination. The process of myelination improves connectivity in neuronal circuits allowing nerve pulses to transmit faster. The region which contains these axonal networks which arise from the neurons is the outermost layer of the developing brain and spinal cord known as the marginal zone (Lagercrantz 2002). This zone later makes up the white matter in the CNS (Schoenwolf 2009).

Further development within the Brain

These three layers discussed (the ventricular zone, the mantle/ intermediate zone and the marginal zone) remain throughout development of the spinal cord and medulla (Lagercrantz 2002). However in development of the mammalian brain these three layers have further evolved to become the six layers of the highly organised neocortex (ventricular, sub-ventricular, intermediate zone, sub plate, cortical plate and marginal plate) (Lagercrantz 2002; Richards, Plachez & Ren 2004).

After mitosis the surviving neurons continue to grow and differentiate (Richards, Plachez & Ren 2004), the initial neurons form layers closet to the ventricular zone and subsequent neurons migrate further through preceding layers to form the external layers of the cerebral cortex (Lagercrantz 2002).

Cells produced within the Ventricular Layer

Once the production of neurons is reduced a new cell type known as glioblast are produced. Glioblasts differentiate into glia which provides structural support for the CNS. The glia found within the CNS are known as astrocytes and oligodendrocytes (Schoenwolf 2009). The interaction between astrocytes and neurons which involves the exchange of metabolites and neurotransmitters are a prerequisite for normal brain function and therefore proper function of the CNS in adulthood depends on proper development of astrocytes during embryogenesis (Lagercrantz 2002).

The ependymal cells are the last cells to be produced by the ventricular layer, these cells line the ventricles and are involved in the production of cerebrospinal fluid (CSF) which protects and supports the brain (Schoenwolf 2009).

Neurotransmitters and Neuromodulators

The action of neurotransmitters and neuromodulators play a role in the detailed wiring of neuronal circuits. Signals passed on to neurons can be promoted, amplified, blocked, inhibited or attenuated by neurotransmitters and neuromodulators and therefore they give rise to signalling patterns between many neuronal networks (Lagercrantz 2002).

Their markers in CNS development generally initially appear in the caudal part of the brain and levels increase at the same time as synapse formation. In the healthy newborn it is hypothesised that there is a surge of excitatory neurotransmitters and a down regulation of inhibitory ones. Early insult such as prenatal stress at a critical period can result in long term changes in the structure and function of the brain. Prenatal and perinatal stress such as hypoxia can affect the expression of neurotransmitters and neuromodulators as well as their receptors, which can lead to long term behavioural effects (Lagercrantz 2002).

Maturation of White Matter

First Trimester

Different parts of the brain mature at different rates (Marshall 1968) demonstrated by differences seen in the degree of myelination among adjacent white matter sites (Brody 1987) and the variation in anisotropy in different white matter regions (Pierpaoli et al. 1996). At the beginning of the 5th week of the embryo's development in the intrauterine environment each cerebral hemisphere arises. As development continues, the cerebral hemispheres grow. First the anterior hemisphere which forms the frontal lobe, followed by laterally and superiorly to form the parietal lobes. Lastly the posterior and inferior hemisphere grow producing the occipital and temporal lobes (Snell 2010). By the end of the first trimester the foetal hypothalamus, pituitary and adrenal glands have been

activated (Okada et al. 2011). At the end of the first trimester/ beginning of the second (around 12 weeks) axons begin to cross between the two hemispheres (Marin-Padilla 1997).

Commissures are a bundle of nerve fibres which cross the midline of the CNS to connect similar structures in the brain from different sides to one another (Martin 1994). The third commissure to develop is the corpus callosum (the second and first being the optic chiasm and anterior commissure) (Huang 2010; Snell 2010). The corpus callosum is the largest fibre tract in the brain and connects neurons in both cerebral hemispheres (Richards, Plachez & Ren 2004) to share learning and memory (Cooke & Abernethy 1999). It connects both sides of the frontal lobe (Snell 2010) and is formed by cortical axons which are attracted to the midline by glial cells, with the genu developing prior to the splenium. At 9 weeks gestation the earliest components of the corpus callosum appear, by 12 weeks it is definable as an independent structure (Volpe 2008) and by 18 can be seen on ultrasound (Marin-Padilla 1997). The corpus callosum then further develops over the next few weeks in both the anterior and posterior directions. By 19 weeks the anterior corpus callosum has become more dominant (Huang et al. 2006) suggesting there is more development within the anterior corpus callosum over the 15-19 week gestation period (Huang 2010). By 20 weeks the basic structure is completed (Volpe 2008) and by 22 weeks the structure has adult features (Marin-Padilla 1997).

While various masses of grey matter develop within the cerebral hemispheres elsewhere in the nervous system neurons are maturing. These maturing neurons send axons to or from the cortex and these axons form the large ascending and descending tracts that make up the internal capsule (Snell 2010) A structure which can be visualised in DTI at 13 weeks gestation. By 13 weeks the limbic fibres are also visible, as are the projection fibres which develop further to the peripheral regions over the second trimester. The next tracts to begin development within the second trimester are some of the commissural and association tracts (Huang 2010).

Second Trimester

During the second trimester the adrenal cortex (Okada et al. 2011), association fibres of the corpus callosum and internal capsule are developing significantly (Huang 2010) and are well visible by 19 weeks (Huang et al. 2006). By contrast the inferior fronto- occipital fasciculus, inferior longitudinal fasciculus and uncinate fasciculus do not and are un-traceable until 19 weeks (Huang 2010) and even at 19-20 weeks they cannot be well appreciated (Huang et al. 2006).

By 19 weeks the limbic fibres are well developed, as is the cerebral peduncle and the anterior commissure. At 19-20 weeks gestational age the temporal lobe is visible and the basal ganglia and hippocampus can be clearly identified, although the brain has a smooth surface and the sylvian fissure is the only sulcus in view. From this time to term the whole brain volume increases 17 times over (Huang et al. 2006).

At 20 weeks the corpus callosum is more advanced in the frontal lobe rather than the occipital lobe where as callosal fibres are not well developed and enlargement of the genu is visible (Huang et al. 2006).

In the cerebral hemisphere the anterior limb of the internal capsule is well developed and the proportion of the internal capsule shows the anterior region to be more developed than the posterior limb of the internal capsule (Huang et al. 2006).

Third Trimester

White matter development continues from 21-24 weeks to term (Huang 2010) and microstructural changes that occur between 28 and 40 weeks gestation include fibre development such as myelination (Huppi et al. 1998; Marin-Padilla 1997). Although myelination may begin as early as 24 weeks gestation in the fibres of the basal ganglia (Snell 2010).

In terms of the development of white matter tracts, the limbic fibres are first to develop, the commissural and projection fibres develop from the core to the

periphery and from anterior to posterior. The association fibres are last to develop (Huang 2006, 2010; Marin-Padilla 1997). As development of white matter tracts follows an antero-posterior gradient, at any prenatal age, the frontal regions of the WM are more developed than the parieto-occipital regions (Marin-Padilla 1997).

Post-natal

The last process to occur is the maturation of white matter (Lagercrantz 2002) which forms from the core of the brain to the periphery and from the anterior to posterior regions (Huang et al. 2006). Therefore maturation proceeds from the frontal region anteriorly to the occipital and temporal lobes.

White matter consists of the axonic fibres that connect the different regions of the cerebral hemispheres. In addition to axonic fibres white matter is made up of myelin sheaths and neurofibrils which make up axonal systems consisting of large groups of myelinated axons (Beaulieu 2002). Although Drobyshevsky et al believe an increase in oligodendrocytes that occurs during the premyelination period may be important in defining the structural and functional maturation of white matter (Drobyshevsky et al. 2005).

Development of Myelination

Preparatory Changes

Prior to myelination there are changes in the development of fibre tracts in central white matter which are related to the 'early wrapping of axons by oligodendroglial processes' (Barrett 2010; Huppi et al. 1998; Remahl & Hildebrand 1990; Snell 2010). Axons make up white matter and are mostly surrounded by myelin sheaths which in part are produced by the neuroglial cells (Barrett 2010; Marshall 1968; Snell 2010). There are four types of neuroglial cells one of which is oligodendrocytes which are believed to be involved in the process of forming myelin in the central nervous system (Barrett 2010; Snell 2010). In the optic nerve of rats, ensheathment (the process by which the axons are surrounded by myelin sheaths) has been found to

begin six days post birth when the axons are large. Prior to myelination clusters of vesiculotubular profiles are present within the axon **(**Hildebrand & Waxman 1984). These vesiculotubular profiles fuse with the membrane in a way that contributes to membrane growth by addition of phospholipids to the axon membrane (Waxman 1985). This is a type of lipid and a component of membrane which can form lipid bilayers. Membrane synthesis involves the transportation of lipid bilayers in vesiculotubular profiles via the axonal system. Vesiculotubular profiles travel to the axon where they fuse with the membrane as mentioned above. Differentiation of the axon membrane may then occur in situ altering the axolemmal structure which may be a basis for the development of myelination into a highly organised structure (Waxman 1985).

Antenatal Myelination

Neuroglial cells are small cells that are present among nerve cells (Marshall 1968; Snell 2010) and they account for nearly half (40%) of the total brain volume (Bayer 2005; Snell 2010). Sites of glial cell growth are present throughout grey and white matter during the second trimester. Within the fibre tracts glial cells develop and divide prior to myelination (Bayer 2005). These sites are known as myelination gliosis and remain present in the third trimester (Bayer 2004). Myelin sheaths start forming before birth and continue to form post birth for the first year. Myelination is a systematic process where different nerve fibres myelinate at different times. It begins around the 16^{th} week of intrauterine life and by 37 weeks gestation the surface of the brain hemispheres show myelin (Snell 2010). At 28 weeks gestation the corpus callosum remains unmyelinated (Huppi et al. 1998) and once myelination begins the process is slow (Dudink 2007; Kinney 1988). Whereas at this time period the internal capsule is in the process of myelination (Huppi et al. 1998) and is still myelinating rapidly at term (Cowana & de Vriesb 2005; Huppi et al. 1998; Kinney 1988; O'Shea et al. 2005; Rutherford et al. 1998). At term age the corticospinal tracts are arising in the postcentral gyrus and enter the posterior limb of the internal capsule (Rutherford et al. 1998). At 44 post-conceptional weeks (gestational plus postnatal) 50% of infants have mature myelin in the posterior limb

and 90% by 103 weeks whereas myelination in the posterior frontal white matter is not present until 47 post-conceptional weeks (Kinney 1988).

Microstructural changes that occur between 28 and 40 weeks gestation include fibre development such as myelination (Huppi et al. 1998)**.** Myelination is the process by which nerve fibres are coated with thin layers of lipid called myelin, the layers of this lipid being disposed in a spiral. The axon must be ensheathed by a secondary cell (Bunge 1968); glial lamellae (Remahl & Hildebrand 1990). Spiralling of the ensheathment process occurs and the layers of this process must be compacted. When myelination has completed and the nerve fibres are coated sufficiently with myelin this coating is known as the myelin sheath. Myelin acts as electrical insulation and increases the efficiency of nerve fibres and the myelinated axon has been referred to as the "superhighway of the nervous system" (Bunge 1968). Large groups of myelinated axons are known as axonal systems which are made up of myelin sheaths, axonal membranes and neurofibrils. These axonal systems connect various regions in the brain and are known collectively as white matter (Beaulieu 2002).

Postnatal Myelination

The myelination process is slow so post-birth the brain is still largely unmyelinated and the corticobulbar, corticospinal, tectrospinal and corticopontocerebellar fibres begin to myelinate (Snell 2010). By three months post-birth; myelination is most advanced in some of the motor and sensory areas followed by auditory and visual areas (Marshall 1968). Six months post-birth the corticospinal fibres start to myelinate and their myelination is mostly complete by the end of two years. Myelination continues until all the major nerve fibres are myelinated which occurs by the time the infant is walking (Snell 2010). Two years post-birth, the progress of myelination throughout the nervous system continues and will continue through puberty, maturity and into old age (Dubois et al. 2006; Marshall 1968; Snell 2010).

Definitions

Infants that are said to be small for gestational age are infants whose birth weight is below the 10th centile. Their growth is low at all gestational ages but they are otherwise healthy. Preterm infants are infants born before 37 weeks gestation. Low birth weight infants are infants born below 2500 grams. Those infants born with an especially low birth weight are said to suffer from intrauterine growth restriction (IUGR) and this can be defined as a birth weight below the 10th percentile predicted for gestational age or a birth weight two standard deviations below the mean. With IUGR growth is normal in the early part of pregnancy but slows in utero by at least two measurements.

Brain Development in Preterm Infants

At 24-40 weeks gestation multiple developmental events take place which involve pre-oligodendrocytes, microglia, axons, subplate neurons, the cerebral dorsal subventricular zone and ventral germinative of the ganglionic eminence, thalamus, cortex and cerebellum. During the premature period cerebral white matter axons such as the projection, commissural and association fibres are rapidly growing and ensheathement of axons by pre-oligodendrocytes occurs although mature myelin producing oligodendrocytes do not become wide spread in cerebral white matter until after term (Volpe 2009). In the development of the preterm infant myelin is evident in numerous grey and white matter tracts under 28 weeks gestational age. Between 30 and 36 weeks this myelination continues to increase in the areas already identified but there is no myelination in new sites until 36 weeks gestation when myelin becomes evident in the posterior limb of the internal capsule (Counsell et al. 2002).

It is well documented that preterm infants are less developed than term infants at term age (Abernethy LJ et al. 2003; Dudink 2008; Huppi et al. 1998; Nosarti 2008; Woodward 2005). In very preterm infants fibre organisation and myelination during the period between preterm birth and term age may not match that of term infants still in the intrauterine environment (Counsell & Boardman 2005; Nosarti 2008).

This slower development in preterm infants continues throughout the first year of life (Cole & Cole 1997).

Brain Development and DTI (see Chapter 3 for more information on DTI)

Maturation of white matter including the development of myelination can be determined by changes in DTI parameters such as the anisotropy parameter of apparent diffusion coefficient (ADC) and fractional anisotropy (FA) (Dubois et al. 2008; Dudink 2008; Huppi et al. 1998; Neil et al. 1998; Wimberger 1995). FA and ADC are sensitive to micro structural abnormalities(Murakami 2007) and increases in anisotropy associated with premyelination are the earliest indications of the beginning processes of myelination (Neil 2002).

ADC

ADC characterises the diffusion of water; diffusion is reduced by biological barriers in the brain and therefore by measuring ADC we get an insight into biological barriers (i.e. structures) in the brain. A high ADC value means more water diffusion, i.e. the higher the ADC value the further the water molecules have travelled per unit time. The further the water molecules are travelling the less brain structure there is acting as a barrier to diffusion (Tofts 2003).

FA

FA looks at the direction of water diffusion and whether it its isotropic i.e. travelling the same distance in all directions. When FA is isotropic (low FA) there are no or limited barriers to diffusion allowing the water to diffuse to the same degree in all directions. When FA is anisotropic i.e. the water molecules are travelling different distances in different directions (high FA) there are barriers limiting the diffusion in certain directions.

ADC and FA During Development

ADC and anisotropy measures are shown to change dramatically during development (Cascio, Gerig & Piven 2007; Drobyshevsky et al. 2005; Dubois et al. 2006; Neil 2002) which reflects the changes in tissue water content and

cytoarchitecture. These changes are occurring more rapidly in early development and in particular in the first year of life. Measurements taken from adult brains differ to those from paediatric brains and the parameters vary with age (Neil 2002). ADC decreases with age whereas FA increases (Drobyshevsky et al. 2005). These changes offer an insight into the maturation of the human brain (Neil 2002). Anisotropy becomes more evident with the increasing maturation of white matter (Wimberger et al. 1995). Thus I hypothesise that ***Infants FA and ADC measures will be significantly correlated with weight and gestational age at birth.***

Gestational Age and Birth weight

Importance in looking at birth weight and gestational age at birth is demonstrated by previous research which found differences in the prevalence of neurodevelopment disability according to gestational age at birth (Hack & Fanaroff 1999). They found 30% to be severely disabled when born at 23 weeks, a range of 17%-45% when born at 24 weeks and a range of 12% - 35% at 25 weeks. They also found cognitive disability to be evident in 15%-31% of preterm infants born at 27 weeks gestation (Hack & Fanaroff 1999). Measures of motor and visual motor skills have been found to significantly correlate with gestational age at birth and birth weight. Children who performed poorly on three different measures of motor skills were found to have lower gestation and birth weight (LA Foulder-Hughes 2003). Furthermore Rose et al argue that gestational age is known to influence brain structure abnormalities and disabilities associated with cerebral palsy (Rose et al. 2009).

FA and ADC in Brain Regions

The different areas/ structures of the brain mature at different times and rates as mentioned earlier. Changes in anisotropy and myelination also occur in this manner (Drobyshevsky et al. 2005; Dubois et al. 2006) with striking differences in degree of myelination in different white matter sites (Brody 1987). Changes in ADC occur more rapidly in projection fibres such as the internal capsule compared to changes in commissural fibres such as the corpus callosum which are slower to change (Drobyshevsky et al. 2005).There is earlier maturation in the motor and sensory

tracts compared to the cortico-cortical tracts (Dubois et al. 2006) and changes in anisotropy occur earlier in white matter underlying the visual cortex than other sub cortical white matter and are low in occipital white matter at 26 weeks but increase at 40 weeks gestation (Neil 2002). In the white matter of rats there is an increase in the number of myelinated and unmyelinated axons towards the posterior regions and a significant difference between the number of myelinated and unmyelinated axons in the frontal compared to occipital regions (Ginus Partadiredja 2003). Although other research has found significant difference between anterior and posterior commissures with significantly more myelin axons in the anterior commissure and a significant difference between myelinated axons in the genu (anterior) and splenium (posterior) of the corpus callosum in the rat brain (Sargon et al. 2003).***This leads to the hypothesis that higher FA and lower ADC will be found in the posterior part of the internal capsule and corpus callosum.***

FA and ADC Prior to Myelination

There seems to be two phases to the increasing anisotropy values in white matter during infant development. The first includes changes to the structure prior to myelination (Huppi et al. 1998; Wimberger 1995) and the second is associated with the appearance of myelin and its maturation. This has been demonstrated in central cerebral white matter where there are increases in anisotropy and decreases in ADC between 28 and 40 weeks gestation (Huppi et al. 1998) even though central cerebral white matter does not begin to myelinate until term (Kinney 1988). Furthermore high anisotropy values were found in the corpus callosum of preterm infants even though it is mostly unmyelinated (Partridge et al. 2004) and both the unmyelinated corpus callosum at 28 weeks gestation and the posterior limb of the internal capsule which at 28 weeks gestation is in the process of myelination demonstrated anisotropy indicating that anisotropy may be linked to myelination processes but myelination is not the only process causing anisotropic diffusion (Huppi et al. 1998). Drobyshevsky et al believe diffusion anisotropy in development relates to the increase in premyelinating oligodendrocytes (Drobyshevsky et al. 2005).

These measures (FA and ADC) relate to behavioural measures such as cognitive and motor abilities in both clinical and healthy children (Cascio, Gerig & Piven 2007).

Brain Development and Stereology in Preterm Infants

Stereology is an unbiased method of estimating the volumes of an irregular shape and can be applied to MRI images using Cavalieri's principle in combination with point counting. It provides accurate estimation of the volume of an arbitrary object from a few systematic sections and has been applied to measure foetal volume in utero (Roberts et al. 1994). Stereology is an efficient method to make a bias free estimation of a structure without needing a detailed assumption of its shape (Roberts, Puddephat & McNulty 2000b).

Stereology has been useful in the measure of foetal development in the womb via an estimation of foetal volume (Roberts et al. 1994) and in the study of brain structure volumes in preterm infants (Allin et al. 2001; Lancefield et al. 2006; Nosarti et al. 2002b). Preterm infants have been found to have a reduction in whole brain volume, grey matter volume and hippocampal volumes (Nosarti et al. 2002b) as well as smaller white matter volume, corpus callosum, cerebellum (Escobar-Morreale et al. 2005) and smaller cerebellar volume although not significantly (Shah et al. 2006). Reduced grey matter included the temporal, frontal, occipital cortices and cerebellum and white matter loss in the brain stem, internal capsule, temporal and frontal regions and the major fasiculi (Nosarti et al. 2008). Other research has found significantly lower cerebral volumes (Allin et al. 2001; Lancefield et al. 2006), premotor, sensorimotor and occipital parietal regions in preterm infants and significantly larger prefrontal regions (Lancefield et al. 2006). Similarly reduced volumes of parieto-occipital grey matter but increased volumes of mid-body, occipital horns and temporal horns of lateral ventricles has been found in preterm infants in addition to larger anterior cortices (including: dorsal prefrontal, orbital prefrontal, premotor, subgenual and mid temporal regions) and smaller sensori motor and posterior cortices (Peterson et al. 2003; Thompson et al. 2007). In addition Thompson et al (2007) found reductions in the orbitofrontal and premotor regions in preterm infants. In general there seems to be a consensus that there are

several regional disruptions to preterm infants' cerebral development by term age, as summarised by Thompson et al (2007). In addition to reductions in grey and white matter volume discussed above other anatomical changes have been demonstrated including an increase in the grey matter in the temporal and frontal lobes, and white matter excess in temporal, parietal and frontal regions (Nosarti et al. 2008).

Stereology and Later Development

The cerebellum is known to be involved in co-ordination of movement and cognitive processes and Allin et al, 2001 found significant associations between cerebellar volume and cognitive test scores. They argue that the cognitive deficits associated with preterm birth may be related to dysfunction in several neural systems including the cerebellum. The thalamus anatomically connects the cerebellum to the dorsal lateral and dorsal medial prefrontal cortex (Allin et al. 2001), thus similar reductions in volume and dysfunction may be also associated with these areas. Furthermore a meta analysis found reductions in volume of preterm infants to be associated with decreased general cognitive functioning (Escobar-Morreale et al. 2005) and the mental subscale of Baileys at 18 to 20 months corrected age correlated significantly with neonatal white matter(Peterson et al. 2003). Disturbances in cerebral development of the preterm infant have also been found to impact later working memory (infants aged two), specifically reductions in total tissue volumes of dorsolateral prefrontal cortex, sensorimotor, parietooccipital and premotor regions (Woodward et al. 2005). Preterm infants that exhibited working memory deficits at age two were found to have smaller hippocampi than children with a intact working memory and children who failed to learn or persist with the working memory task (Beauchamp et al. 2008). Aged two, preterm infants demonstrated differences in cognitive and psychomotor development compared to term controls with less preterm infants scoring within the normal range and increases in preterm infants scoring mild and serious cognitive and motor delay. This poor performance in mental and motor performance of preterm infants was significant (Woodward et al. 2005). Very preterm infants scored lower on measures of language and executive function and were more likely to show cognitive

impairment in adolescence than term controls and this cognitive impairment was associated with decreased grey and white matter volume (Nosarti et al. 2008).

Gestational Age and Birth weight

Associations between gestational age and brain structural volume have been found with a reduction in myelinated white matter fibres in older adults (74 years) and younger adults (female 38 years, males 21.8 years) (Tang 2003). Similarly elder subjects have been found to have a lower volume of white matter and myelinated fibres in comparison to young subjects but this difference was not significant (Tang et al. 1997) and an inverse relationship between age and number of neurons in the hippocampus has been found (West & Gundersen 1990). Similar associations with gestational age were found in preterm infants with the more premature the infant the smaller the white matter volume (Nosarti et al. 2002b) and increasing immaturity was found to be associated with poorer multisearch multilocation task performance which is associated with working memory (Woodward et al. 2005). Cognitive impairment in very preterm adolescents has been found to be associated with gestational age and language and executive function score was positively associated with gestational age (Nosarti et al. 2008). Contradictory to this other research in preterm infants found regional volumes to not correlate with age (Kesler et al. 2008; Peterson et al. 2003) and there was no relationship between cerebellar volume and gestational age (Shah et al. 2006). Despite claiming degree of immaturity was not related to regional brain structure in their abstract Thompson et al (2007) did find a small effect of immaturity and region specific differences according to gestational age. ***I hypothesise that smaller brain structural volumes will be found in infants with lower birth weight and gestational age at birth and with which they will significantly correlate.***

Prefrontal Cortex Regions

In the prefrontal cortex dorsal regions have been found to be larger than orbital regions with dorsal lateral being the largest sub-region of the prefrontal cortex (Howard 2003).

Sex Differences

In addition to changes in ADC and FA measures during development and differences between structures in the brain, sex differences have been observed. There is numerous evidence of gender differences in the maturation of the foetal and young child's brain (Petty 1999). Sex differences were found in the splenium of the corpus callosum, right posterior limb of the internal capsule and frontal lobes, males had lower FA in the splenium of the corpus callosum (Rose et al. 2009; Schmithorst, Holland & Dardzinski 2008) higher ADC in the splenium of the corpus callosum, lower FA and higher ADC in the right posterior limb of the internal capsule suggesting delayed development of these structures in males (Rose et al. 2009), although they had higher FA in associative white matter regions including the frontal lobes (Schmithorst, Holland & Dardzinski 2008).Other research has also found sex differences in brain abnormalities including the splenium of the corpus callosum and right posterior limb of the internal capsule. Males had a lower FA and a higher ADC suggesting delayed development of these structures in males. Furthermore males were more likely to have abnormal neurodevelopment and had a higher incidence of cerebral palsy. Neurodevelopment was abnormal in more than four times as many males than females. Gestational age at birth has been found to be lower in males rather than females (Kesler et al. 2008; Rose et al. 2009). In a rat model of preterm infant brain injury males were found to be more sensitive to insult (NuÑEz & McCarthy 2003). Other earlier research has demonstrated a smaller corpus callosum in males compared to females (Kesler et al. 2008; WITELSON 1989) and later research found greater volume loss in mouse male hippocampus compared to females (Mayoral, Omar & Penn 2009). Sex differences in the sub regions of the corpus callosum have also been found in preterm infants (Peterson Bs & et al. 2000). Research has found male brains to be larger in volume, females had less cerebral white matter but larger cortical volume than males (Filipek et al. 1994). Similarly Thompson et al (2007) found males to have larger volumes in all

brain regions and in particular in posterior regions whilst females had lower total tissue volumes in the inferior occipital and dorsal prefrontal region. Furthermore larger cerebellar volumes in male compared to female preterm infants has been found (Shah et al. 2006). However other research has found preterm males to have significantly lower white matter volumes (bilateral cingulum, corpus callosum, corticospinal tract, prefrontal cortex superior and inferior longitudinal fasiculi) and grey matter volumes (prefrontal cortex, basal ganglia and temporal lobe) whereas females did not differ significantly between term and preterm (Kesler et al. 2008). Contradictory to the above others have found no sex difference in prefrontal cortex volume (Gur Re & et al. 2000) and regional volumes (Eckerman, Sturm & Gross 1985). Thus I hypothesise that there will be a difference in DTI and stereology measures between males and females**.**

Asymmetry

Functional asymmetries such as handedness have been well established and researched (Petty 1999; Westerhausen et al. 2004; WITELSON 1989), however in addition there is structural differences between the two sides of the brain (Petty 1999). In a study of East African skulls asymmetry was found in 98% of the skulls with the largest asymmetry present in the parietal and occipital regions with the right frontal being larger than left and left occipital larger than right (Gundara & Zivanovic 1968). The left occipital lobe is often wider than the right and the right frontal lobe usually extends further than the left (Le May & Kido 1978) i.e. the left occipital lobe is larger than the right and the right frontal lobe is larger than the left (Petty 1999). This has been confirmed by Weinberger et al who also found the right frontal lobe and left occipital lobe to be larger in the majority of brains measured. These asymmetries were observed in foetuses as young as 20 weeks gestation indicating that this asymmetry is an early manifestation of human brain development (Weinberger et al. 1982). Another area of marked asymmetries is in parts of the dorsal lateral prefrontal cortex (Petty 1999) with right dorsal lateral larger than left (Gur Re & et al. 2000; Howard 2003). Right prefrontal, premotor and sensorimotor cortices have been also been found to be larger in the right compared to left in preterm infants (Lancefield et al. 2006). Furthermore altered lateralisation

of white matter in the parietal occipital region of preterm infants has been found with larger left and smaller right sided structures (Eckerman, Sturm & Gross 1985). By contrast in the whole sample from one study including both premature and term born infants the cerebellar volume was larger in the left hemisphere (Shah et al. 2006).

In contrast to this other research has found brain structures to be symmetrical, and asymmetry to be a sign of abnormality. Snook et al found very little hemispheric asymmetry in ADC and FA values (Snook et al. 2005) and other research has reported all structures to be symmetric or nearly symmetric in volume (Filipek et al. 1994). Furthermore hemispheric weight was found to not be significantly different between the left and right hemispheres and there was no significant difference between right and left sides in any region (Ginus Partadiredja 2003). Patients with schizophrenia and delusional disorder had a larger left lateral ventricle than the right which is not seen in controls (Howard et al. 1994), furthermore Petty argue disturbances in asymmetry are particularly striking in patients with schizophrenia (Petty 1999) suggesting asymmetry to be a sign of abnormality. Furthermore asymmetry of the posterior limb of the internal capsule has been associated with hemiplegia (Cowana & de Vriesb 2005). Rose et al found FA in the right posterior limb of the internal capsule to be lower than left in children with abnormal neurodevelopment and the opposite to be true in children with normal development (Rose et al. 2009). This result has not been replicated and is not in agreement with other research which has found asymmetry to be a sign of abnormal development such as hemiplegia and schizophrenia (Cowana & de Vriesb 2005; Howard et al. 1994; Petty 1999).

I hypothesise that generally there will be no asymmetries present in the structures measured.

<u>Motor Difficulties in Preterm Infants</u>

In the prematurely born infant, white matter injury is often seen (Drobyshevsky et al. 2005) and the brain tissue most susceptible to injury includes the fragile area

surrounding the ventricles. The white matter tracts related to motor functions travel through this vulnerable region of the brain, and movement difficulty affecting the legs is a frequent consequence of brain injury in the preterm infant. It has been postulated that injuries of the motor white matter tracts are due to an incomplete state of development of the vascular supply to the cerebral white matter and the maturation-dependent vulnerability of the oligodendroglial precursor cells (Huppi et al. 1998; Neil 2002; Volpe 2001).

DTI has been recently applied to imaging the newborn brains and can be used to identify damages to various areas of the brain, which contributes to movement difficulties (Neil et al. 1998) [for reviews see (Neil 2002)]. The major form of perinatal brain injury is periventricular leukomalacia (PVL) (Hoon Jr et al. 2009) which is most often found in preterm infants (Neil 2002). PVL is strongly associated with impaired movement, especially in the legs. The medical diagnosis given when the significant movement problems do not resolve as the child develops is cerebral palsy (CP) (Hoon Jr et al. 2009). Preterm infants are most at risk for CP (Drobyshevsky et al. 2005).

Previous DTI study of PVL patients revealed that limbic fibres are well preserved, while posterior regions of the corpus callosum, corona radiate and sagittal stratum are most severely affected (Hoon Jr et al. 2009). By comparing the DTI derived metrics, FA and ADC values, Thomas et al have found the significant difference of these values between CP patients and normal controls is mainly associated with the white matter tracts related to motion, for example, corticospinal tract and corticobulbar tract (Thomas 2005).

Other structures have also been linked to motor development. Abnormalities within the posterior limb of the internal capsule have been associated with motor deficits. Rutherford et al found that an abnormal signal within the posterior limb of the internal capsule is able to predict poor neurodevelopment outcome in infants with Hypoxic-Ischemic Encephalopathy (HIE), although the exact relationship between the posterior limb of the internal capsule and motor deficit is unclear (Rutherford et al. 1998). Furthermore an asymmetrical posterior limb of the internal capsule has

been shown to predict an abnormal neurological assessment (L. S. De Vries F. Groenendaal I. C. van Haastert 1999).

The Role of Thyroxine in Development

Thyroxine (T4) is necessary for normal growth, development of the central nervous system and normal brain development (Caroline Delahunty 2001; Morreale de Escobar 2001; Ng et al. 2008; Van Wassenaer 2002; Williams, Visser & Hume 2006). The reactions and products of thyroxine in animals and humans influence carbohydrate metabolism, protein synthesis/ breakdown, cardiovascular, renal and brain function (Wilson 1995). T4 levels increase with increasing gestational age (Bernard, Oddie & Fisher 1977; Oddie et al. 1977; Rapaport, Rose & Freemark 2001) and when gestational age is constant T4 levels increase with birth weight, particularly when gestational age is 39 weeks or higher (Bernard, Oddie & Fisher 1977).

Thyroxine levels are important for the development of the hippocampus, cerebellum, caudate and corpus callosum as shown from animal studies (Abernethy LJ et al. 2003) and have been found to be lower in preterm infants compared to controls (den Ouden 1996; Osborn 2001; VAN WASSENAER et al. 1997b). Low levels of thyroxine results in transient hypothyroxinemia, a common finding in premature infants (Caroline Delahunty 2001; Reuss et al. 1996; Van Wassenaer 2002; Williams, Visser & Hume 2006). Transient hypothyroxinemia in preterm infants has been associated with developmental problems (Briet et al. 2001) including neurodevelopmental deficits and long term effects for brain development (Caroline Delahunty 2001; Ng et al. 2008; Van Wassenaer 2002; Williams, Visser & Hume 2006).

However although thyroid dysfunction is common among preterm infants Chung et al argue that it generally does not affect neurodevelopmental outcome (Chung et al. 2011). Despite this Van Wassenaer et al (2008) found thyroxine treatment to be associated with improved mental, motor and neurological outcomes in infants <28 weeks gestation, but with worse mental and neurological outcome in infants of 29 weeks gestation (van Wassenaer & Kok 2008). However Sze et al (2013) found

supplementing all babies below 28 weeks' gestation with LT4 had no apparent effect on brain size and argued their results do not support routine supplementation with LT4 for all babies born below 28 weeks' gestation (Sze M Ng. 2013).

Generally research on thyroxine supplementation has found it to have no effect and claimed its unsuccessful (van Wassenaer et al. 1997a; Williams, Visser & Hume 2006) although thyroxine supplementation has been found to raise TT4 levels in comparison to placebo infants early on during supplementation but by week 3 levels were close between levothyroxine and placebo groups (Vanhole et al. 1997) and FT4 levels have been found to be significantly higher in a thyroxine compared to placebo group (van Wassenaer et al. 1997a).

Benefit has been found in administering thyroxine supplementation in the more premature infants (under 27 weeks) (Briet et al. 1999; van Wassenaer et al. 1997a). Possibly infants born earlier are more likely to have a lack of thyroxine and be more likely to benefit from supplementation.

Williams et al argued that thyroxine substitution therapy should only be applied to infants who suffer with hypothyroxinemia (Williams, Visser & Hume 2006). The sample of premature infants receiving levothyroxine supplementation was under 28 weeks gestation so within the age range of the subgroup of premature infants that were shown to have benefit from thyroxine supplementation (van Wassenaer et al. 1997a). Thus I hypothesise that ***FT4 levels will show a temporary increase following thyroxine supplementation in comparison to the placebo group but that levels will stabilise between groups over time. T4 levels will positively correlate with gestational age and birth weight. Higher FA and lower ADC will be found in infants treated with levothyroxine and smaller brain structural volumes will be found in infants receiving placebo.***

Intrauterine Growth Restriction

Preterm infants are smaller than term infants at birth and a low birth weight is classified as below 2500 grams. Those infants born with an especially low birth

weight for their gestational age are said to suffer from foetal or IUGR (Cole 1997). IUGR is defined as a birth weight below the 10th percentile predicted for gestational age (Cole 1997; Guellec et al. 2011; Merialdi & de Onis 2005; Resnik 2002) or a birth weight that is at least two standard deviations below the mean (Fang 2005).

It was the World Health Organisation that first made a distinction between low birth weight (less than 2500g) and born prematurely (born at less than 37 weeks gestation). Small for gestational age (SGA) was later introduced and better describes infants with intrauterine growth restriction (IUGR) (Merialdi & de Onis 2005).

IUGR and Later Development

Infants born preterm or with a low birth weight are of an increased risk for later developmental problems (Cole 1997) and IUGR infants are said to be of higher vulnerability (Esteban et al. 2010). IUGR can be caused by maternal disease such as infection, malnutrition, high blood pressure, smoking and alcoholism, poor socioeconomic conditions, multiple pregnancy and foetal disease. These have also been associated with preterm birth (Martin 1994). Effects of IUGR may persist after infancy and have been associated with the development of disorders in adulthood such as cardiovascular disease (Merialdi & de Onis 2005; Ozanne, Fernandez-Twinn & Hales 2004), hypertension, osteoporosis, schizophrenia, depression, breast cancer, polycystic ovary syndrome (Ozanne, Fernandez-Twinn & Hales 2004), high blood pressure, obstructive lung disease, diabetes, high cholesterol concentrations and renal damage (Sanz-Cortes et al. 2010a). Birth weight has also be established as a risk factor for autism although the causal effect is unknown i.e. it is unknown whether low birth weight is a cause or consequence of autism (Burd 2005).

FA and ADC in IUGR Infants

IUGR presents a complex pattern of brain reorganisation which is already present at birth (Eixarch et al. 2012; Zimine 2002). It has specific structural and functional consequence for cortical brain development (Tolsa et al. 2004). A study of IUGR in rabbits reported that the normal inverse relationship between FA and ADC was not

seen. The authors claim the lack of significance in the higher ADC may be due to the sample size. FA differences were observed in multiple regions of grey and white matter including the frontal, insular, occipital and temporal cortex, the hippocampus, putamen, thalamus, claustrum, medial septal nucleus, anterior commissure, internal capsule, fimbria of hippocampus, medial lemniscus and olfactory tract (Eixarch et al. 2012). Similarly a significant increase in ADC values in the internal capsule and thalamus and a decrease in relative anisotropy in the corpus callosum, thalamus and unmyelinated white matter has been found at term age in IUGR infants (Zimine 2002). Despite this relative anisotropy and ADC changes between the first and second examination were similar to that of normal development (Drobyshevsky et al. 2005) with a reduction in ADC in the internal capsule and thalamus of IUGR and preterm infants and an increase in ADC between the first and second examination (Zimine 2002).

IUGR and Stereology

IUGR has been associated with significant reductions in brain volume (Eixarch et al. 2012) and especially in posterior regions(Thompson et al. 2007). Specific structural brain and developmental differences in IUGR infants has been found (Esteban et al. 2010). In particular IUGR was associated with deficits in the inferior and and parieto-occipital and cerebellar regions (Thompson et al. 2007). In terms of global grey matter volume, IUGR infants were found to have non-significant reductions in comparison to appropriate for gestational age infants and term infants (Sanz-Cortes et al. 2010a). Significant reductions in intracranial volume and cerebral cortex grey matter has been found within two weeks of birth with IUGR and these changes persisted at term age (Tolsa et al. 2004). In addition infants with IUGR have been found to have significantly reduced volumes of insular and temporal lobes compared to term infants (Padilla et al. 2011; Sanz-Cortes et al. 2010a), reduced grey matter in temporal, parietal, frontal and insular regions but increases in white matter in temporal regions compared to appropriate for gestational age (AGA) infants and increases in white matter in frontal, parietal, occipital and insular regions compared to term. Reductions in white matter in IUGR infants were observed in the cerebellum. Differences in volumes of brain structures between the

different groups (preterm-IUGR, preterm-AGA and term) are demonstrated in table 2.1 (Padilla et al. 2011). However Tolsa et al (2004) found white matter to not be affected by IUGR. Other research has found significant reduction in fractional dimension of grey and white matter in an IUGR group compared to preterm and term groups where as there was no significant difference between preterm and term groups. Fractional dimension was related to gestational age and IUGR (Esteban et al. 2010). ***I hypothesise higher FA and lower ADC will be found in Preterm IUGR infants compared with preterm non-IUGR infants.***

FA, ADC, Volume and Later Development

Changes in FA measures found in IUGR correlated with poor outcome in neurobehavioral tests (Eixarch et al. 2012). Similarly IUGR infants showed a reduction in neurodevelopment which positively correlated with grey matter in various regions (Padilla et al. 2011). They were also found to score less maturely on a measure of behaviour (Lodygensky et al. 2008). Volume of the temporal and insular lobe was found to significantly positively correlated with neonatal birth weight, length and head circumference (Sanz-Cortes et al. 2010a). At term age cerebral cortical grey matter volume correlated with attention interaction capacity score which was significantly less mature in infants with IUGR (Tolsa et al. 2004). Grey matter volume of IUGR infants in the left precuneus and right superior frontal gyras positively correlated with motor scores. Grey matter volume of IUGR infants in the right superior parietal gyrus positively correlated with fine motor subscale and the adaptive behaviour score. There were no correlations between structural volume and neurodevelopmental scores in the preterm cohort (Padilla et al. 2011). Sanz-Cortes et al (2010) also found IUGR infants to score lower on adaptive behaviour as well as on motor and fine motor compared to a term group.

Preterm-IUGR compared to term.		Preterm-IUGR compared to preterm-AGA.	
Decreased GM Volume:	Bilateral temporal lobe	Decreased GM Volume:	Bilateral distribution involving left temporal lobe
	Bilateral insular lobe		Bilateral insular lobe
	Bilateral parietal lobe		Bilateral parietal lobe
	Right frontal lobe		Left frontal lobe
Decrease white matter:	Cerebellum (significant)		
	Right hippocampus (not significant)		
Greater white matter:	Left parietal lobe		
	Left occipital lobe		
	Left insular lobe		

Table 2.1 Table of changes in white and grey matter in IUGR infants compared to term and preterm appropriate for gestational age infants. Adapted from Padilla et al 2011 (Padilla et al. 2011).

In a rabbit model of IUGR it has been associated with significantly poorer neurobehavioral performance (Eixarch et al. 2012) and an assessment of preterm behaviour found less mature scores in IUGR infants compared to controls (Tolsa et al. 2004). Furthermore IUGR infants have been found to have significant reductions in neurodevelopmental scores including motor, fine motor and adaptive behaviour scores (Esteban et al. 2010; Padilla et al. 2011; Sanz-Cortes et al. 2010a) and these scores positively correlated with grey matter in various regions (Padilla et al. 2011),

including correlations between adaptive behaviour and the insular lobe (Sanz-Cortes et al. 2010a). The significant reductions seen in IUGR infants were not present in the preterm (without IUGR) compared to term infants and thus it seems IUGR adds to the effects of prematurity on brain structure and makes the differences more pronounced i.e. reductions went from not significant in preterm compared to term to significant comparing IUGR preterm to term infants (Esteban et al. 2010). Sanz-Cortez et al (2010) concluded that IUGR causes structural changes regardless of prematurity that persist age one and are associated with developmental difficulties and Eixarch et al (2012) concluded that IUGR has a complex pattern of brain reorganisation which is already present at birth. ***I hypothesise that smaller brain structural volumes will be found in preterm IUGR infants compared with preterm non-IUGR infants.***

IUGR and Later Education

Children born very preterm after IUGR are at an increased risk of cognitive impairment at early school age compared to other preterm infants (Morsing et al. 2011) and are associated with mortality, behavioural outcomes and school difficulties. Even infants born between 29 and 32 weeks gestation when combined with a birth weight that classifies them as small for gestational age have a significant increase in educational disadvantages (Guellec et al. 2011). Similarly infants with a birth weight under 750 grams were found to obtain lower scores than term controls on measures of reading and mathematics and they also scored more poorly than infants with a birth weight between 705 and 1499 grams on perceptual planning, reading and mathematics. Furthermore the 750 gram group had lower IQs and scored less well on neuropsychological and achievement measures than controls (Taylor et al. 2002). Further down the line in education Very low birth weight (VLBW) young adults had lower mean IQ and lower academic achievement scores. Significantly more VLBW had repeated a grade at school and significantly fewer had graduated high school. Thus the educational disadvantage associated with VLBW persists into early adulthood. VLBW young adults also had higher rates of neurosensory impairments, subnormal height but demonstrated less risk taking

behaviours including less alcohol and drug use and lower rates of pregnancy (Hack et al. 2002).

Cognitive impairment, defined as a full scale IQ below 70, was found to be significantly more prevalent in preterm infants with IUGR than preterm infants that are appropriate for gestational age (Morsing et al. 2011), although birth weight has been found to not be associated with any cognitive, behavioural or motor outcomes at the age of 5 or school performance outcome at age 8. Despite this children born between 29 and 32 weeks gestation that were small for their gestational age were found to be at a higher risk of mortality, minor cognitive deficits, inattention-hyperactive symptoms and school difficulties compared to appropriate-for-gestational-age children. This study also had a group of infants which they classified as mildly small for gestational age (birth weight between the 10^{th} and 20^{th} centile) who demonstrated an increased risk of minor cognitive difficulties and behavioural difficulties (Guellec et al. 2011).

IUGR has been found to have later academic effects such as significantly lower scores on verbal and full scale IQ when compared to a preterm appropriate for gestational age (AGA) group and a term AGA group. The preterm AGA group also differed in IQ from the term AGA group. Performance IQ was found not to differ between IUGR and AGA preterm infants but was significantly lower than the term AGA (Morsing et al. 2011).

Gestational age was found to not be related to full scale IQ in either the preterm AGA or the preterm IUGR group (Morsing et al. 2011).

Sex Differences

As with research into preterm infants discussed earlier similar sex differences have also been found in infants born with IUGR. Boys have been found to score lower in verbal and full scale IQ than females within a preterm-IUGR group and the authors concluded that boys may be especially vulnerable to the influence of IUGR and preterm birth (Morsing et al. 2011). Furthermore VLBW males but not females were

significantly less likely to be enrolled in post secondary study and scores on applied problems had a greater difference in males compared to females (Hack et al. 2002).

Causes of IUGR

Maternal factors that can cause IUGR include both alcohol abuse and smoking (Eixarch et al. 2012; Fang 2005). Nicotine exposure during in utero development can influence foetal growth (Sizonenko et al. 2006) and maternal smoking may decrease foetal weight by 135-300g as can alcohol use (Resnik 2002).

Summary of Hypothesis

I hypothesise that ***Infants FA and ADC will be significantly correlated with weight and gestational age at birth.***

I hypothesis that ***higher FA and lower ADC will be found in the posterior part of the internal capsule and corpus callosum***

I hypothesise that ***smaller brain structural volumes will be found in infants with lower birth weight and gestational age at birth and with which they will significantly correlate.***

I hypothesise that ***there will be a difference in DTI and stereology measures between males and females.***

I hypothesise that ***generally there will be no asymmetries present in the structures measured.***

I hypothesise that ***FT4 levels will show a temporary increase following thyroxine supplementation in comparison to the placebo group but that levels will stabilise between groups over time. T4 levels will positively correlate with gestational age and birth weight. Higher FA and lower ADC will be found in infants treated with levothyroxine and smaller brain structural volumes will be found in infants receiving placebo.***

I hypothesise that ***higher FA and lower ADC will be found in Preterm IUGR infants compared with preterm non-IUGR infants.***

I hypothesise that ***smaller brain structural volumes will be found in preterm IUGR infants compared with preterm non-IUGR infants.***

CHAPTER 3

Magnetic Resonance Imaging (MRI) and Image Analysis

Aim of Chapter

This chapter aims to explore the workings of an MRI machine and goes on to discuss the MRI techniques and image analysis used in this study:

a) MRI Physics
b) Diffusion Weighted Imaging and Diffusion Tensor Imaging
c) Stereology

MRI Physics

MRI is an imaging technique used in hospitals and research facilities to produce images of internal tissues in the human body non-invasively (McRobbie 2005). MRI contains three electromagnetic components; a set of magnet coils, three gradient coils and an integral radiofrequency transmitter coil. These coils generate different types of magnetic field and are applied to a patient in combination. Resultantly they produce magnetic resonance signals that form MR images (Ridgway 2010).

The human body is made up of trillions of atoms which act as tiny magnetic fields which interact with each other. MRI uses the resonance of these atoms within the body. The atoms resonate due to the applied radio waves from the MRI scanner. The resonating atoms produce an MR signal that produces anatomical information (McRobbie 2005).

The Atom

The atom consists of a nucleus spinning on its own axis and electrons that orbit the nucleus and spin on their own axis. The nucleus consists of two particles: protons that have a positive charge and neutrons that have no net charge. Because the nucleus is positively charged and is spinning, it develops magnetic properties and has magnetic moment (figure 3.1) which means it will interact with an external magnetic field (Chavhan 2007; Haacke et al. 1999).

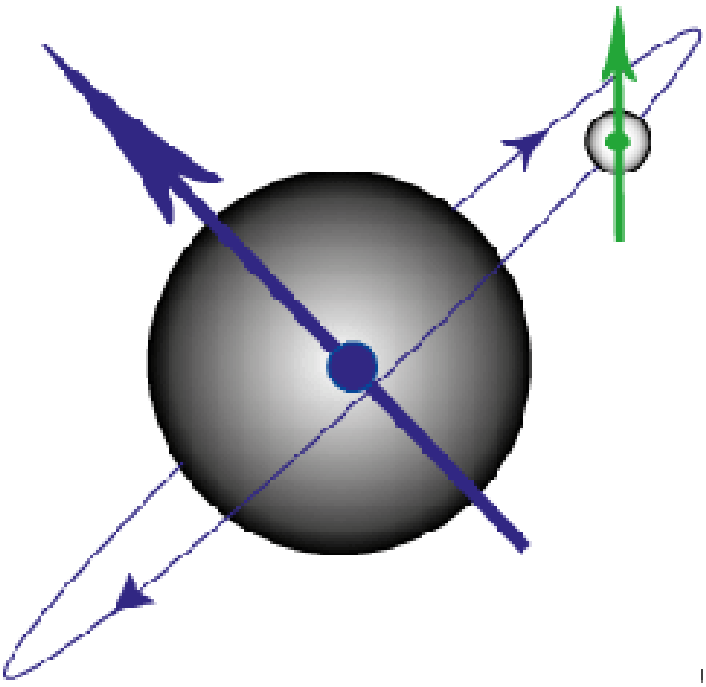


Figure 3.1. Illustration of the magnetic moment produced by an electron orbiting the nucleus and that produced by the spin of the electron taken from http://www.doitpoms.ac.uk/tlplib/ferromagnetic/why_magnetic.php?printable=1

In the majority of MRI and this thesis, we only consider the MR signal arising from the hydrogen nuclei in water, which contains a single proton. Because of the spin characteristics of the hydrogen proton, when it is placed in the bore of the external magnetic field, the magnetic moment of the proton will either align parallel or anti-parallel with the direction of the magnetic field (B_0) (Chavhan 2007; Haacke et al.

1999). The magnetic moment of the proton will also precess at some frequency around the direction of the external field (Sands & Levitin 2004).

The Precessional Frequency

The frequency of the proton's precession depends on the strength of the surrounding magnetic field. The precession frequency ω is directly proportional to the strength of the external magnetic field (B_0) and is defined by the Larmor Equation:

$$\boldsymbol{\omega_0 = \gamma B_0}$$

Where w_0 is known as the precessional, Larmor or resonance frequency. The symbol γ refers to the gyromagnetic ratio, which is a constant unique to every atom. For hydrogen protons, γ = 42.56 MHz per Tesla. Therefore the precessional frequency of the hydrogen proton at 3 Tesla is ω_0 = 128 MHz. The precessional frequency can be increased by increasing the strength of the magnetic field (Chavhan 2007; Haacke et al. 1999).

When placing many protons in a magnetic field, the majority align parallel and slightly less align anti parallel (Sands & Levitin 2004). The parallel protons have a slightly lower energy state. Increasing the strength of the magnetic field will increase the proportion of protons in the parallel direction. It is this excess of protons in the lower energy state that are accessible to us in an MRI experiment. Using an MRI system with a stronger magnetic field will allow us to gain access to more protons, so increasing the signal in our measurements (Chavhan 2007; Haacke et al. 1999).

The magnetisation of the protons in the antiparallel direction will cancel an equal amount of magnetisation from protons in the parallel direction. The remaining magnetisation will be parallel to the direction of (B_0). This is the net magnetization vector (NMV) (Chavhan 2007). The NMV is composed of two components of magnetization; one the magnitude of the magnetization in the direction of B_0 is referred to as the longitudinal magnetization, and the other one in the

magnetization orthogonal to B_0 is known as the transverse magnetization (Haacke et al. 1999).

Pulse Sequences

In simple terms a pulse sequence consists of pulses, signals and recovery periods of precession. The main characteristics of a pulse sequence are repetition time (TR) and echo time (TE) (McRobbie 2005; Westbrook 2005). TR is the time between RF pulses and it determines the amount of precession. TE is the time from the RF pulse and the peak of the signal (i.e. echo) (McRobbie 2005; Westbrook 2005). Contrast in MR images is produced by applying the RF pulse at certain TR times and receiving the signal at certain TE times (Westbrook 2005).

Diffusion Weighted Imaging and Diffusion Tensor Imaging

Diffusion Weighted Imaging (DWI) is an MRI technique that uses the motion of magnetic moments to produce signal changes (McRobbie 2005). Diffusion can be influenced by molecular weight, intermolecular interactions and temperature. The cellular microstructure of human tissues influences the diffusion of the molecules as intracellular, extracellular, neurons, glial cells and axons within the tissue act as barriers (Beaulieu 2002; Hajnal et al. 1991).

In DWI gradients with equal amplitude and opposite polarity are applied over a set period of time (Liney 2006). Magnetic moments that have moved during the time period will dephase and lose signal. Those which remained stationary had equal dephasing and rephasing. As gradients with high amplitude are used the sequence is sensitive to the diffusion of water and other motion at the microscopic level. The reduction in signal will depend on the extent of diffusion and the gradient strength and timing known as the b- factor (Liney 2006).

Images can be acquired with at least two different values of b so that apparent diffusion coefficient (ADC) can be calculated. A Tensor also has to be calculated to receive a three-dimensional image of diffusion. The Tensor requires B=0 and at least

6 gradient pairs. This is known as Diffusion Tensor Imaging and it is able to distinguish between anisotropy as there is more diffusion along structures and fibres such as in white-matter tracts (Liney 2006). DTI images can be obtained from the eigenvalues of the diffusion tensor. The eigenvalues (λ1, λ2, and λ3) are the lengths of the longest, middle and shortest axis and the eigenvectors (V1, V2 and V3) are there orientations (Huang 2010).

DTI enables imaging of unique structural information which may make it possible to detect white matter injuries in specific tracts much earlier (Huang 2010; Neil et al. 2002) especially with the advancement of tractography where the trajectory between brain regions of white matter fibres are predicted (Cascio, Gerig & Piven 2007).

DTI is more advanced than DWI due to its lack of dependence on patient positioning in the scanner. With DWI the ADC measurement is dependent on the individual's orientation; in the scanner for example measuring the Corpus Callosum on the x direction can give slightly different measurements if the individual's head is tilted to one side. This makes comparisons between individual's and scan-rescan tests not as accurate as one would like (Tofts 2003). DTI is not dependent on exact positioning of the individual in the scanner or the applied magnetic field gradient coordinate system and therefore can be reproduced more accurately than DWI (Tofts 2003). DTI looks at anisotropy in different directions more than in DWI. This does result in longer scanning times but allows measures of FA as well as ADC to be acquired. The direction of the diffusion can be observed which is usually along white matter tracts as the tracts act as a barrier to diffusion. DTI can map out white matter tracts and neurology and damage to white matter tracts can also be observed (Liney 2006).

In diffusion imaging the b- factor controls how much the ADC of a tissue contributes to the image weighting. With a long TR and TE and B=0 the image becomes T2 weighted, whereas if the B factor is increased the weighting becomes diffusion weighted which means areas with high signal have a high signal because they have a low ADC rather than because there is a long T2 time. Different gradients in DWI can map out the anatomy of white matter pathways (Westbrook 2005) and DWI and

other quantified diffusion measurements are being used as diagnostic and research tools (Tofts 2003). It has been used to map out myelination in preterm infants (Westbrook 2005) and demyelination in intracranial disease processes. High diffusion is shown as dark areas on the DWI but as bright on the diffusion map (Tofts 2003).

With the diffusion of molecules, the distance travelled may not be equal in all directions due to barriers. Where no barriers exist or barriers are not coherently orientated, the diffusion is equal in all directions and is termed isotropic. Where there are highly orientated barriers to diffusion, the diffusion is not equal and termed anisotropic. This anisotropy is related to the geometry of fibres. The size, shape, composition of barriers and spacing between barriers will determine the degree of diffusion (Beaulieu 2002).

FA is a measure used to characterise the anisotropy of the tensor, i.e. how directionally dependent it is. The formula for this is (Huang 2010; Westin et al. 2002)

FA =

$$\frac{\sqrt{(\lambda 1-\lambda 2)^2+(\lambda 1-\lambda 3)^2+(\lambda 2-\lambda 3)^2}}{\sqrt{2}\ \sqrt{\lambda 1^2+\lambda 2^2+\lambda 3^2}}$$

FA values range from 0 to 1 (Huang 2010). When FA is 0 water diffusion is isotropic, equal in all directions implying there are no barriers to its diffusion. A high FA (the highest being 1) means the water diffusion is directionally dependent suggesting there are barriers such as brain structures preventing equal water diffusion.

ADC is defined as the average of the three eigenvalues (Huang 2010):

$ADC = (\lambda 1+ \lambda 2+\lambda 3)/3$

ADC is a physical value and usually has the unit of 10^{-3} mm^2/s (Huang 2010) that represents the number of directions the water diffuses, a high ADC the more directions the water diffuses in suggesting there is less structural barriers. The ADC

value is dependent on the interactions of the diffusing molecule with the cellular structures and active processes within the tissue (Beaulieu 2002).

Water molecules are more likely to diffuse along the length of axons, as when diffusing perpendicular to them there are barriers such as the myelin sheath (Beaulieu 2002; Hajnal et al. 1991). Thomsen et al reported regional variations in ADC in normal human white matter and suggested these differences may be due to the degree of myelination or the orientation of myelin sheaths in the white matter tracts (Thomsen, Henriksen & Ring 1987). In normal development where highly ordered structures are present diffusion should be anisotropic as found in the human spinal cord (Hajnal et al. 1991) and corpus callosum in the rat (King et al. 1991). Mean diffusivity and ADC decrease whereas anisotropy increases with maturation (Beaulieu 2002; Huppi et al. 1998; Neil et al. 1998; Nomura et al. 1994; Sakuma et al. 1991).

DTI and Myelin

Changes in ADC and anisotropy accompany brain maturation (Dubois et al. 2008; Neil et al. 2002) and are useful for assessing for cerebral white matter development (Kroenke et al. 2005). It is clear that diffusion occurs along orientated fibres due to less resistance but the specific causes for the anisotropic diffusion was unknown (Beaulieu 2002; Cascio, Gerig & Piven 2007; Dubois et al. 2006; Most-ley, Wendland & Kucharczyk 1991; Prayer et al. 1997; Westin et al. 2002). Hindrance to diffusion could be by myelin sheaths or axonal membranes (Dubois et al. 2006). Myelination in the nerve fibres and white matter are undoubtedly related to the anisotropic diffusion (Beaulieu 2002; Drobyshevsky et al. 2005; Kroenke et al. 2005; Most-ley, Wendland & Kucharczyk 1991; Westin et al. 2002). The lipid bilayers of myelin have limited permeability to water so they are expected to hinder diffusion across fibres. This led to the thought that the myelin sheath encasing the axons is the cause of lack of diffusion perpendicular to fibres. (Beaulieu 2002; Most-ley, Wendland & Kucharczyk 1991; Westin et al. 2002). However it is also clear myelin is not the only cause of anisotropic diffusion. In assumption that myelin is the only source of anisotropic diffusion it was hypothesised that diffusion would be more isotropic in

fibre tracts without myelin (Beaulieu 2002). Beaulieu and Allen refuted this hypothesis by finding anisotropic diffusion in a non-myelinated nerve of the garfish (Beaulieu 2002; Beaulieu & Allen 1994a). More recently research has supported the finding that myelin is not the only component of anisotropic diffusion. In central cerebral white matter where there are increases in anisotropy and decreases in ADC between 28 and 40 weeks gestation (Huppi et al. 1998) even though central cerebral white matter does not begin to myelinate until term (Kinney 1988). High anisotropy has been found in the corpus callosum (Huppi et al. 1998; Partridge et al. 2004) and the anterior limb of the internal capsule (Beaulieu 2002; Neil et al. 1998) of preterm and term infants even though it is mostly unmyelinated. Furthermore Drobyshevsky et al found increases in anisotropy to be most rapid prior to myelination in rabbits (Drobyshevsky et al. 2005). Other microstructural components of axons within white matter such as the axonal membrane and the neurofibrils could contribute to the anisotropic diffusion in reducing diffusion perpendicular to fibres (Beaulieu 2002). Although other research has found the neurofibrils (specifically neurofilaments) to not have a significant role in anisotropy thus axonal membranes or myelin are the primary determinant in anisotropy (Beaulieu 2002; Beaulieu & Allen 1994b).

Myelination may still contribute to the anisotropic diffusion and it has been demonstrated that myelination modulates the degree of anisotropy (Thomsen, Henriksen & Ring 1987). Furthermore when comparing myelinated and unmyelinated fibre tracts of similar size and density it's expected that anisotropy would be higher in the myelinated fibre and that myelination would have caused a greater hindrance of diffusion perpendicular to the fibre. However the extent of the myelination contribution to anisotropy is unknown (Beaulieu 2002).

Development

Anisotropy in white matter develops prior to myelination (Beaulieu 2002; Prayer et al. 1997; Wimberger et al. 1995). The development of anisotropy prior to myelination could be due to changes in the development of fibre tracts in central white matter which could be related to the 'early wrapping of axons by

oligodendroglial processes' (Barrett 2010; Huppi et al. 1998; Remahl & Hildebrand 1990; Snell 2010). Anisotropy continues to develop in white matter as myelin forms although at some point anisotropy levels seem to reach an optimum level and do not continue to increase further with increasing age and myelination (Beaulieu 2002).

Changes in diffusion are related to the increases in myelination, reduction in brain water, increasing compactness of fibre tracts and reduced extra-axonal space that are involved in white matter maturation. Assumptions that anisotropy relates only to myelination are oversimplified and ignore the many different processes that effect the diffusion of water within the brain (Beaulieu 2002; Wimberger et al. 1995).

In Summary

Although the specifics have been argued there is a consensus that there is an increase in anisotropy and decrease in overall diffusion with increasing age and therefore it seems increased anisotropy and decreased diffusion represent further matured white matter bundles (Cascio, Gerig & Piven 2007) whether it be due to increased myelination (Beaulieu 2002; Drobyshevsky et al. 2005; Kroenke et al. 2005; Most-ley, Wendland & Kucharczyk 1991; Westin et al. 2002), axonal membrane changes (Beaulieu 2002; Neil 2002) compactness of fibre tracts or multiple factors (Beaulieu 2002; Wimberger et al. 1995).

Stereology

Stereology is an efficient method for quantifying three dimensional structures and provides reliable data from sections of three dimensional structures (Gundersen et al. 1988). This structure may be of biological matter such as brain tissue or could be pieces of steel or flake of rock. Stereology is widely used not only in biological sciences but also by material scientists and geologists to investigate the internal structure of objects aided by microscopy (Reed 1998). Stereology can be applied to T1 weighted and T2 weighted MRI imaging discussed earlier in the chapter. It involves applying mathematical theory specifically Cavalieri method to estimate the

volume of arbitrary structures by splitting the structures into slices equal distance apart and measuring the area of each section then multiply by number of slices and distance apart. Bonaventura Cavalieri was the first to consider measuring volume by dividing a three dimensional object into equal sections and analysing these sections The Cavalieri method provides estimates of a shapes volume that are mathematically unbiased (Roberts, Puddephat & McNulty 2000b) and guarantees accurate estimation of the volume of an arbitrary object (Roberts et al. 1994).

$$V = T (A1, A2 + \ldots\ldots + An)$$

where V equals volume, T equals distance between each slice and A1, A2..... equals the area of each section summed (figure 3.3).

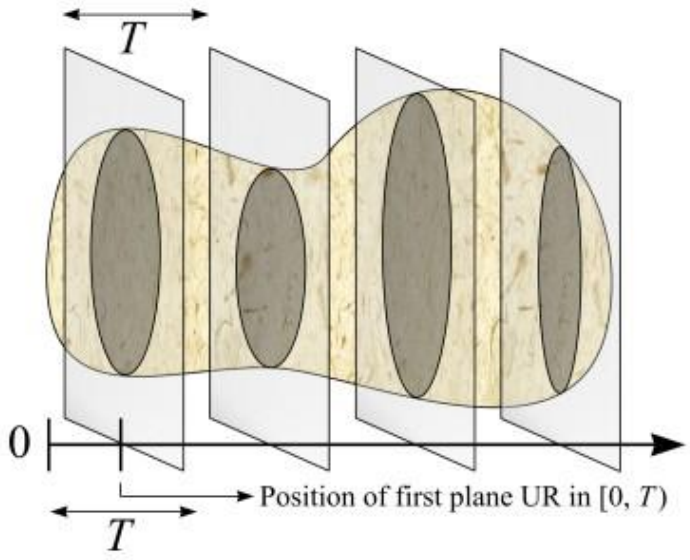


Figure 3.2 Diagram of Cavalieri method taken from http://www.mikepuddephat.com/Page/1599/Single-object-stereology-(part-1)

MRI is a useful tool for sectioning the brain into the various slices/sections and in combination with tools such as analyse, the sections can be demarcated for the specific structures of interest. The imaging software Easymeasure which was

developed by Puddephat can be used to apply point counting method to each of the slices. The image is overlayed with an array of test points (figure 3.3) and thenumber of points which fall within the boundary of the region of interest are counted (Puddephat 1999). The point counting method is demonstrated within the structures of interest in the followingMethodology section.

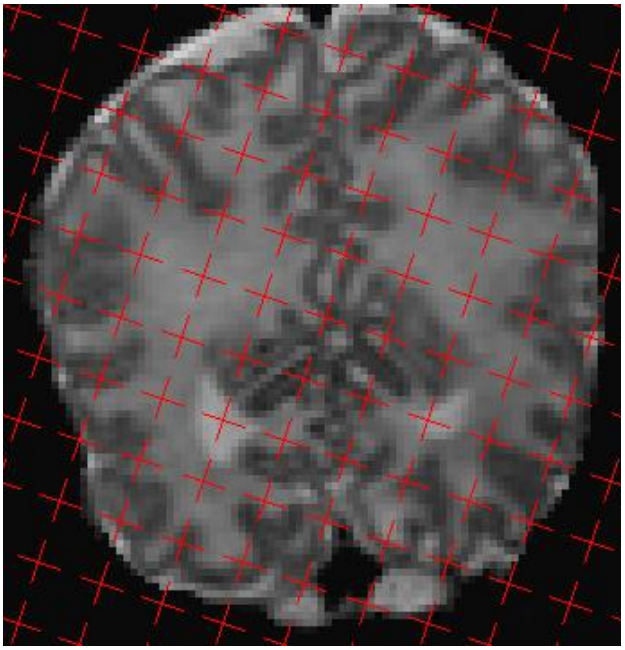


Figure 3.3 Array of test points arranged over a T2 weighted image.

With the application of point counting the equation for volume becomes:

$$V = T \left(\frac{a}{p}\right) (P_1 + P_2 + ...P_n)$$

whereas before V equals volume, T equals distance between each slice and P_1, P_2..... represents the points counted and (a/p) represents the area of each point. Volume calculation involves a two step process involving sectioning and point counting (Roberts, Puddephat & McNulty 2000b).

Coefficient of error is the margin of error made when estimating a volume using Cavalieri and point counting methods, and in stereology the aim is to maintain the coefficient of error below 5%. Coefficient of error when stereology has been performed with point counting can be predicted by the following formula:

$$CE(est_2V) = \left(\sum_{i=1}^{m} P_i^{\ -1} \right) \cdot \left\{ \frac{1}{12} \left(3 \sum_{i=1}^{m} P_i^{\ 2} + \sum_{i=1}^{m-2} P_i \cdot P_{i+2} - 4 \sum_{i=1}^{m-1} P_i \cdot P_{i+1} \right) + 0.0543 \frac{\overline{B}}{\sqrt{A}} \left(\sum_{i=1}^{m} P_i \right)^{1/2} \right\}^{1/2}$$

(Cruz-Orive 1999).

The parameters for stereology of the prefrontal cortex (PFC) in order to maintain a coefficient error below 5% are represented in table 3.1 (Howard 2003; Keller 2004).

Structure	Grid Size	Pixels	Slice Gap	Shape Coefficients
Medial Orbital PFC	4	7x7	2	5.19
Lateral Orbital PFC	4	7x7	2	5.48
Dorsal Medial PFC	6	10x10	2	5.99
Dorsal Lateral PFC	6	10x10	2	5.65

Table 3.1 Parameters for volume estimation.

The different areas of the prefrontal cortex (PFC) have been well demonstrated inlateral, frontal and medial views in Howard et al (2003) demonstrated below in figure 3.5 and table 3.2.

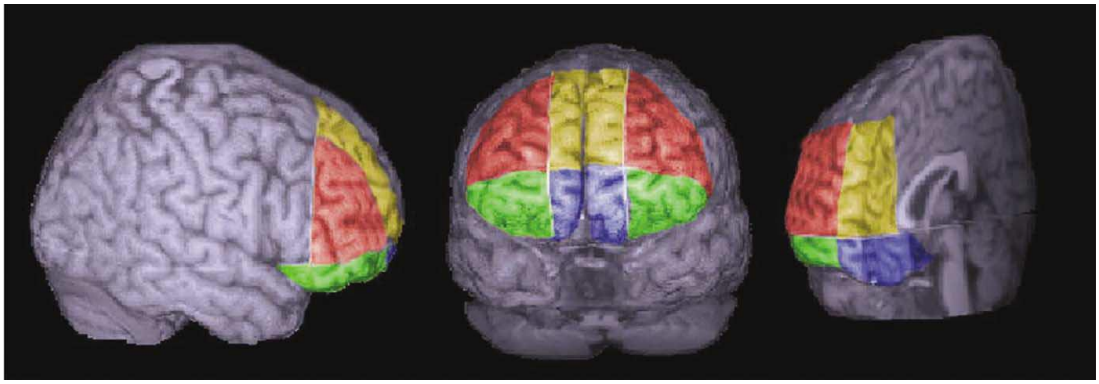


Figure 3.4 Demonstration of PFC subfields in lateral, frontal and medial views. Dorsal lateral is represented in red, dorsal medial in yellow, orbital lateral in green and orbital medial in blue.

Prefrontal cortex subfield	Gyri	Brodmann area
Dorsal medial	Superior frontal gyrus, frontal pole, cingulated gyrus	8,9, 10,24 ,32,6
Dorsal lateral	Middle and inferior frontal gyri, superior frontal gyrus, frontal pole	8,9, 10, 44, 45,46
Orbital medial	Gyrus rectus, medial orbital gyrus, anterior and posterior orbital gyri, cingulated gyrus, frontal pole	10,11, 12,24,25, 32
Orbital lateral	Lateral, anterior and posterior orbital gyri, inferior and middle frontal gyri, frontal pole	10,11,47

Table 3.2 Gyral neuroanatomy and Brodmann areas for the prefrontal cortex sub regions.

Stereology technique in combination with point counting methods is a well established and used methodology in autopsy (Ginus Partadiredja 2003; Tang 1997; Tang et al. 1997; Yang 2008) and MRI investigations (Howard 2003). The Cavalieri principle with point counting is more efficient than region drawing (Howard 2003) and provides unbiased volume estimation (Howard 2003; Roberts, Puddephat & McNulty 2000a). Advantages of the Cavalieri priniciple include high efficiency and easy implementation (Howard 2003).

CHAPTER 4

Methodology

Aim of Chapter

This chapter presents the general design of the study. Demographics of the infants taking part of the study are presented with details of the MRI procedure and image analysis.

Participants

Sixty nine infants (51 preterm; 9 infants born with intrauterine growth restriction and 9 controls) for whom consent was obtained to perform MRI (Figure 4.1). Preterm infants were recruited as part of a wider study (Ng et al. 2008) from five centres (Liverpool Women's Hospital, Saint Mary's Hospital Manchester, Arrowe Park Hospital, Hope Hospital and Royal Preston Hospital) through the time period of September 2007 to May 2009. Ethical approval was granted by the North West Research Ethics Committee, and approved by the Research Governance Group at Liverpool Women's Hospital. The IUGR infants were also part of a larger research trial and ethical approval was approved by Oldham Local Research Ethics Committee.

Infants born under 28 weeks gestation, who reached the inclusion criteria for the TIPIT protocol (Figure 4.2) and whose parents consented to MRI at 40 weeks corrected gestation were included (N= 51).

Inclusion criteria were infants born with gestational age under 28 weeks at birth based on the estimate available in obstetric notes at the time of delivery, infants

were recruited within five days of birth and both single and multiple births were included.

Infants born to a mother with known thyroid disease or on antithyroid medications during pregnancy and mothers on amiodarone during pregnancy were excluded. Infants diagnosed with major congenital or chromosomal abnormalities known to affect thyroid function or brain development and maternal death during or within five days after childbirth were excluded.

Treatment

Infants were randomized and allocated to treatment with two forms of the active medication: intravenous levothyroxine (trade name: Levothyroid) and oral levothyroxine solution (trade name: Evotrox) or corresponding placebos.

In the initial phase, infants received either intravenous Levothyroid or placebo (5% dextrose) within the first 5 days after birth. The treatment regime followed the dosage at 8mcg/kg birth weight/day single daily dose.

In the next phase, once enteral feeds were fully established, oral solution Evotrox or placebo (carrier solution without active drug) was given at 8mcg/kg birth weight/day single daily dose until the baby reaches 32 weeks CGA.

Randomization codes were computer generated using the statistical package STATA with random variable block size of two and four. Randomisation was stratified by centre, and gestational age at birth (under 25 weeks' gestation, 25-26 weeks' gestations, 27-27weeks 6 days gestation). Allocation concealment was provided by pharmacy departments of the respective hospitals. Multiple births followed the randomisation process as per singleton births, such that siblings would receive the same allocation.

Parents, clinicians involved in patient care and individuals assessing study end-points were blinded to the study treatments. Levothyroxine and its placebo were presented identically.

Intrauterine Growth Restriction

Intrauterine Growth Restriction infants were part of a wider study where 31 babies born between 28 and 36 weeks were studied (19 IUGR and 12 Controls). Individualised birth weight of IUGR infants was below the 3rd centile and the 12 infants who were appropriately grown individualized birth weight were between the 25th and 75th centile. The infants whom MRI was performed were included in this thesis and MRI was performed at term equivalent age.

MRI

TIPIT

All TIPIT infants were scanned on the same a 1.5 Tesla Achieva Nova MRI Scanner (Phillips Medical Systems, Best, Netherlands) using a phased-array SENSE head coil (8 channel) to facilitate the production of high quality MR images with high signal-to-noise ratio (SNR). The preterm cohort were scanned at term equivalent age in natural sleep following a feed using a "feed and wrap" technique. Sedation was not used. Careful positioning by the radiographer ensured the comfort of the infants. Hearing protection with President putty and Mini-muffs was used. This helped keep the infants soundly asleep and still during the scan, reducing image degradation from motion artefacts. Additional scanner-inbuilt acoustic noise reduction techniques were also employed. Experienced dedicated paediatric radiographers performed the scans; as we did not use any sedation, it was not necessary to have a paediatrician present. Diffusion images were acquired using a spin echo sequence with 24 contiguous axial slices of 3 mm thickness with in-plane isotropic resolution of 2 mm. Thirty-two gradient directions were applied using a b-factor of 700 s/mm^2, and one image at b=0. Two averages were collected, giving a total scan time of 3 minutes and 47 seconds. T2 weighted images were acquired using a turbo spin echo (TSE) sequence with a total scan time of 5 minutes (see table 4.1 for MRI sequences).

Figure 4.1: MRI Participant Flow Chart

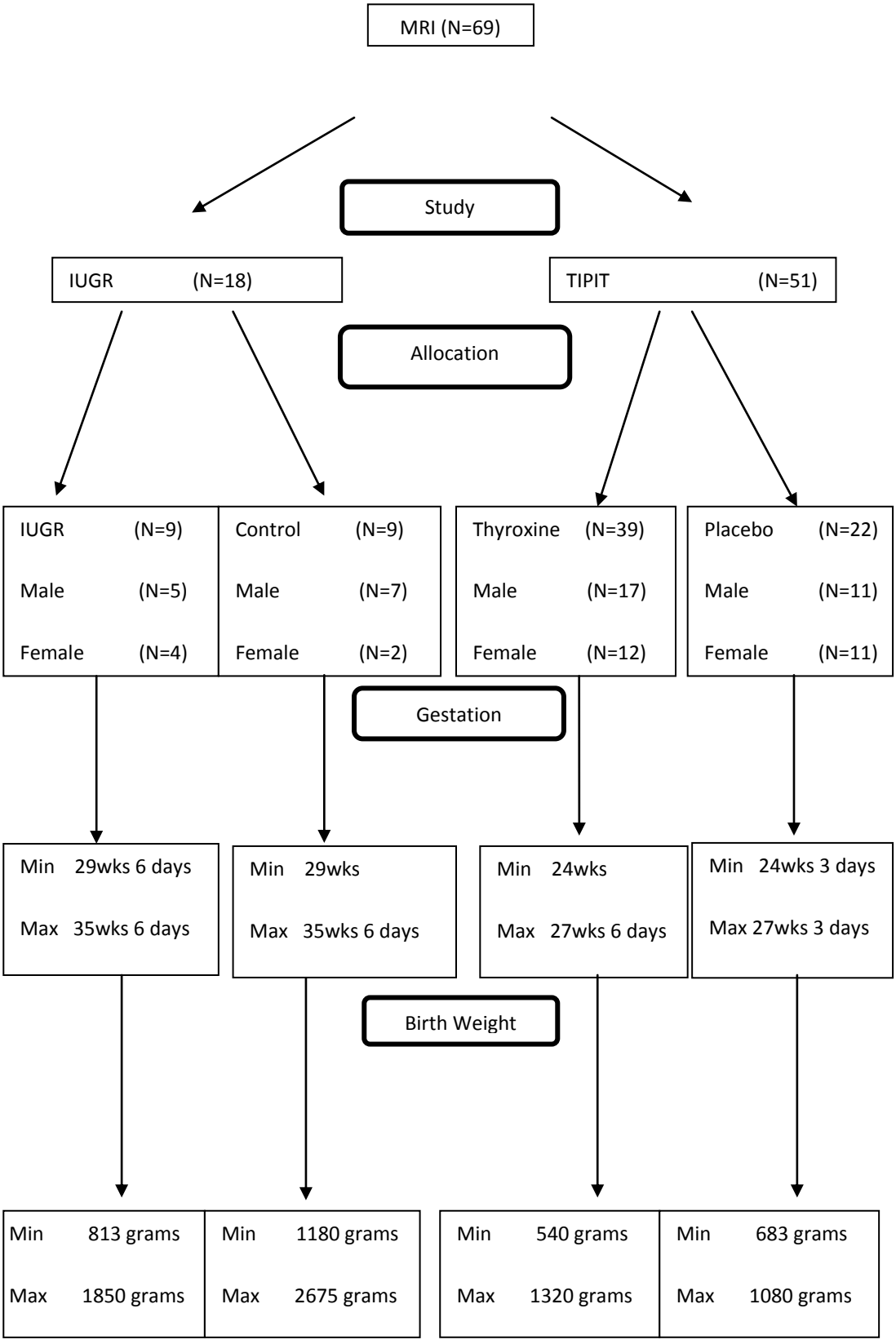


Figure 4.2: TIPIT Consort Flow Chart

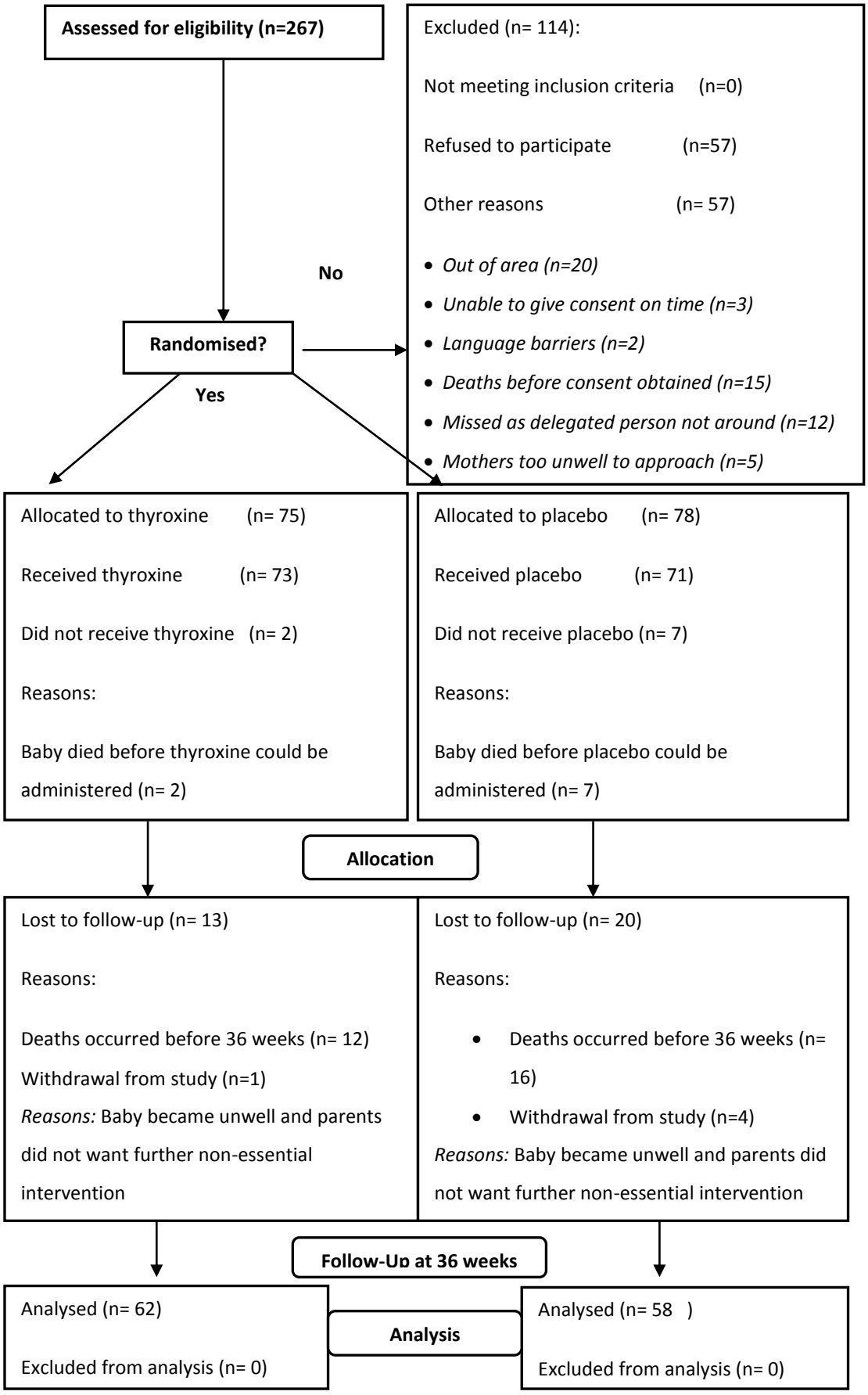


Table 4.1: MRI Sequences

	Sequence	Scan Parameters	Target Area
1	Midline sagittal T1 weighted Turbo Spin Echo (TSE)	TR/TE = 337/ 18ms Slice thickness = 3 mm, 0.3 mm gap Scan Duration = 2 minutes	Anatomical alignment
2	Coronal heavily T2 weighted TSE (whole brain)	TR/TE = 5750 /150 ms Slice thickness = 1.5 mm, contiguous Scan Duration = 5 minutes	Measurement of whole brain volume (cerebrum/cerebellum) and measurement of specific brain structures using stereology: • prefrontal cortex
3	Diffusion tensor imaging 2D single shot spin-echo Echo-Planar Imaging (SE-EPI) with SENSE factor of 2.5. 24 contiguous axial slices of 3 mm thickness with in-plane isotropic resolution of 2 mm.	TR/TE = 3350/74 ms. 32 gradient directions with a bfactor of 700 s/mm2. 2 averages. Scan Duration = 3 minutes 47 s.	Measurement of degree of mean diffusivity and fractional anisotropy (as markers of water content and myelination) in: • posterior limb of internal capsule • anterior and posterior corpus callosum • frontal white matter • occipital white matter

4	Axial T2-weighted TSE	TR/TE = 3023/150 ms. Axial slices thickness= 3mm In-plane isotropic resolution of 0.7mm. Scan duration = 3minutes	Same slice location as DTI to aid region drawing.

IUGR

The IUGR cohort were scanned at term equivalent age in natural sleep following a feed. Sedation was not used. Infants were scanned using 1.5 T (Philips Medical System) MR system based at the Wellcome Trust Clinical Research Facility, Manchester following discharge from the neonatal unit. A pillow was used for head immobilisation and ear putty was used for ear protection. Heart rate and oxygen saturation was monitored throughout the scan. The MRI protocol included a 2D turbo spin echo sequence to provide T_2-weighted images for the estimation of brain volumes, with scan parameters: TR 5760 ms, TE 150 ms, 70 contiguous coronal slices of 1.5 mm thickness with in-plane isotropic resolution of 0.9 mm, scan duration 5 minutes. Diffusion-weighted images were also acquired using a 2D single shot spin-echo Echo-Planar Imaging sequence with SENSE factor of 2.5 and scan parameters: TR 3650 ms, TE 74 ms, 32 isotropic gradient directions with b-factor 700 s/mm^2, and one image at b = 0, 24 axial slices of 3mm thickness and 0.3mm gap with in-plane isotropic resolution of 2mm, 2 averages, scan duration just under 4 minutes.

Image Analysis

DTI

DTI Studio was used to analyse diffusion images. Maps of apparent diffusion coefficient and fractional anisotropy were produced and regions of interest were drawn by hand (Table 4.2, Figure 4.3).

Table 4.2 DTI Image Analysis

Structure	**ROI**	**ROI Size**
corpus callosum (anterior and posterior) frontal lobes occipital lobes	Oval Oval	20-25 25-30 25-30
posterior limb of internal capsule	Free	25-30

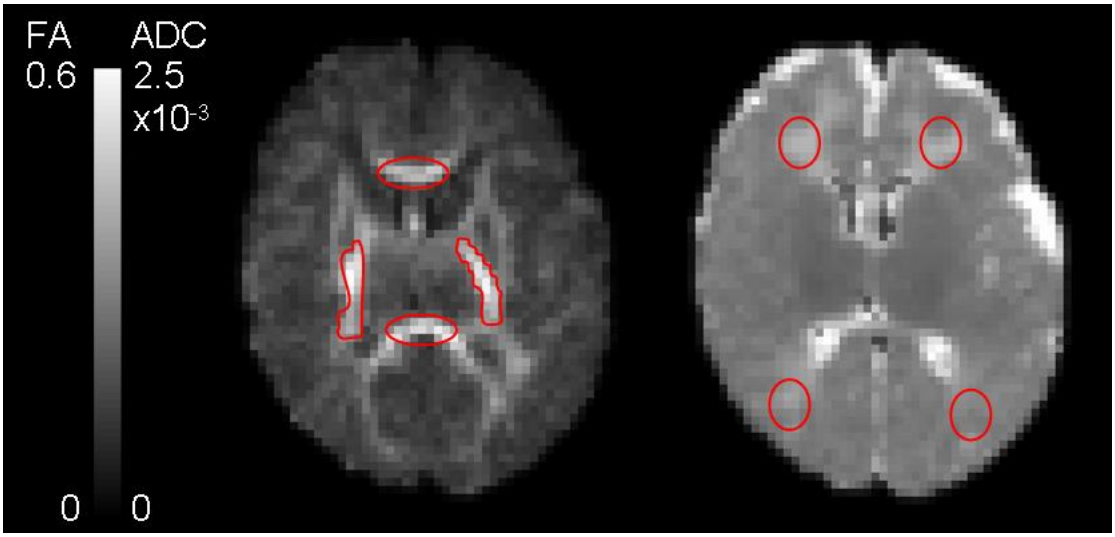


Figure 4.3: ROI'S shown in red

Stereology

The software Brain Voyager QX (version 1.9.10) was used for image realignment and demarcation of T2 weighted images. The images were aligned to correct for any differences in positioning of the head during scanning. When loading the data the

image is inverse (Figure 4.4) and was rotated 90 degrees on the X plane (Figure 4.5). For measurement of the prefrontal cortex the image was rotated on the Z field so that the subject's nose was facing the left and the cerebellum is near the right of the screen (Figure 4.6). The image was rotated to correct for positioning differences during scanning (Figure 4.7).

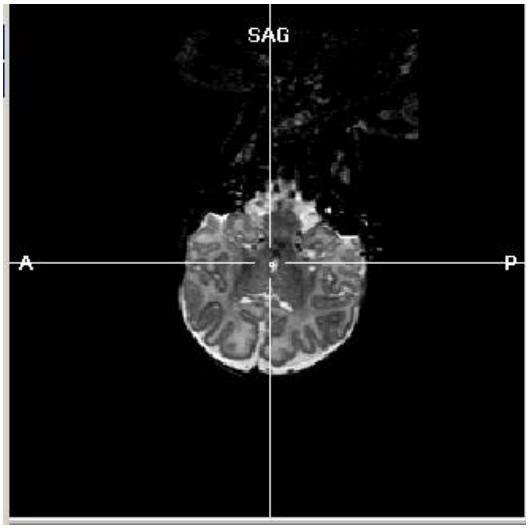


Figure 4.4 the inverse image.

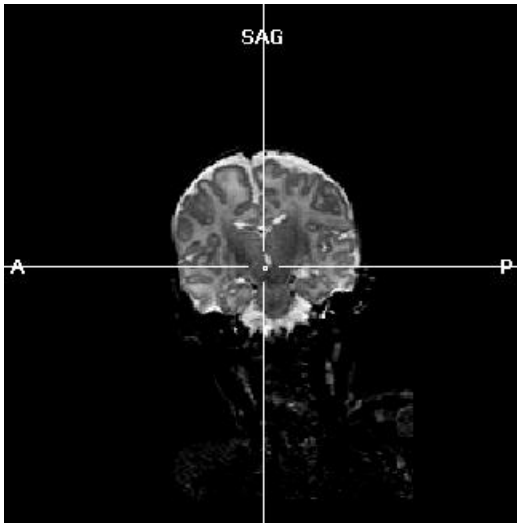


Figure 4.5 the rotated image.

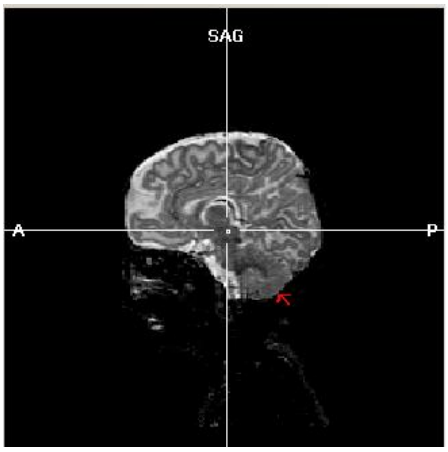


Figure 4.6 the rotated image used for measurement of the prefrontal cortex, the red arrow indicates the cerebellum.

The prefrontal cortex was demarcated for measurement of the four subfields (dorsolateral, dorsomedial, orbitolateral, orbitomedial). A line was added to the AC/PC line just below the corpus callosum to demarcate lateral and medial fields. An additional line was added in front of the corpus callosum to demonstrate the dorsal/orbital boundary (Figure 4.8). The location of the sulcus was used to demarcate medial and lateral subfields (Figure 4.9).

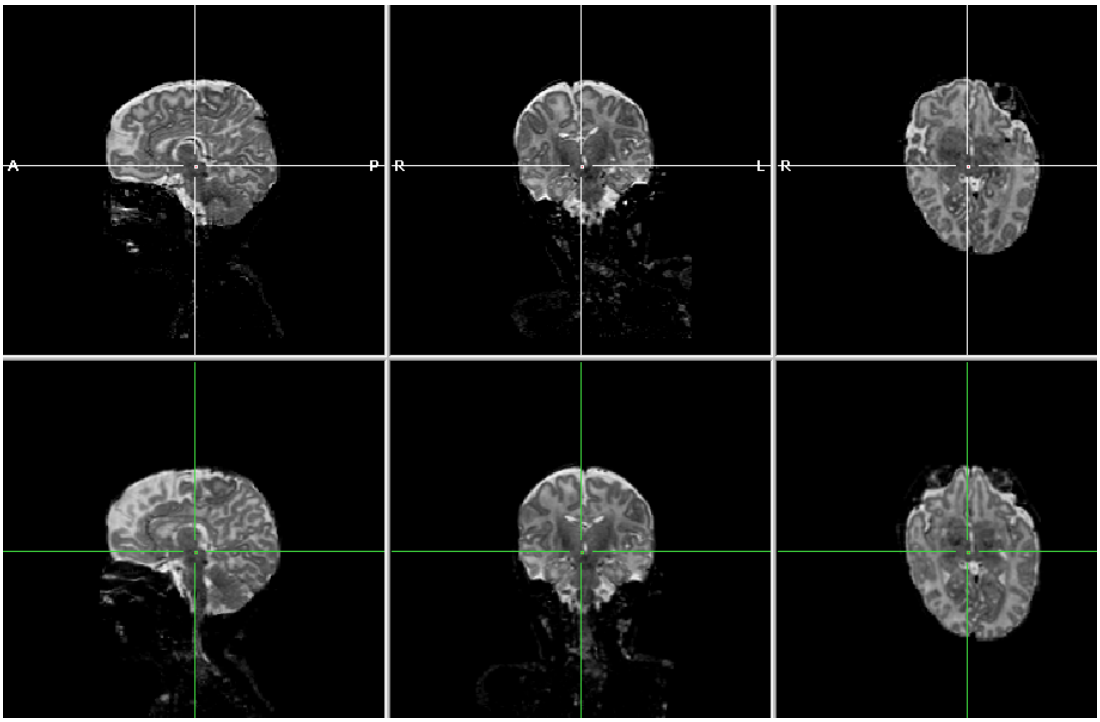


Figure 4.7 re-alignment for measuring the prefrontal cortex. The top row shows before and the bottom row after re-alignment.

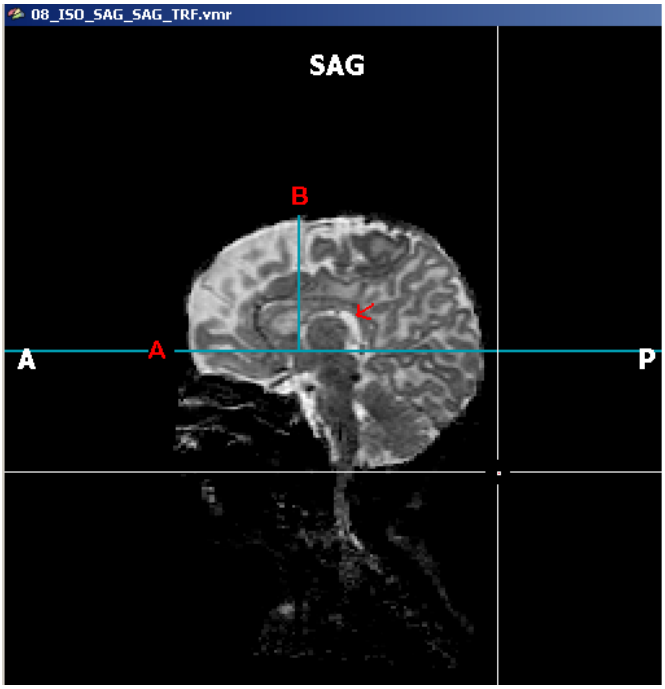


Figure 4.8 demarcation of dorsal and ventral (A) and posterior boundary for the dorsal subfield (B). The red arrow indicates the corpus callosum (CC).

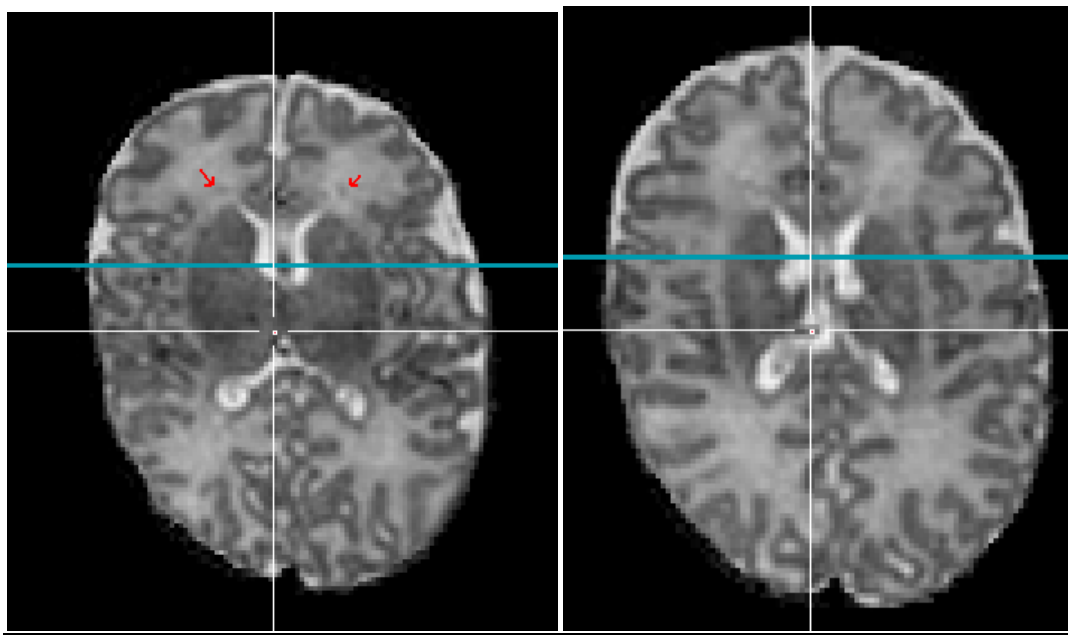


Figure 4.9 left: sulcus is visible indicated by the red arrows, Right: sulcus is no longer visible on both sides of the brain.

The brain was rotated differently for measurements of the hemisphere volumes i.e. the cerebrum (Figure 4.10) and no demarcation was needed.

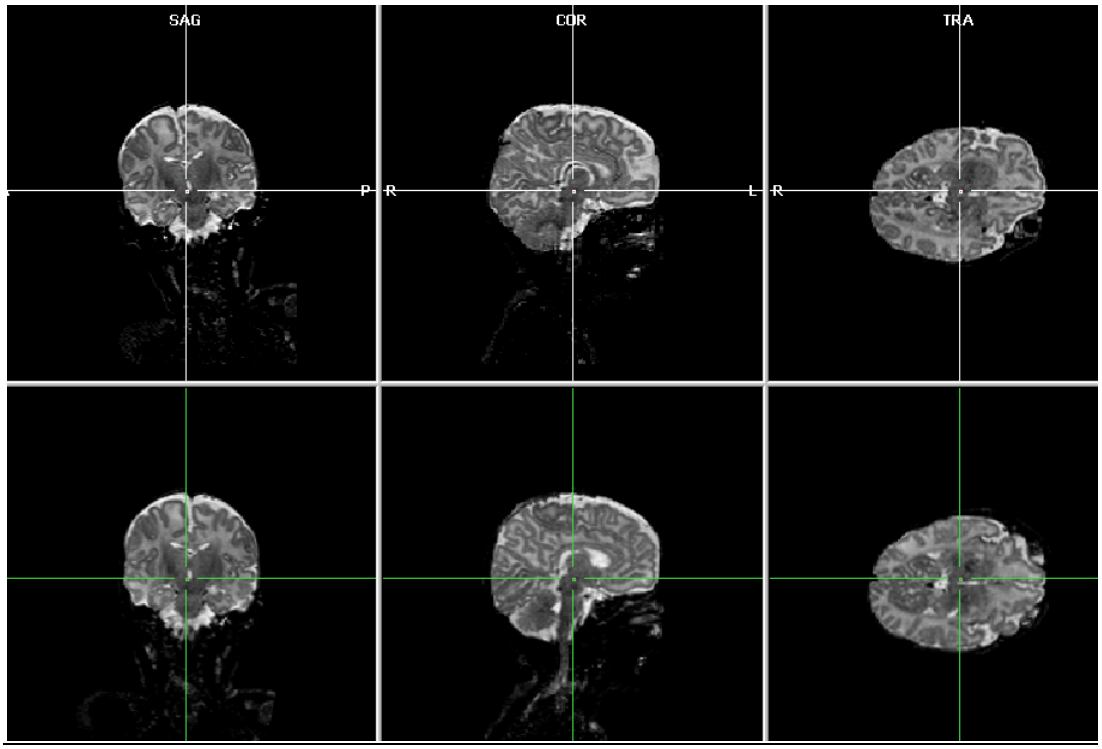
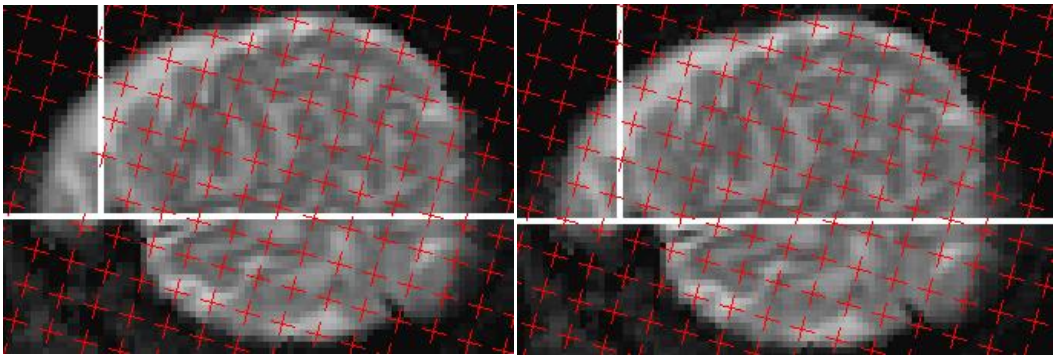


Figure 4.10 re-alignment for measuring the cerebrum. The top row shows before and the bottom row after re-alignment.

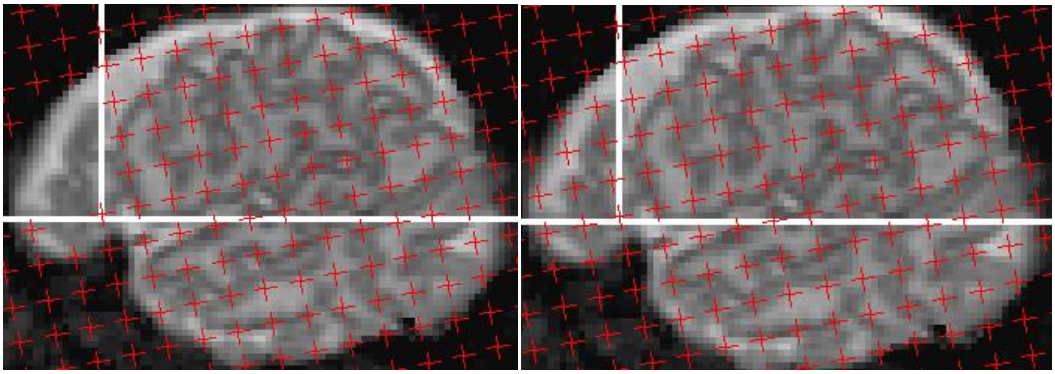
T2 weighted images were analysed using medical imaging software for structure-specific brain volume measurements (Easymeasure). Volume of the brain structures were estimated using Stereology (see chapter 3), a technique that uses Cavalieri and point counting methods (Ng et al. 2008).

Figure 4.11 Point Counting in the Dorsolateral Subfield of the Prefrontal Cortex

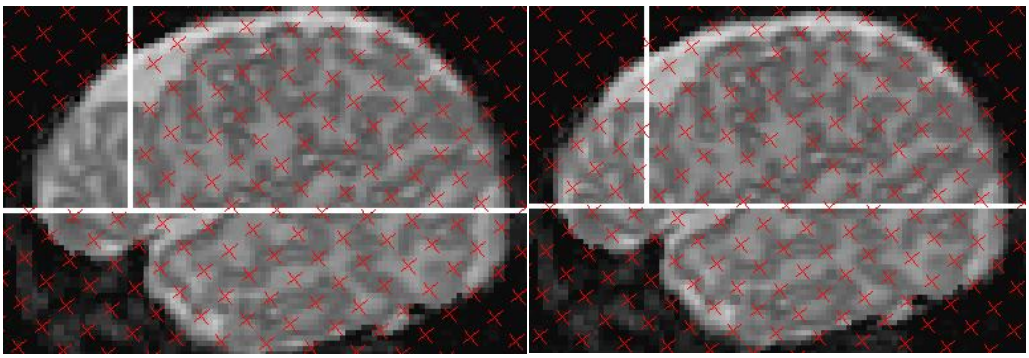
After Counting Before Counting



SLICE 48



SLICE 49



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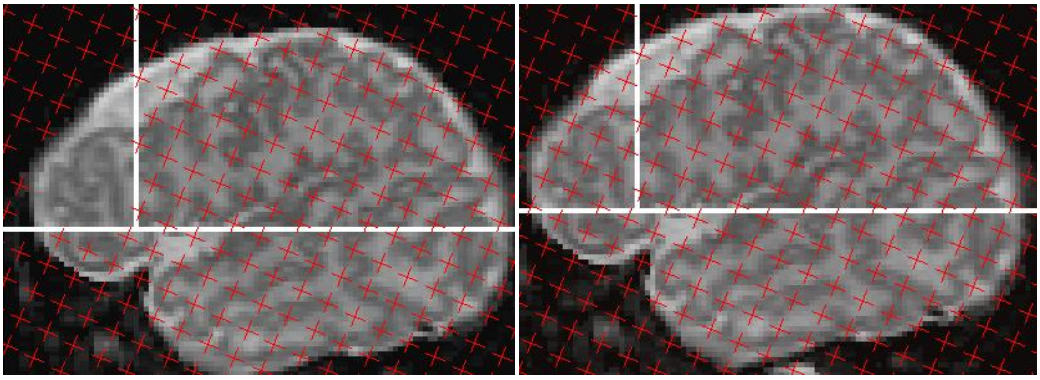
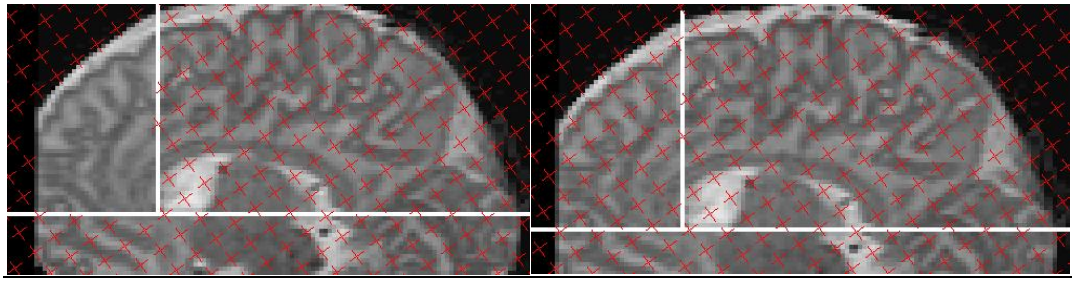


SLICE 51

Figure 4.12 Point Counting in the Dorsomedial Subfield of the Prefrontal Cortex

After Counting Before Counting



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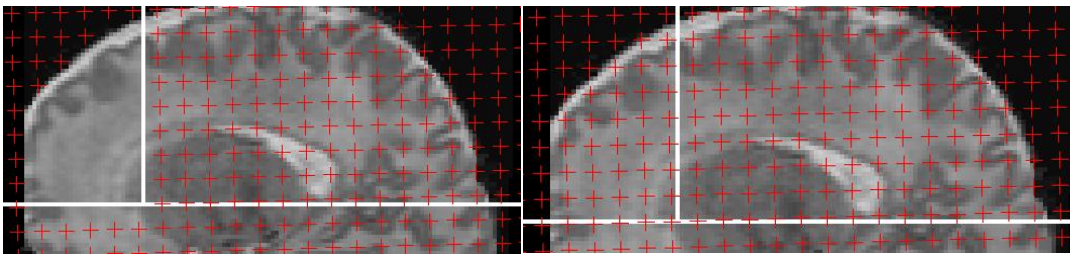

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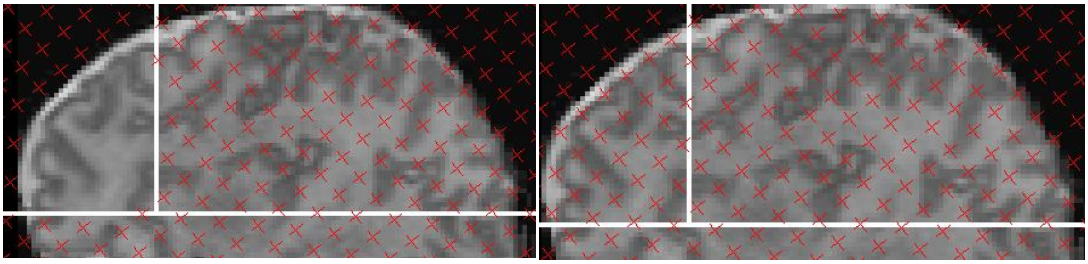

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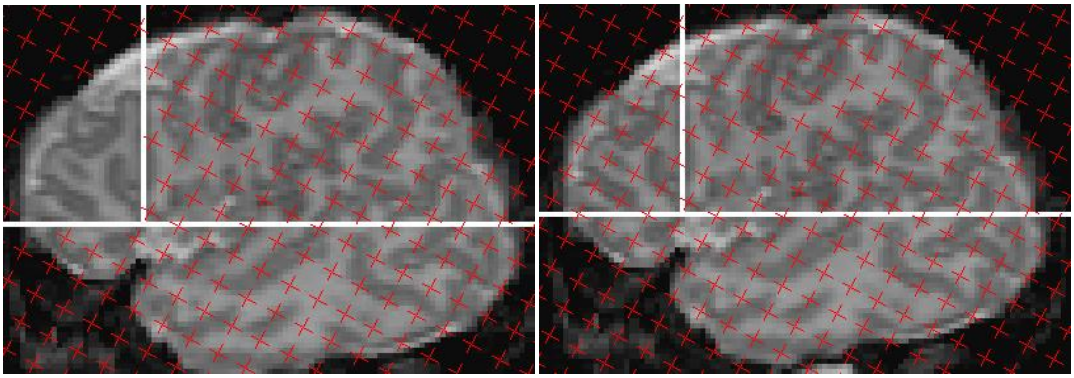
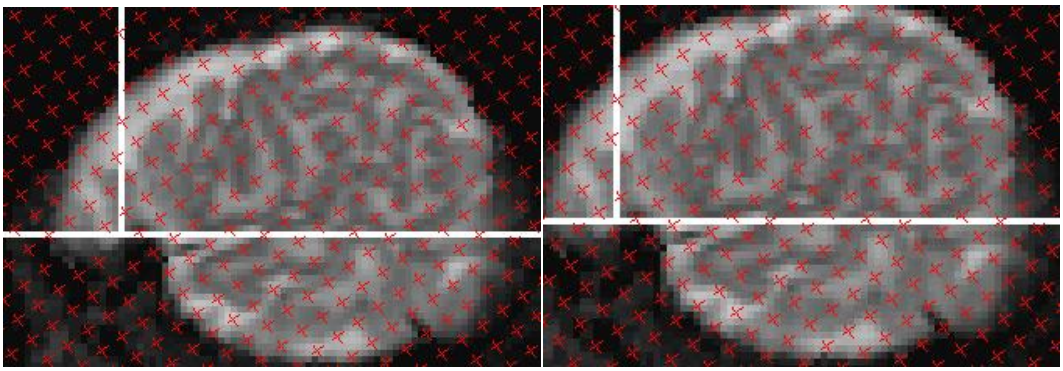


SLICE 80

Figure 4.13 Point Counting in the Orbitolateral Subfield of the Prefrontal Cortex

After Counting Before Counting



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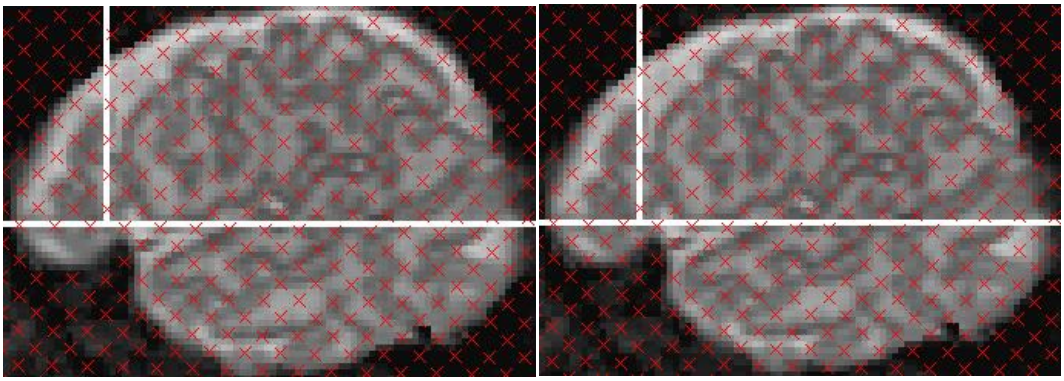

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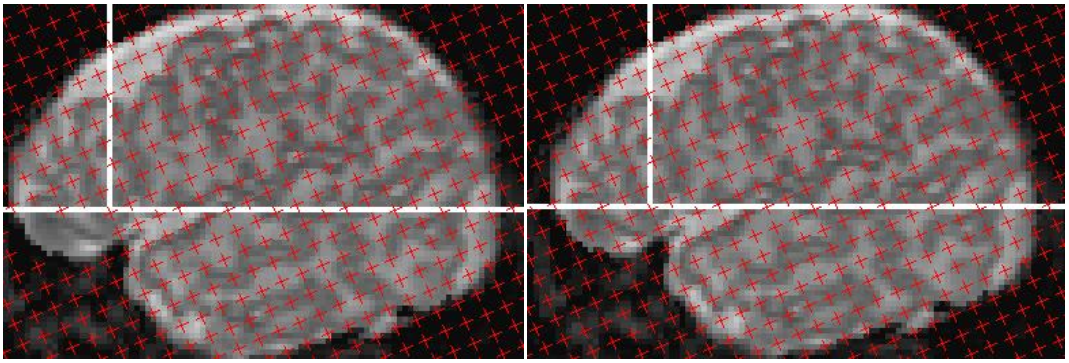

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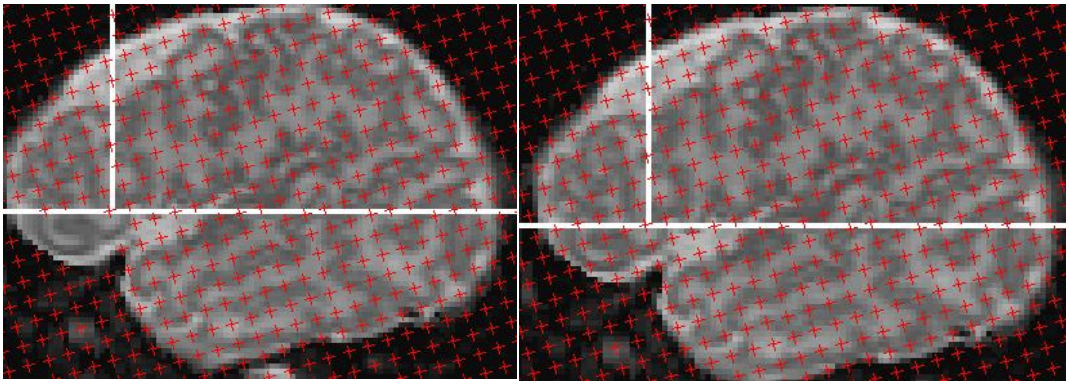
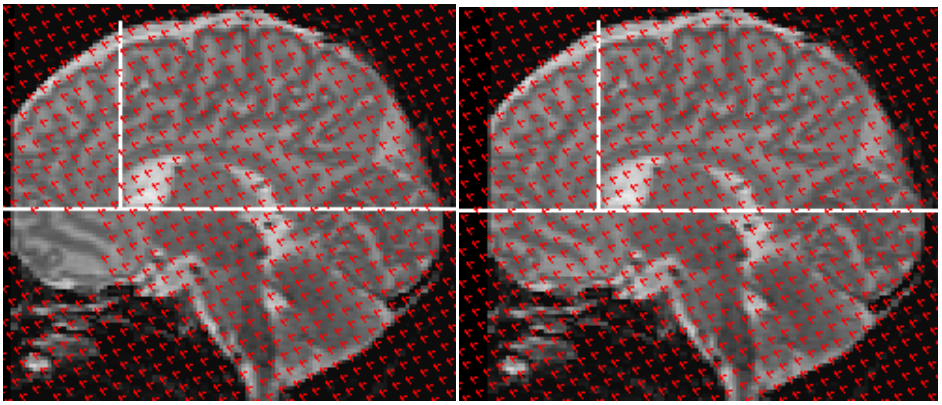


SLICE 51

Figure 4.14 Point Counting in the Orbitomedial Subfield of the Prefrontal Cortex

After Counting Before Counting



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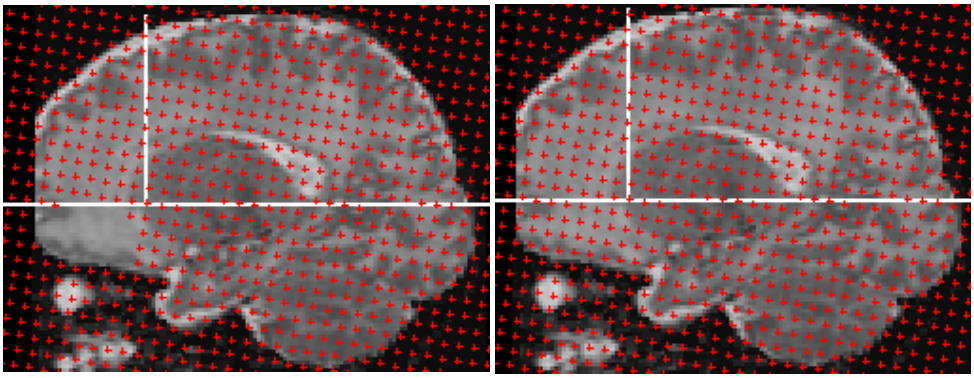
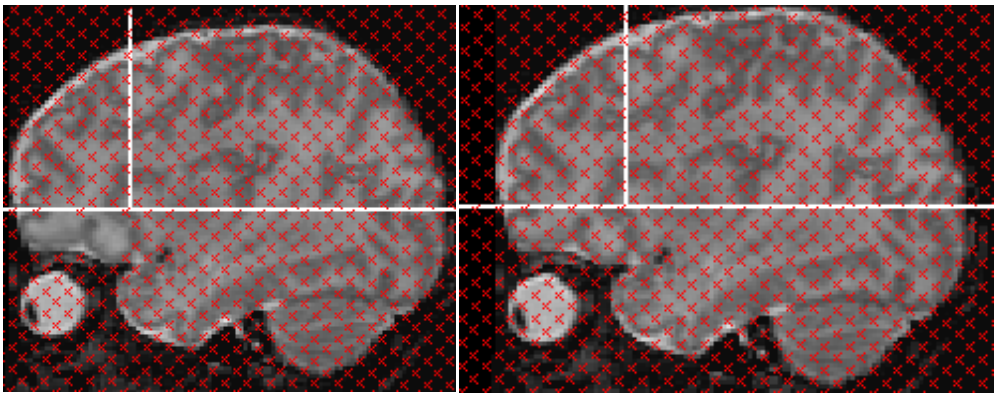


SLICE 71



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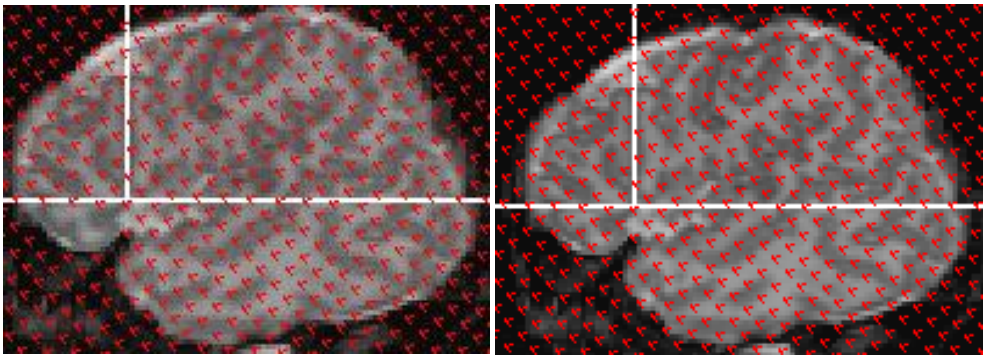
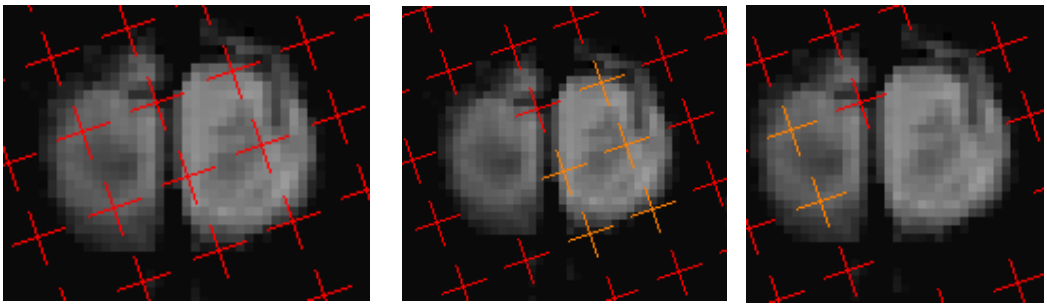


SLICE 80

Figure 4.15 Point Counting of the Cerebrum

Before Counting | Counting on the Right | Counting on the Left



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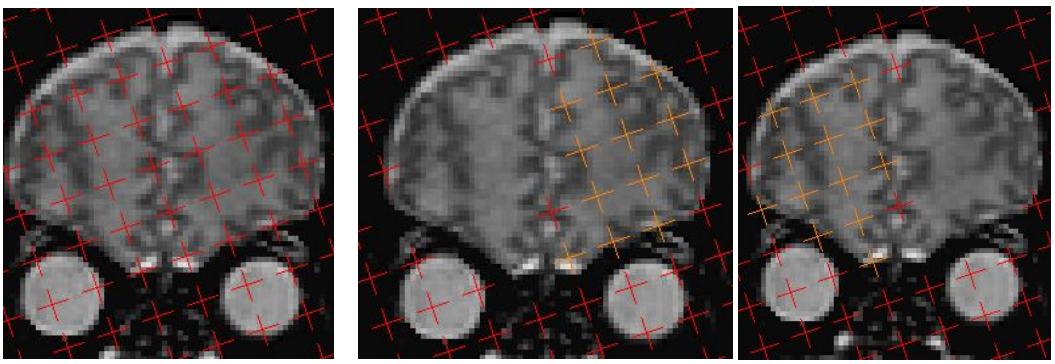

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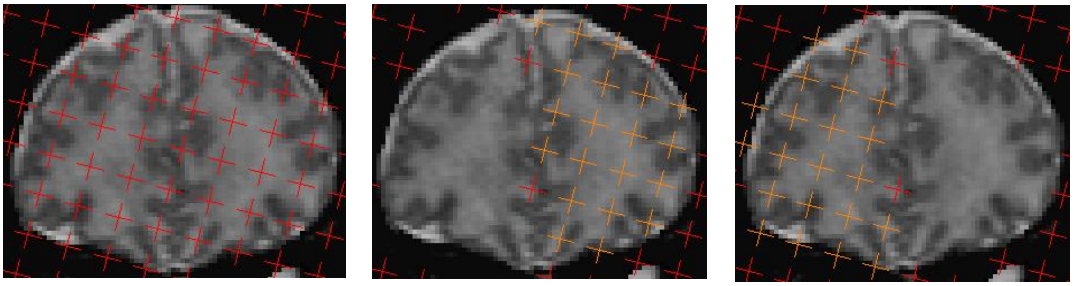

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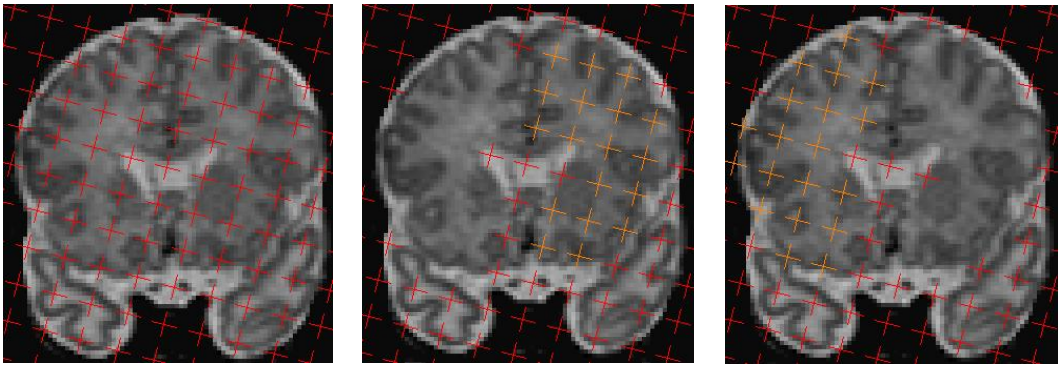

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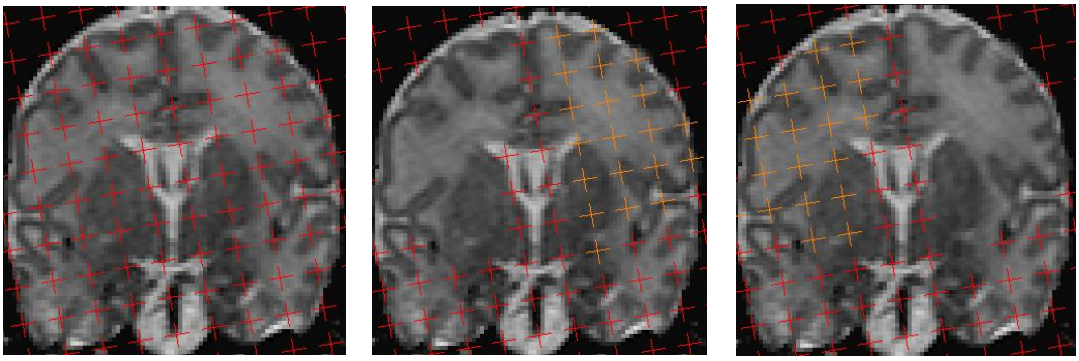

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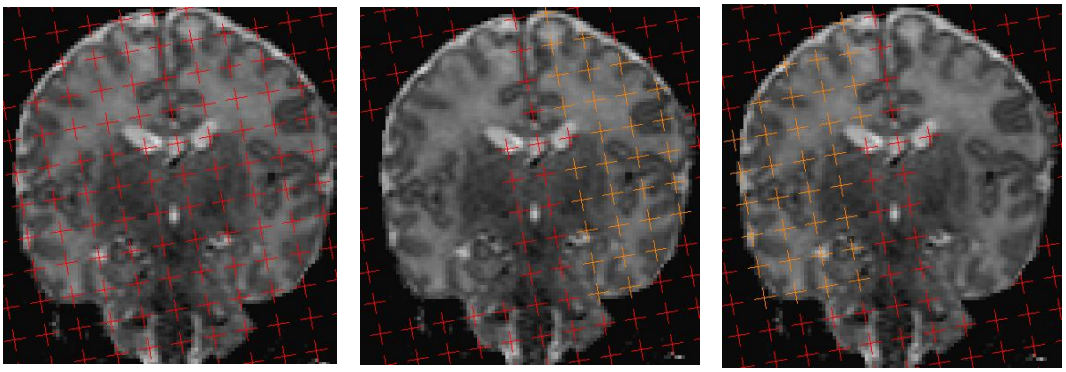

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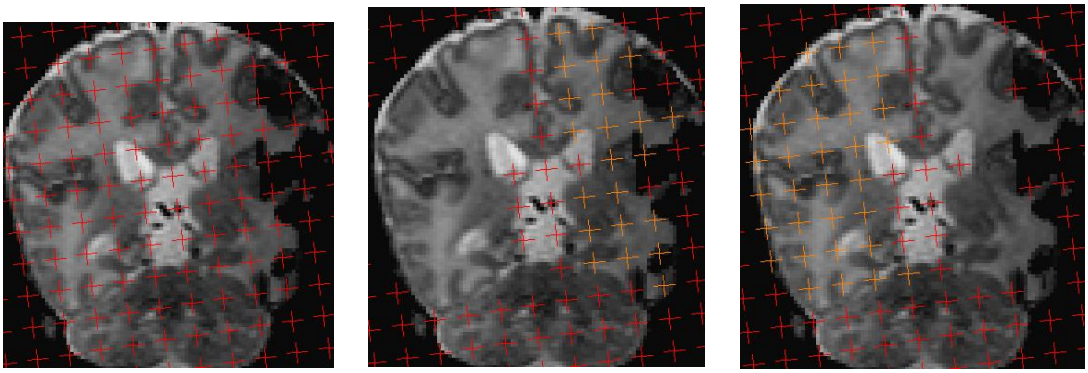

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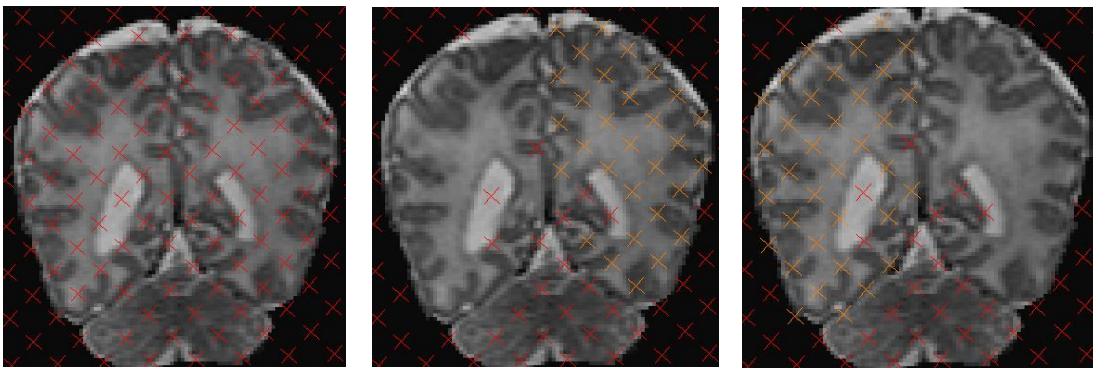

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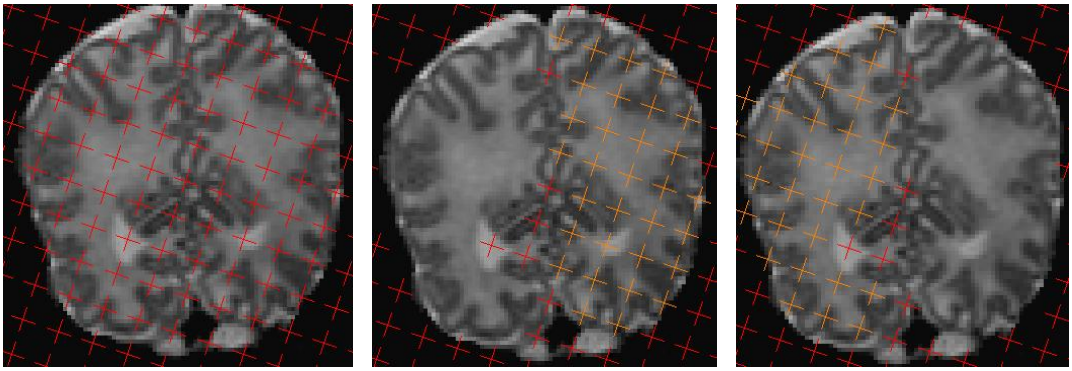

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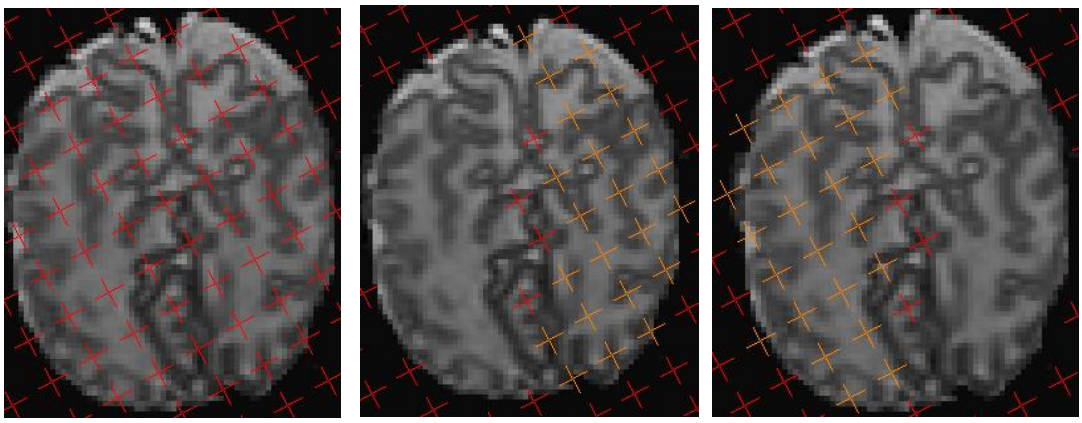
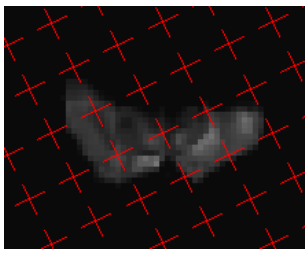


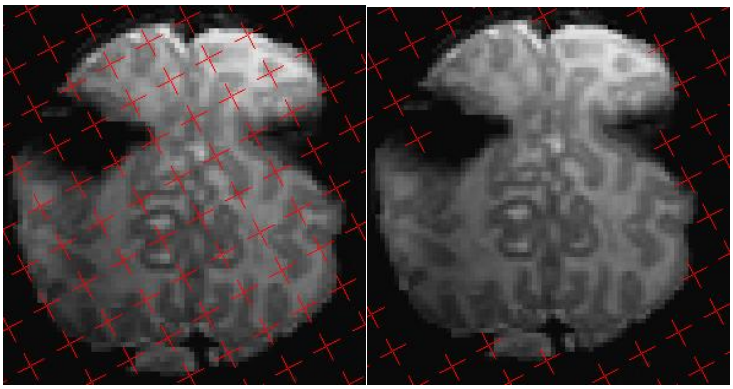
SLICE 15

Figure 4.16 Point Counting of Intracranial Volume



SLICE 6

Before Counting After Counting



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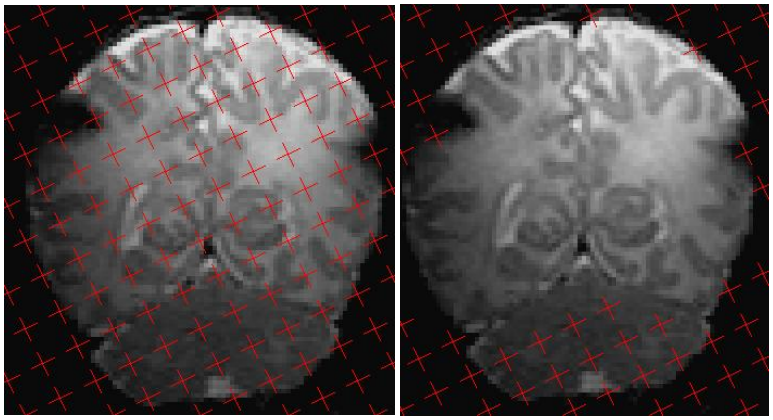

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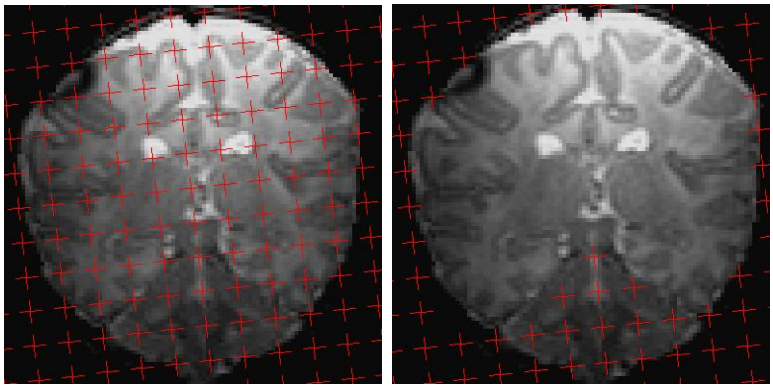

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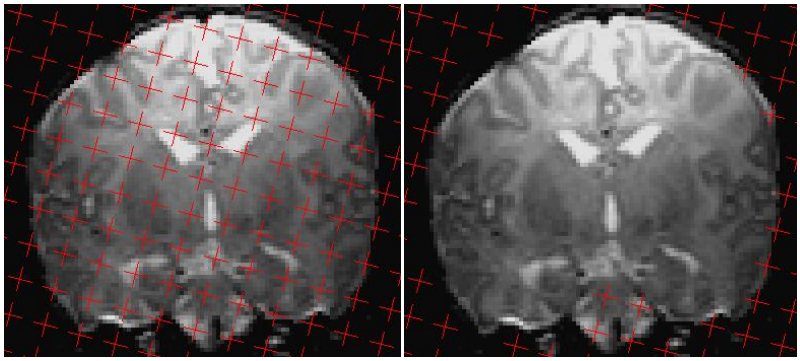

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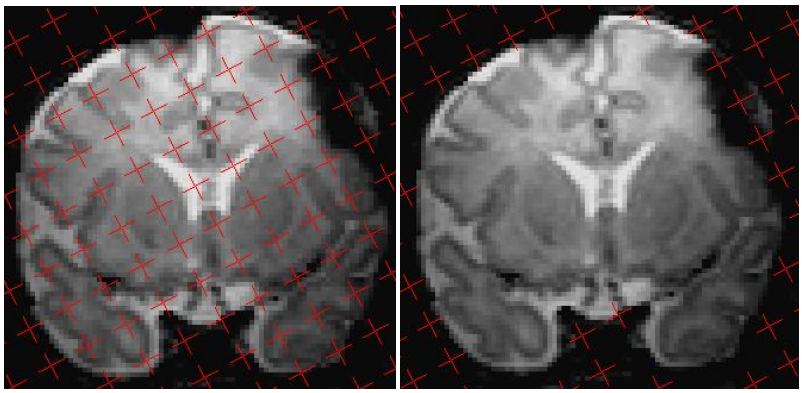

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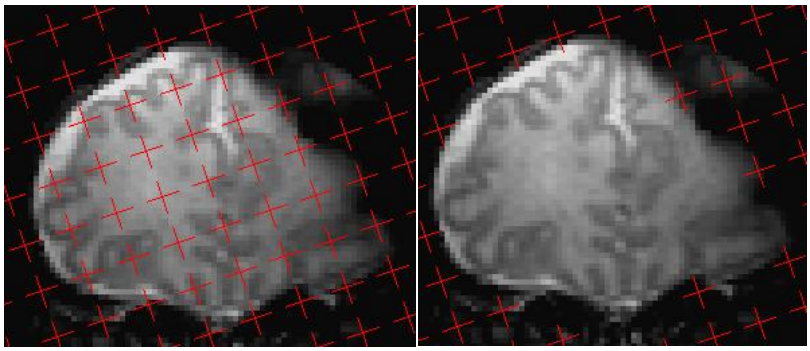

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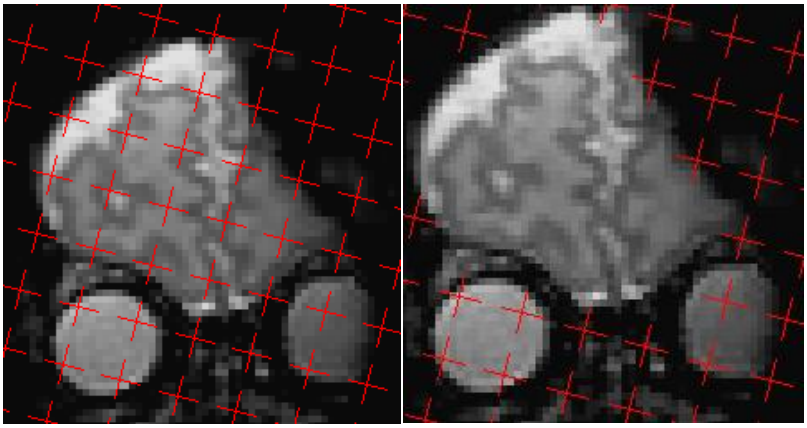

SLICE 13

Statistical Analysis

Statistical analysis was performed using software package SPSS version 19. The following hypotheses were tested (with a p value of <.05 considered significant):

Summary of Hypotheses

White Matter

Higher FA/lower ADC will be found in:

a) Females compared with males

b) Infants with higher birth weight and gestational age at birth and with which they will significantly correlate.

c) Infants treated with levothyroxine.

d) The posterior part of the internal capsule and corpus callosum measured in both thyroxine and placebo groups.

e) Preterm non-IUGR infants compared with preterm IUGR infants.

Hemispheric and Regional Volumes

1) Smaller brain structural volumes will be found in:

f) Males compared with females

g) Infants with lower birth weight and gestational age at birth and with which they will significantly correlate.

h) Infants receiving placebo

i) Preterm IUGR infants compared with preterm non-IUGR infants.

2) There will be no asymmetries present in the structures measured although asymmetry may be found in the occipital and frontal lobes and dorsal lateral prefrontal cortex and with which the right frontal lobe and left occipital lobe having higher FA and lower ADC.

Thyroxine levels

3) FT4 levels to have a temporary increase following thyroxine supplementation in comparison to the placebo group but that levels will stabilise between groups over time. T4 levels will positively correlate with gestational age and birth weight.

CHAPTER 5

DTI Results and Thyroxine

Aim of Chapter

This chapter presents the results of the DTI study for:

a) The preterm cohort
b) The IUGR cohort

The Preterm Cohort

Descriptive statistics are shown in Table 5.1 and 5.2 for the preterm cohort.

Structure		Min FA	Max FA	Mean FA	Std.Deviation
FLR	Placebo	.10	.66	.17	.13
	Thyroxine	.11	.32	.15	.04
FLL	Placebo	.10	.58	.19	.12
	Thyroxine	.10	.48	.15	.08
OLR	Placebo	.09	.58	.20	.12
	Thyroxine	.11	.40	.16	.06
OLL	Placebo	.09	.63	.20	.13
	Thyroxine	.09	.42	.15	.07
RPLIC	Placebo	.34	.48	.40	.04
	Thyroxine	.32	.47	.40	.04
LPLIC	Placebo	.33	.46	.39	.04
	Thyroxine	.30	.49	.41	.04
CCA	Placebo	.23	.46	.34	.06
	Thyroxine	.24	.42	.34	.05
CCP	Placebo	.25	.49	.38	.07
	Thyroxine	.25	.51	.38	.07

Table 5.1 FA Table of Descriptive Statistics (FLR= right frontal lobe, FLL= left frontal lobe, OLR= right occipital lobe, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left

posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Structure		Min ADC	Max ADC	Mean ADC	Std.Deviation
FLR	Placebo	1.17	2.03	1.69	.21
	Thyroxine	1.35	2.00	1.67	.16
FLL	Placebo	1.42	2.39	1.75	.22
	Thyroxine	1.39	1.98	1.65	.15
OLR	Placebo	1.33	2.18	1.68	.23
	Thyroxine	1.48	1.93	1.74	.14
OLL	Placebo	1.38	2.05	1.72	.20
	Thyroxine	1.42	2.09	1.73	.16
RPLIC	Placebo	.97	1.17	1.09	.05
	Thyroxine	1.01	1.27	1.10	.06
LPLIC	Placebo	.96	1.24	1.10	.06
	Thyroxine	1.06	1.23	1.12	.04
CCA	Placebo	1.22	1.84	1.50	.15
	Thyroxine	1.30	1.85	1.51	.17
CCP	Placebo	1.14	1.90	1.59	.19
	Thyroxine	1.16	1.90	1.52	.18

Table 5.2 ADC Table of Descriptive Statistics (FLR= right frontal lobe, FLL= left frontal lobe, OLR= right occipital lobe, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Comparison of Structures: FA

FA is lower in the frontal and occipital lobes compared to the posterior limb of the internal capsule and corpus callosum (Figure 5.1).

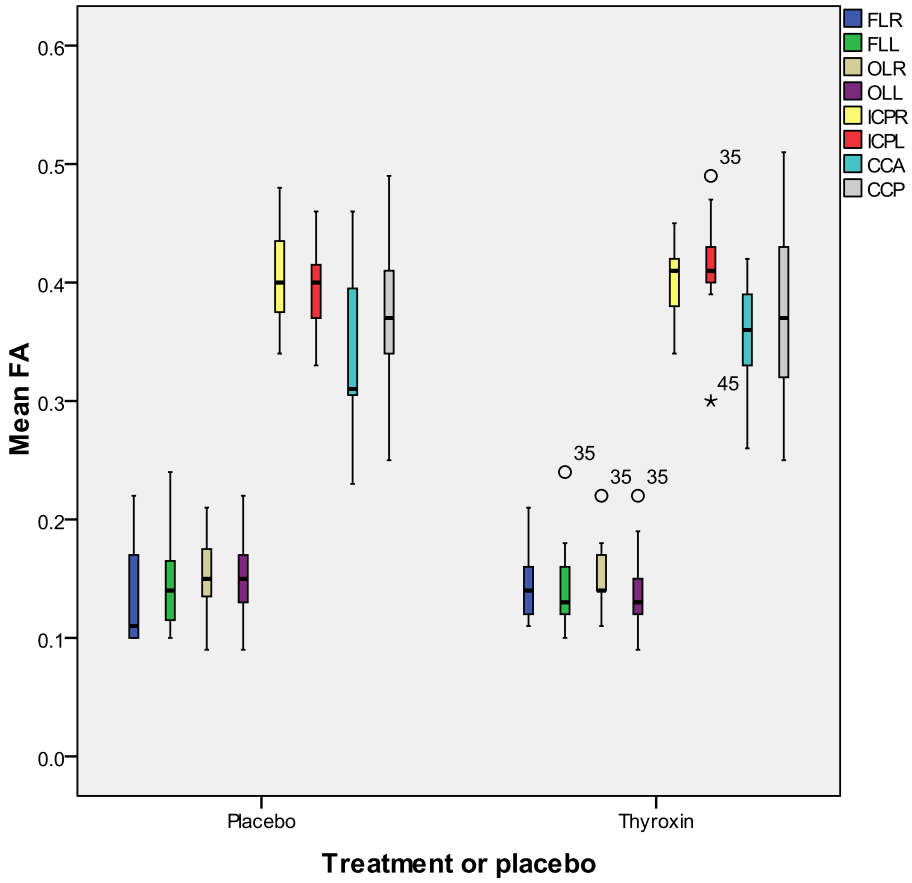

Figure 5.1. Box Plot demonstrating FA means, the inter-quartile range and outliers (FLR= right frontal lobe, FLL= left frontal lobe, OLR= right occipital lobe, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Comparison of Structures: ADC

ADC is higher in the frontal and occipital lobes compared to the posterior limb of the internal capsule and corpus callosum (Figure 5.2).

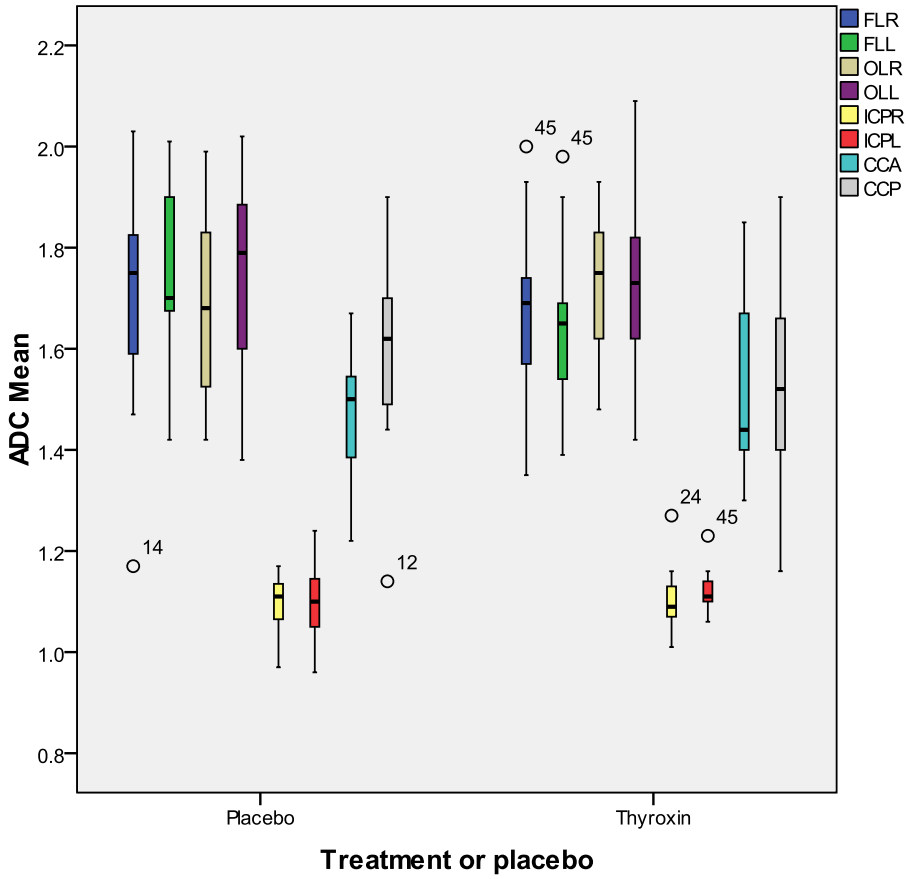

Figure 5.2 Box plot demonstrating ADC means, the inter-quartile range and outlier (FLR= right frontal lobe, FLL= left frontal lobe, OLR= right occipital lobe, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

FA and ADC measures were entered into a repeated measures ANOVA with region and anterior/posterior as within subject factors and treatment group as between subject factor. As there was significant 3 way interaction (Table 5.3), the analysis was re-run for anterior and posterior structures separately.

DTI Measure	Interaction		Mean Square	F	P
FA	Region *Treatment	Linear	.000	.052	.821
		Quadratic	3.334	.027	.871
	Ant_Post*Treatment	Linear	4.77	.003	.957
	Region*Ant_Post*Treatment	Linear	.000	.170	.682
		Quadratic	.005	5.45	.026
ADC	Region *Treatment	Linear	.000	.005	.944
		Quadratic	.011	1.31	.260
	Ant_Post*Treatment	Linear	.001	.037	.849
	Region*Ant_Post*Treatment	Linear	.054	4.80	.035
		Quadratic	.078	16.66	.000

Table 5.3 Repeated Measures Anova for Region and Anterior/Posterior (Ant_Post= Anterior_Posterior).

FA is significantly lower in the anterior compared to posterior regions in both the thyroxine and placebo group (Table 5.4 and Figure 5.3).

	Anterior Structures	Posterior Structures	Wilks' Lambda	P
FA	CCA	CCP	.734	.001
	FLL	ICPL	.033	.000
	FLR	ICPR	.034	.000
ADC	CCA	CCP	.906	.069
	FLL	LPLIC	.062	.038
	FLR	RPLIC	.079	.000

Table 5.4 FA and ADC in anterior vs. posterior structures. Occipital lobes were excluded due to a lack of anterior comparison (FLR= right frontal lobe, FLL= left frontal lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

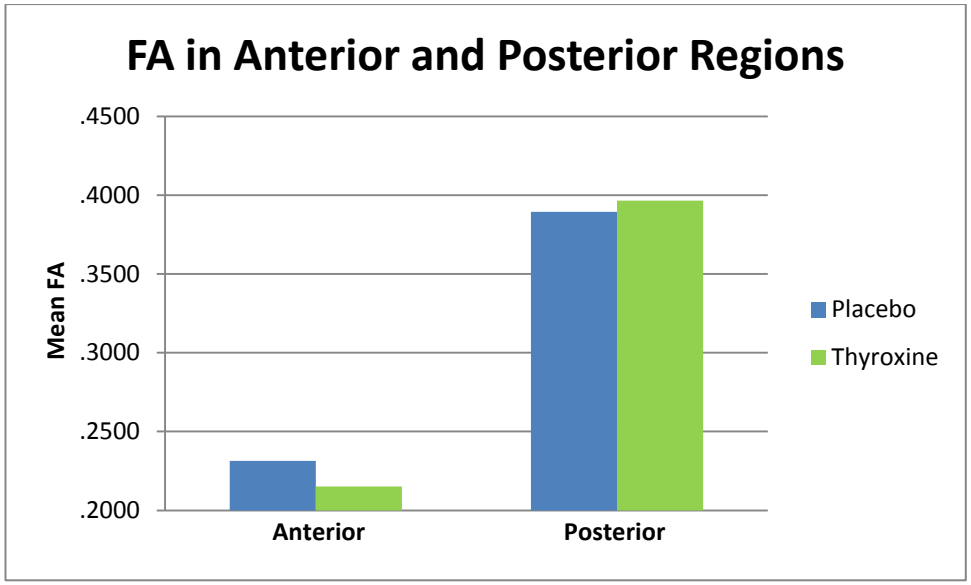

Figure 5.3 Mean FA for the three anterior structures and posterior structures in the two groups.

ADC is higher in the anterior compared to posterior regions, this difference is more pronounced in the thyroxine group (Figure 5.4).

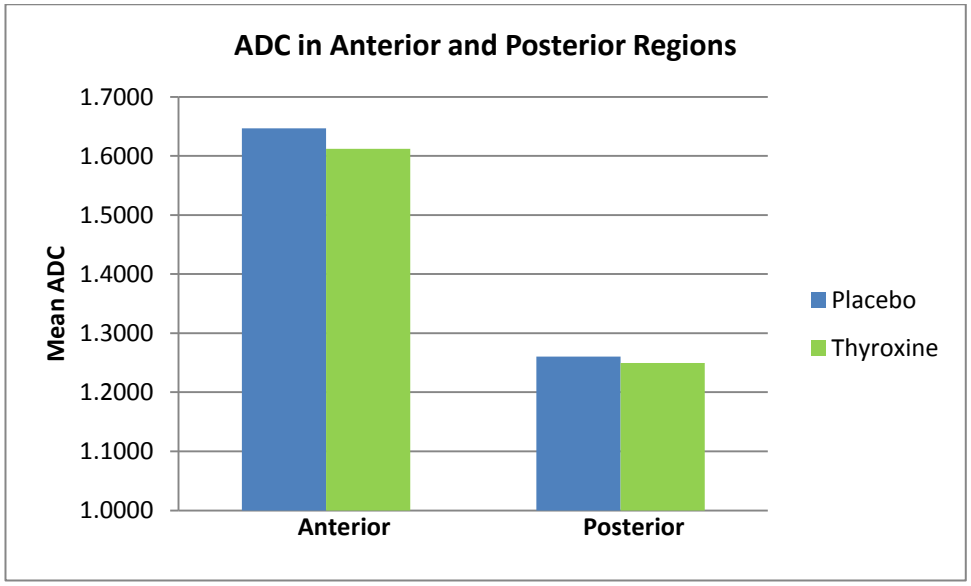

Figure 5.4 Mean ADC for the three anterior structures and posterior structures in the two groups.

The ADC differences in the corpus callosum only reached borderline significance and showed a different finding to the other structures which had significant differences between anterior and posterior regions (Table 5.4).

FA in the anterior corpus callosum is significantly lower than posterior corpus callosum (Figure 5.5).

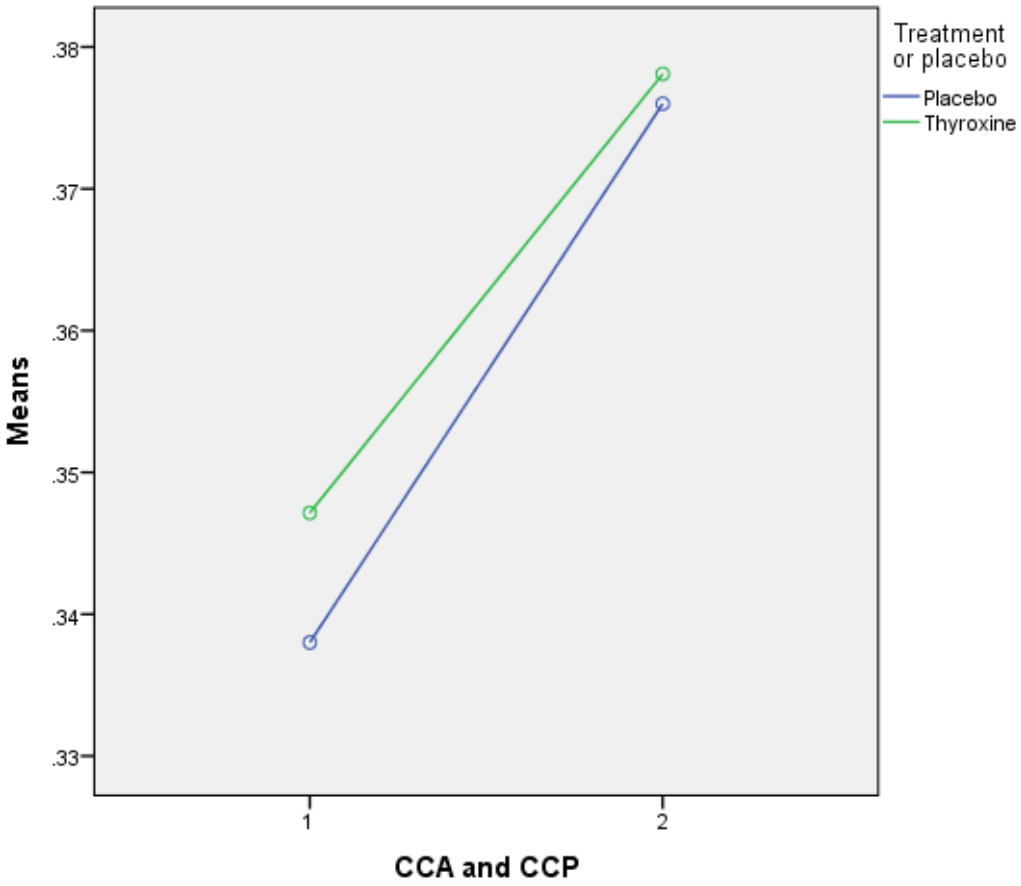

Figure 5.5 Mean FA in the anterior and posterior corpus callosum (CCA= anterior corpus callosum, CCP= posterior corpus callosum).

ADC was very similar in the thyroxine group and lower in the anterior corpus callosum in the placebo group (Figure 5.6).

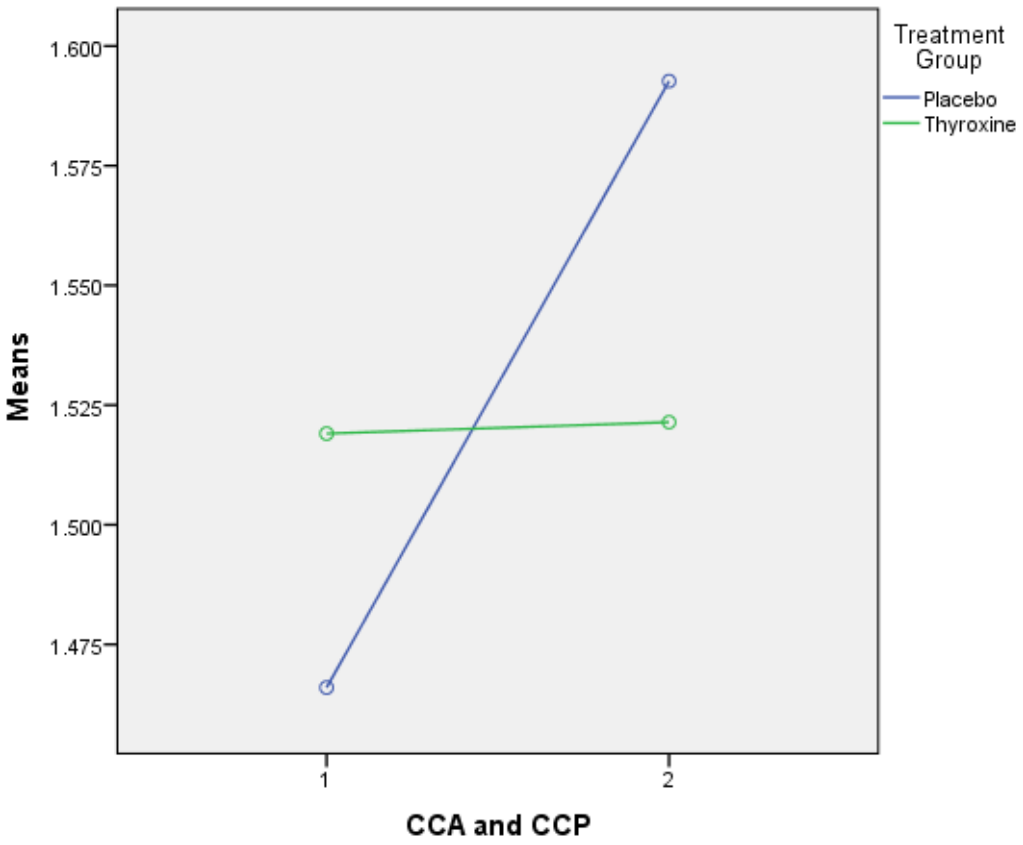

Figure 5.6 Mean ADC in the anterior and posterior corpus callosum (CCA= anterior corpus callosum, CCP= posterior corpus callosum).

FA is significantly lower in the left frontal lobe compared to the ipsilateral posterior limb of the internal capsule (Figure 5.7).

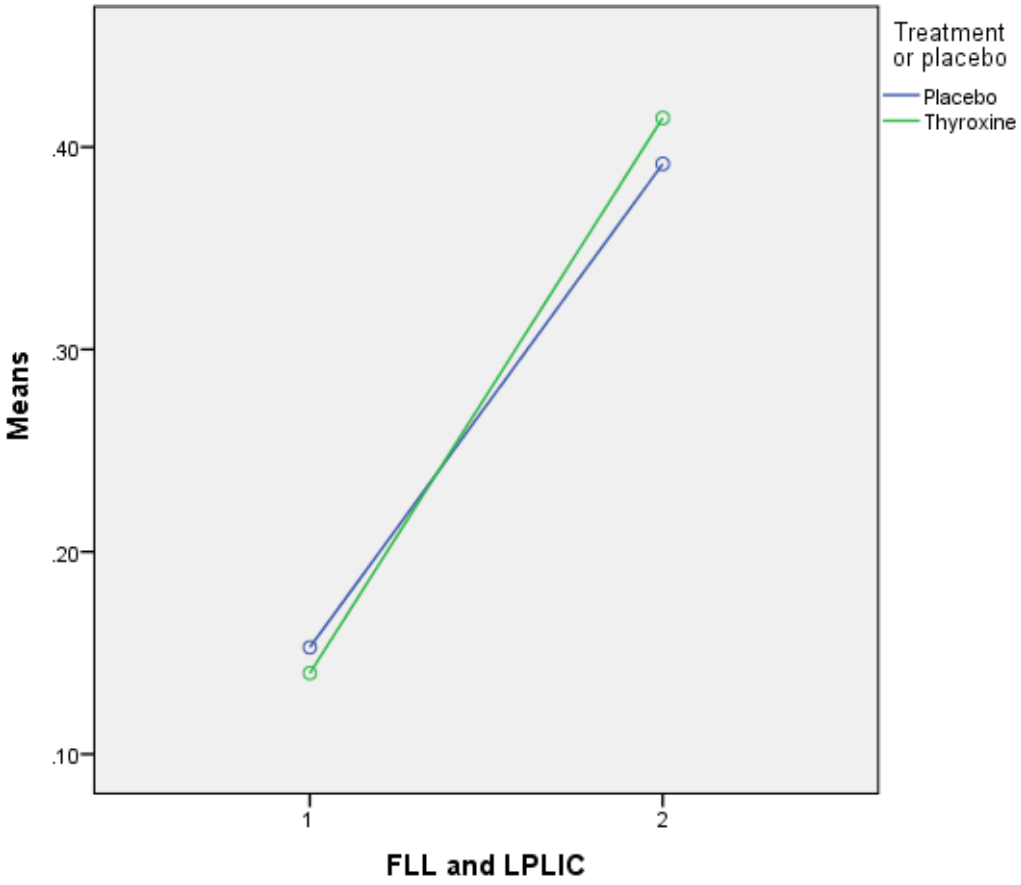

Figure 5.7 Mean FA in the left frontal lobe and ispilateral posterior limb of the internal capsule (FLL= left frontal lobe, ICPL= left posterior limb of the internal capsule).

ADC is significantly higher in the left frontal Lobe compared to the ipsilateral posterior limb of the internal capsule (Figure 5.8).

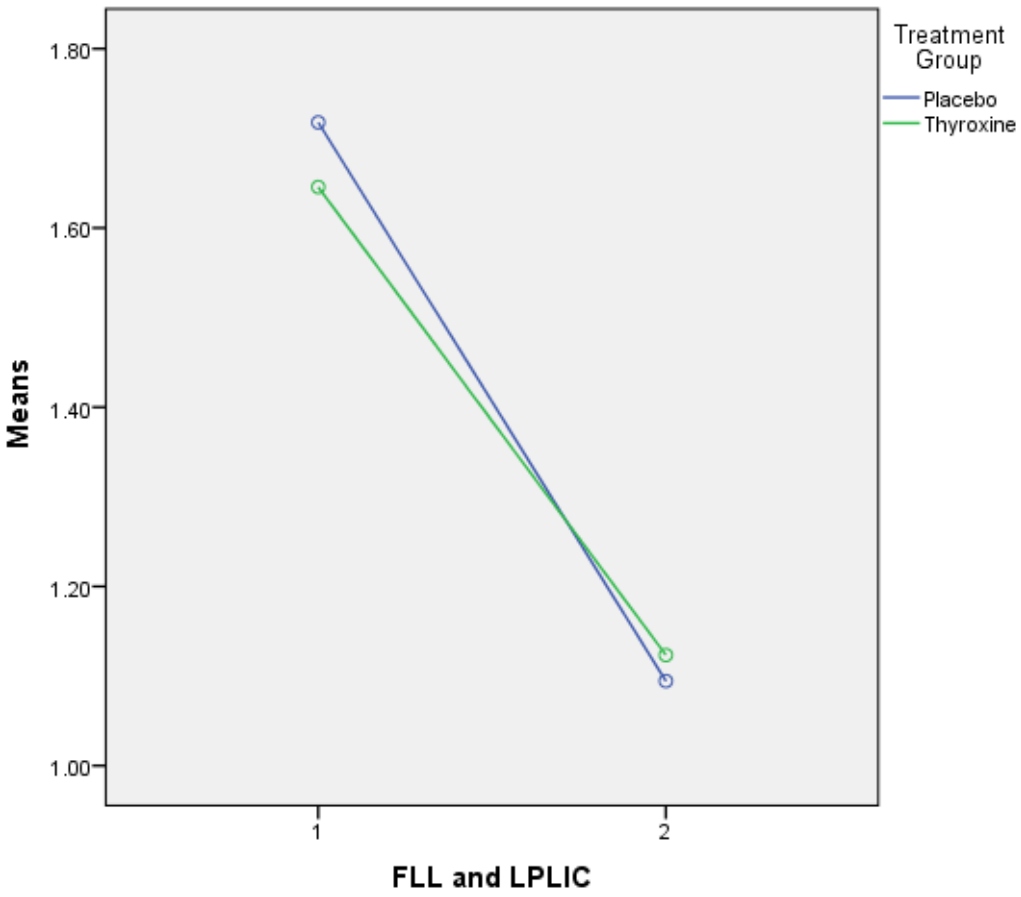

Figure 5.8 Mean ADC in the left frontal lobe and ipsilateral posterior limb of the internal capsule (FLL= left frontal lobe, ICPL= left posterior limb of the internal capsule).

FA is significantly lower in the right frontal lobe compared to the ipsilateral posterior limb of the internal capsule (Figure 5.9).

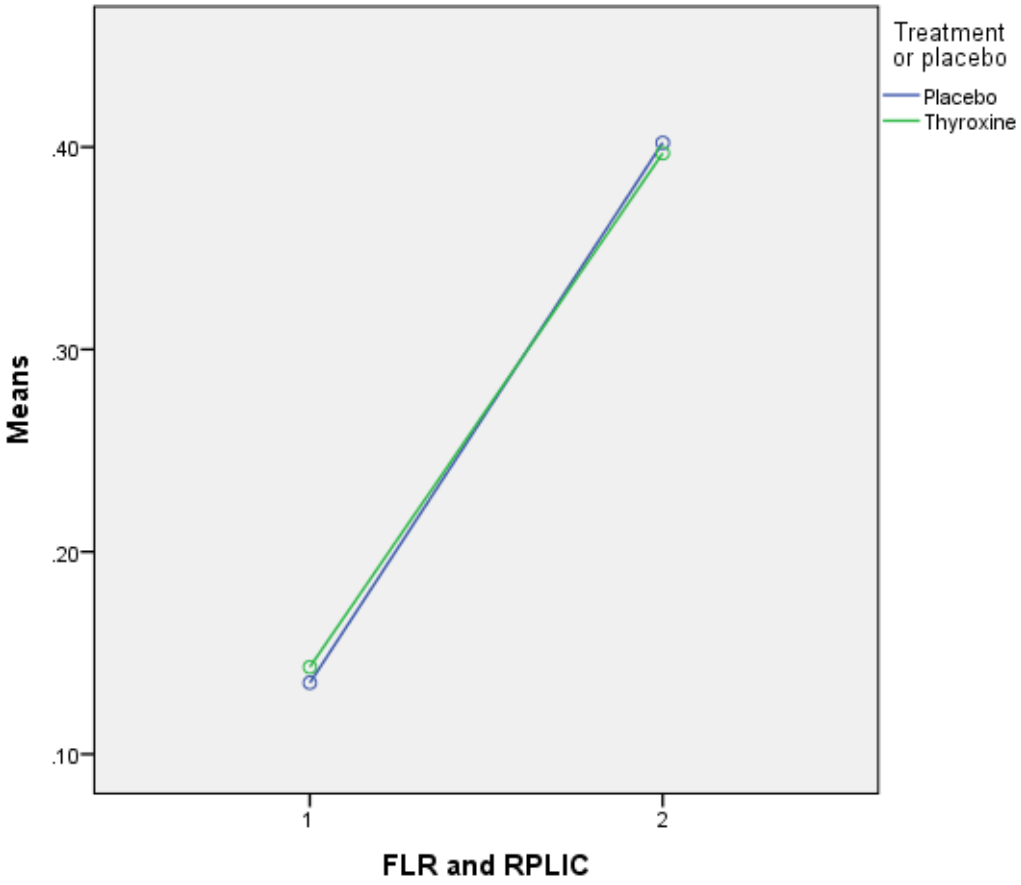

Figure 5.9 Mean FA in the right frontal lobe and ipsilateral posterior limb of the internal capsule. (FLR= right frontal lobe, posterior limb of the internal capsule).

ADC is significantly higher in the right frontal lobe compared to the ipsilateral posterior limb of the internal capsule (Figure 5.10).

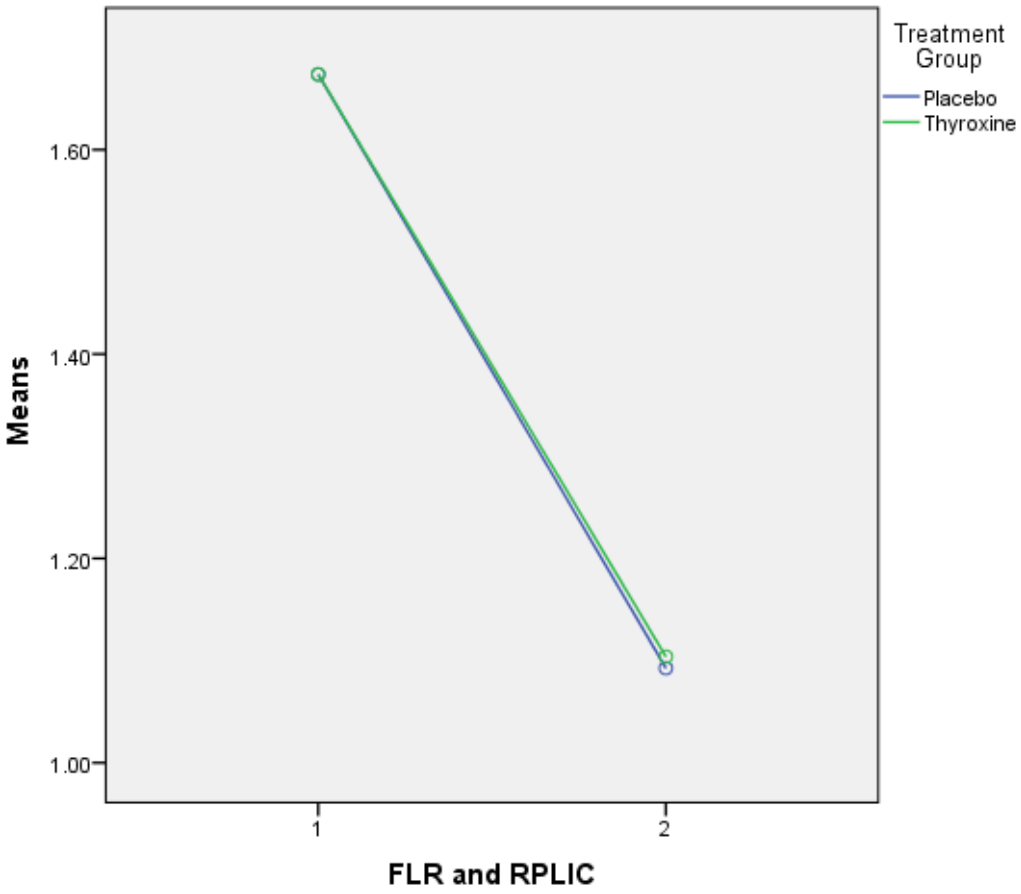

Figure 5.10 Mean ADC in the right frontal lobe and ipsilateral posterior limb of the internal capsule (FLR= right frontal lobe, RPLIC right posterior limb of the internal capsule).

Interaction with Treatment

A significant interaction with treatment was seen when looking at FA and ADC in the left Frontal Lobe and Posterior Limb of the Internal Capsule (Table 5.5).

	Structures	Value	F	P
FA	CCA and CCP	1	.13	.72
	Left FL and PLIC	.88	5.39	.03
	Right FL and PLIC	.98	.71	.41
ADC	CCA and CCP	.91	3.28	.08
	Left FL and PLIC	.89	4.60	.04
	Right FL and PLIC	1	.04	.84

Table 5.5 Interaction with treatment (FL= Frontal Lobe, PLIC= Posterior Limb of the Internal Capsule, CCA= Anterior Corpus Callosum, CCP= Posterior Corpus Callosum).

A further one-way ANOVA found no significant difference between FA in the placebo and thyroxine group for left frontal lobe and a borderline significance between placebo and thyroxine groups in the left posterior limb of the internal capsule. One-way ANOVA for ADC measures found borderline significant difference between treatments groups in the left frontal lobe and no significant differences in the other structures (Table 5.6).

	Structures	Treatment	Means	F	P
FA	FLL	Placebo	.19	1.32	.26
		Thyroxine	.15		
	LPLIC	Placebo	.39	3.87	.06
		Thyroxine	.41		
ADC	FLL	Placebo	1.75	3.00	.09
		Thyroxine	1.65		
	LPLIC	Placebo	1.10	2.77	.10
		Thyroxine	1.12		
	CCA	Placebo	1.50	.01	.91
		Thyroxine	1.51		
	CCP	Placebo	1.59	1.35	.25
		Thyroxine	1.52		

Table 5.6 A Table of Differences in DTI measures between groups (FLL= left frontal lobe, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Laterality

FA and ADC measures were entered into a repeated measure ANOVA with region and laterality as within subject factors and treatment group as between subject factor (Table 5.7).

DTI Measure	Interaction		Mean Square	F	P
FA	Region *Treatment	Linear	.00	.15	.70
		Quadratic	.00	3.70	.06
	Laterality*Region	Linear	.00	.81	.38
		Quadratic	.00	6.44	.02
	Region*Laterality*Treatment	Linear	.01	16.49	.00
		Quadratic	.00	1.17	.29
ADC	Region *Treatment	Linear	.04	1.68	.20
		Quadratic	.06	3.24	.08
	Laterality*Region	Linear	.00	.31	.58
		Quadratic	.01	1.95	.17
	Region*Laterality*Treatment	Linear	.01	2.7	.10
		Quadratic	.01	.88	.36

Table 5.7 Repeated Measures ANOVA for Region and Laterality.

As there was a three way interaction the analysis was re-run for right and left structures separately, and as there was a 2 way interaction with laterality and region the analysis was also run separately for the regions. There was no significant difference in laterality although FA in the frontal and occipital Lobes and ADC in the posterior limb of the internal capsule were close to significance (Table 5.8). After splitting for treatment groups, differences between FA measures in the frontal lobe contra lateral were significant in the placebo group, borderline significant between occipital lobes contra lateral in the thyroxine group and significant between posterior limb of the internal capsule contra lateral in both the placebo and thyroxine group. Differences in ADC measures were significant between the occipital lobes contra lateral in the placebo group and borderline between posterior limb of the internal capsule contra lateral in the treatment group.

DTI Measure	Right Structures	Left Structures	Wilks' Lambda	Sig	Treatment Group	Wilks' Lambda	P
FA	FLR	FLL	.92	.08	Placebo	.74	.03
					Thyroxine	.99	.59
	OLR	OLL	.92	.07	Placebo	.98	.56
					Thyroxine	.84	.05
	RPLIC	LPLIC	.99	.56	Placebo	.75	.02
					Thyroxine	.78	.02
ADC	FLR	FLL	1.00	.97	Placebo	.98	.54
					Thyroxine	.92	.18
	OLR	OLL	.95	.17	Placebo	.66	.01
					Thyroxine	1	.87
	RPLIC	LPLIC	.93	.09	Placebo	.99	.69
					Thyroxine	.86	.07

Table 5.8 FA and ADC in right vs. left structures (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule).

Laterality of the structure did not interact with the groups mean FA or ADC with the exception of FA in the frontal lobes and posterior limb of the internal capsule (Table 5.9, Figure 5.11 and 5.12).

DTI Measure	Structures	Value	F	P
FA	FLR and FLL	.87	5.87	.02
	OLR and OLL	.97	1.07	.31
	RPLIC and LPLIC	.79	10.86	.00
ADC	FLR and FLL	.96	1.59	.22
	OLR and OLL	.93	2.79	.10
	RPLIC and LPLIC	.96	1.55	.22

Table 5.9 Interaction with group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule).

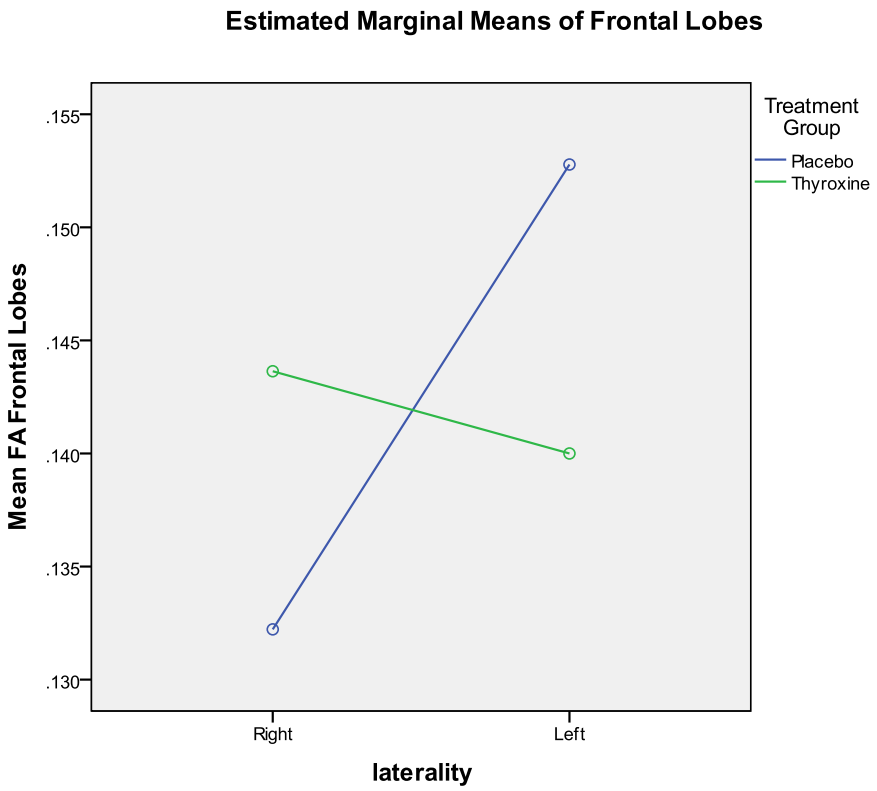

Figure 5.11 Interaction between laterality and frontal lobes.

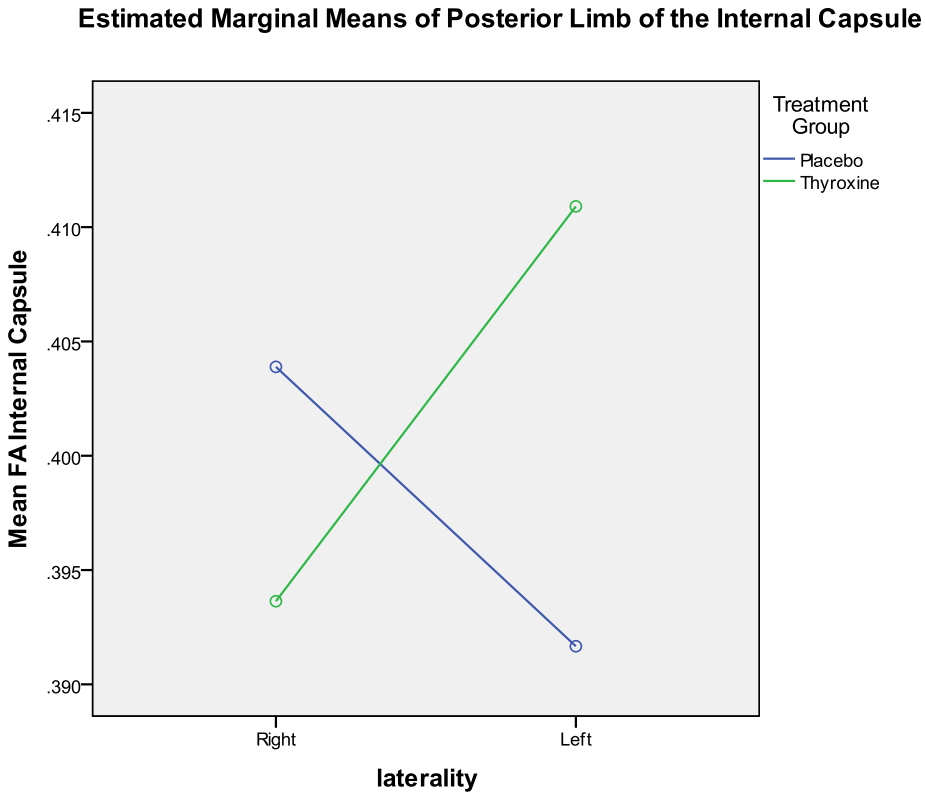

Figure 5.12 Interaction between laterality and posterior limb of the internal capsule.

Further analysis revealed only borderline significant differences between groups in the left posterior limb of the internal capsule with FA higher in the thyroxine group (Table 5.10).

Structure	Group	Means	Std Deviation	T	Sig
Right FL	Placebo	.14	.04	-.77	.45
	Thyroxine	.14	.03		
Left FL	Placebo	.15	.05	1.04	.30
	Thyroxine	.14	.03		
Right PLIC	Placebo	.40	.04	.43	.67
	Thyroxine	.40	.04		
Left PLIC	Placebo	.39	.04	.76	.06
	Thyroxine	.41	.04		

Table 5.10 Group Difference (FL= frontal lobe, PLIC= posterior limb of the internal capsule).

Thyroxine

Total thyroxine levels (TT4) and free thyroxine levels (FT4) are generally higher in the thyroxine group, by week 36 levels in the placebo group increase and are higher in the placebo group (Table 5.11, Figure 5.13 and Figure 5.14).

		Mean	Std Dev	Treatment	Mean	Std Dev
TT4	Screening	66.36	30.53	Placebo	62.71	28.34
				Thyroxine	70.01	33.15
	14 Days	111.90	54.39	Placebo	64.86	19.14
				Thyroxine	147.87	43.76
	21 Days	107.86	55.20	Placebo	72.79	44.08
				Thyroxine	140.41	43.92
	28 Days	107.14	62.55	Placebo	102.52	75.11
				Thyroxine	110.33	55.32
	36 weeks	106.16	33.51	Placebo	109.70	30.08
				Thyroxine	102.61	37.41
FT4	Screening	11.19	4.70	Placebo	9.77	3.84
				Thyroxine	12.41	5.12
	14 Days	19.11	9.37	Placebo	10.97	2.93
				Thyroxine	23.99	8.45
	21 Days	17.71	10.11	Placebo	10.89	4.18
				Thyroxine	23.33	10.17
	28 Days	14.14	6.15	Placebo	11.03	2.83
				Thyroxine	16.38	6.61
	36 weeks	15.21	3.51	Placebo	15.66	3.59
				Thyroxine	14.67	3.44
TT3	Screening	.95	.25	Placebo	.88	.22
				Thyroxine	1.02	.28
	14 Days	1.12	.17	Placebo	1.13	.19
				Thyroxine	1.11	.15
	21 Days	1.15	.32	Placebo	1.18	.35
				Thyroxine	1.13	.30
	28 Days	1.31	.37	Placebo	1.43	.38
				Thyroxine	1.2	.34
	36 weeks	2.0	.47	Placebo	2.08	.48
				Thyroxine	1.92	.47

Table 5.11 Mean thyroxine and triiodothyronine levels (TT4= total thyroxine, FT4= free thyroxine, TT3= total triiodothyronine).

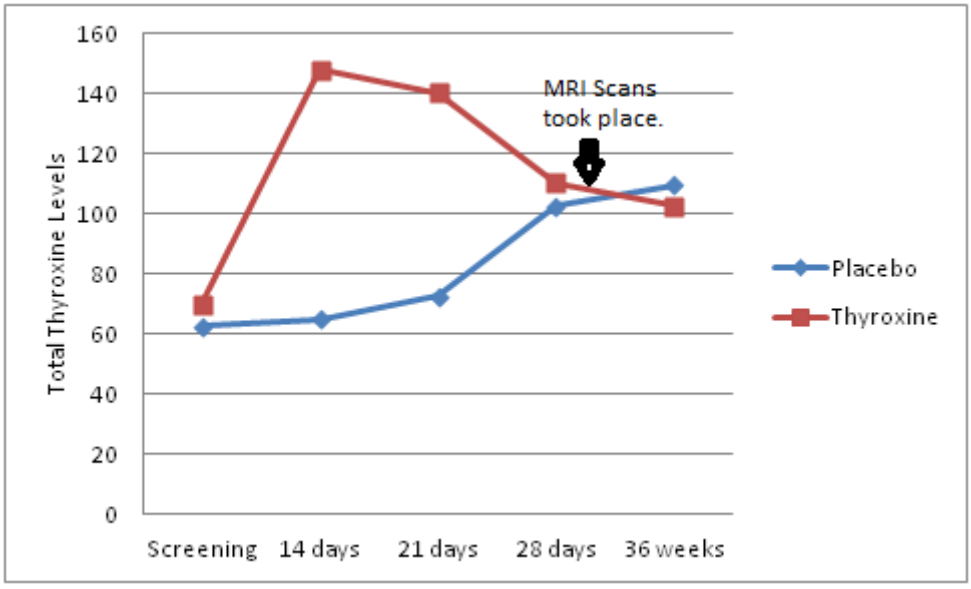

Figure 5.13 Mean total thyroxine levels for placebo and thyroxine group.

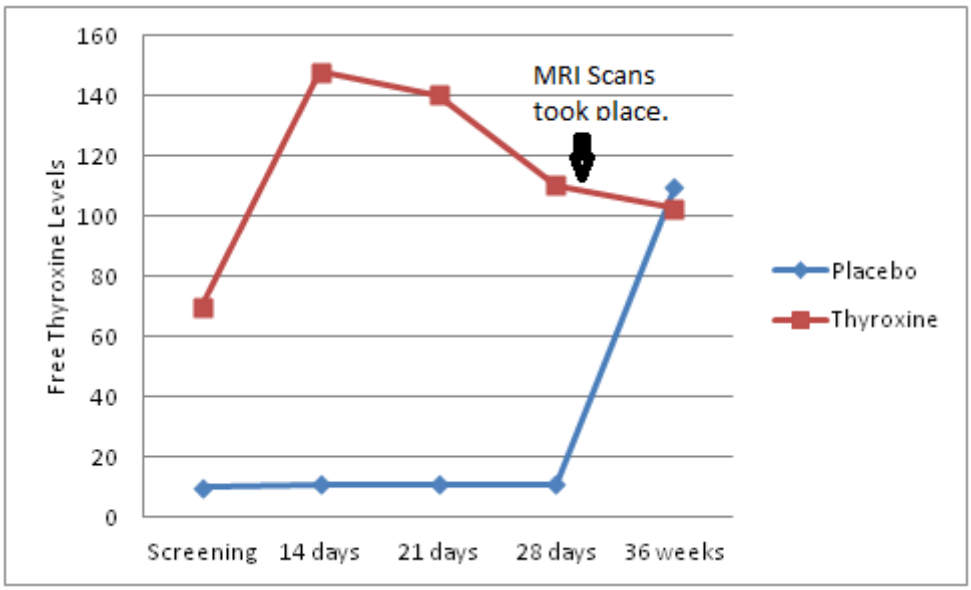

Figure 5.14 Mean free thyroxine levels for placebo and thyroxine group.

Total triiodothyronine levels (T) are generally lower in the thyroxine group (Table 5.11 and Figure 5.15).

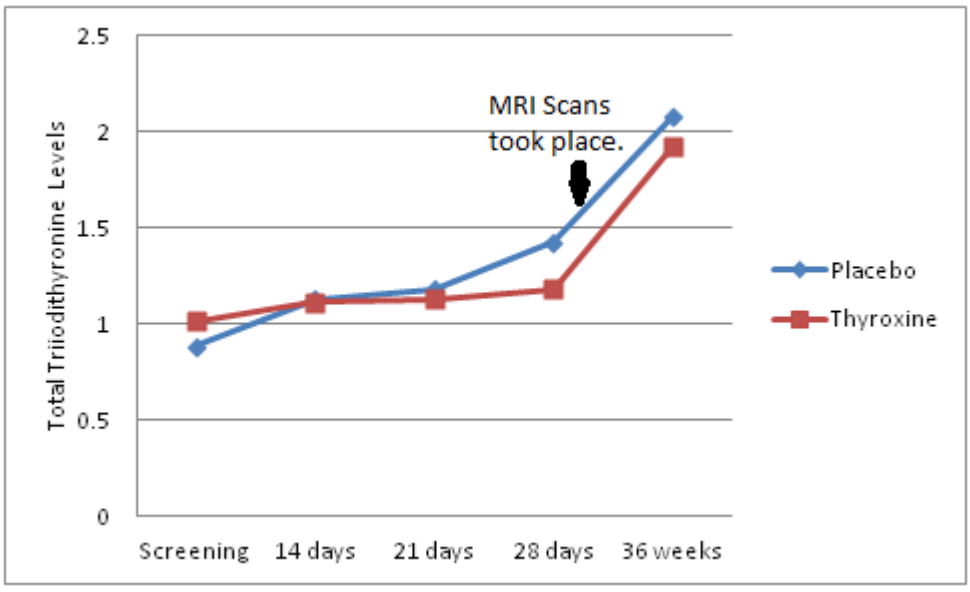

Figure 5.15 Mean triiodothyronine levels in the placebo and thyroxine group.

Differences between the placebo and thyroxine group were not significant at screening for TT4, FT4 and TT3. At day 14 and 21, TT4 and FT4 were significantly

higher in the infants treated with thyroxine. At day 28 FT4 was significantly higher in the thyroxine group but by week 36 there was no significant difference between groups for FT4, TT4 and TT3 levels (Figures 5.13-5.15 and Table 5.12).

		Mean Square	F	P
TT4	Screening	400.41	.42	.52
	Day 14	**50760.03**	**40.56**	**.00**
	Day 21	**30823.6**	**15.93**	**.00**
	Day 28	324.27	.08	.78
	Week 36	352.16	.31	.59
FT4	Screening	67.17	3.21	.08
	Day 14	**1272.05**	**26.29**	**.00**
	Day 21	**1188.82**	**18.31**	**.00**
	Day 28	**216.26**	**6.83**	**.01**
	Week 36	8.5	.69	.41
TT3	Screening	.053	.84	.38
	Day 14	.001	.03	.86
	Day 21	.015	.14	.72
	Day 28	1.83	1.41	.26
	Week 36	.13	.57	.46

Table 5.12 Difference between thyroxine and placebo group (TT4= total thyroxine, FT4= free thyroxine, TT3 = total triiodothyronine).

Infants in the thyroxine group split to high and low levels of TT4, FT4 and TT3 at screening, had no significant difference between the two groups at day 14, day 21, day 28 and week 36 (Table 5.13).

	Screening Group	N	Mean	Std Deviation	T	P
TT4 Screening	Low	9	46.3	13.2	-7.77	.00
	High	6	105.58	16.33		
TT4 Day 14	Low	5	154	61.76	.21	.84
	High	6	147.47	43.05		
TT4 Day 21	Low	4	128	21.21	-1.72	.13
	High	5	157	28.72		
TT4 Day 28	Low	3	82.7	57.75	-.59	.57
	High	6	97.57	20.9		
TT4 Week 36	Low	5	85.2	29.1	-.66	.53
	High	4	95.95	15.79		
FT4 Screening	Low	11	8.64	3.17	-5.65	.00
	High	10	16.55	3.25		
FT4 Day 14	Low	8	24.13	10.37	-.11	.91
	High	9	24.6	7.28		
FT4 Day 21	Low	6	19.93	6.96	-.62	.55
	High	8	23.61	13.11		
FT4 Day 28	Low	7	16.4	8.94	-.09	.93
	High	7	16.71	3.91		
FT4 Week 36	Low	6	13.85	2.46	-.50	.63
	High	7	14.91	4.69		
TT3 Screening	Low	3	.80	.17	-3.25	.03
	High	3	1.23	.15		
TT3 Day 14	Low	3	1.13	.15	-.49	.66
	High	2	1.20	.14		
TT3 Day 21	Low	3	1.03	.12	-1.19	.43
	High	2	1.40	.42		
TT3 Day 28	Low	2	1.15	.07	2.89	.21
	High	1	.90			
TT3 Week 36	Low	3	1.67	.25	-1.45	.24
	High	2	2.05	.35		

Table 5.13 Thyroxine group split for low and high levels at screening (TT4= total thyroxine, FT4= free thyroxine levels, TT3= total triiodothyronine).

Correlations

DTI measures in the preterm population did not correlate with birth weight, gestational age at birth or gestation at scanning, with the exception of ADC measures in the right occipital lobe where there was a significant negative relationship with gestational age at birth, FA in the right frontal lobe where there was a significant positive relationship with gestation at scanning and ADC in the left frontal lobe where there was a significant negative relationship with gestation at scanning (Table 5.14 and Figure 5.16, 5.17 and 5.18).

	Birth weight		Gestation at Birth		Gestation at Scan	
	Pearson Correlation	P	Pearson Correlation	P	Pearson Correlation	P
FLR FA	-.21	-.17	-.28	.06	.38	**.01**
FLL FA	-.11	-.47	-.19	.21	.23	.13
OLR FA	-.11	-.49	-.15	.33	.16	.29
OLL FA	-.09	.58	-.13	.41	.15	.35
RPLIC FA	.10	.51	.06	.73	.19	.23
LPLIC FA	-.10	.53	.11	.51	.11	.51
CCA FA	.08	.65	.21	.19	.08	.63
CCP FA	.04	.83	.05	.79	.08	.66
FLR ADC	.09	.57	.07	.66	-.27	.07
FLL ADC	.11	.49	.09	.56	-.39	**.01**
OLR ADC	-.11	.48	-.30	**.05**	-.11	.47
OLL ADC	.01	.94	-.13	.39	-.19	.22
RPLIC ADC	-.04	.82	-.24	.12	-.36	.02
LPLIC ADC	-.10	.52	-.27	.08	-.15	.36
CCA ADC	-.02	.92	-.13	.42	.03	.87
CCP ADC	.12	.49	-.03	.87	.33	.05

Table 5.14 Correlations between DTI measures, birth weight and gestational age (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum, Sig= significance).

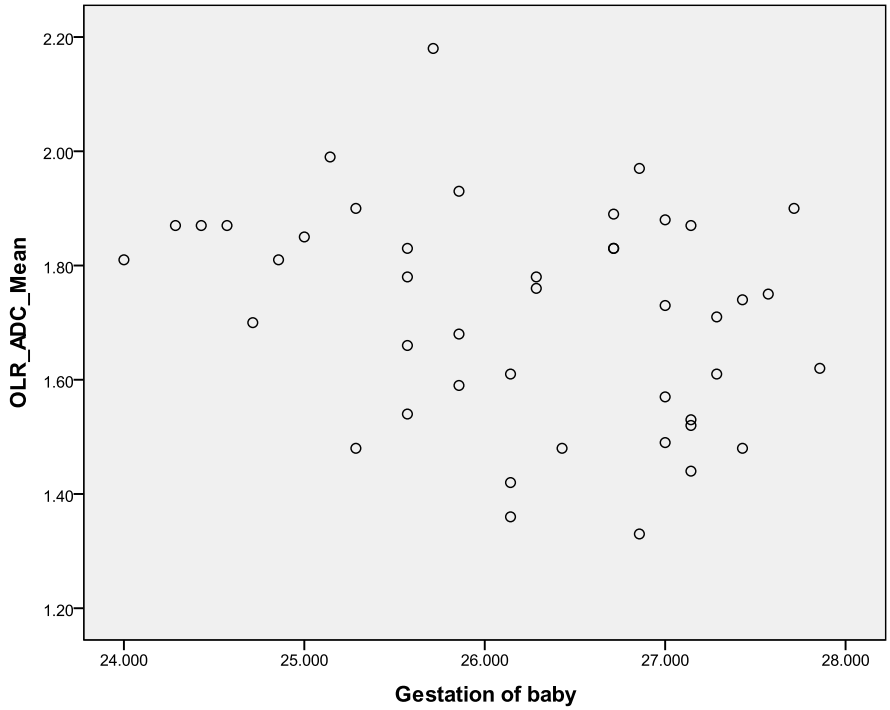

Figure 5.16 Correlation between ADC in the right occipital lobe and gestation at birth (OLR= right occipital lobe).

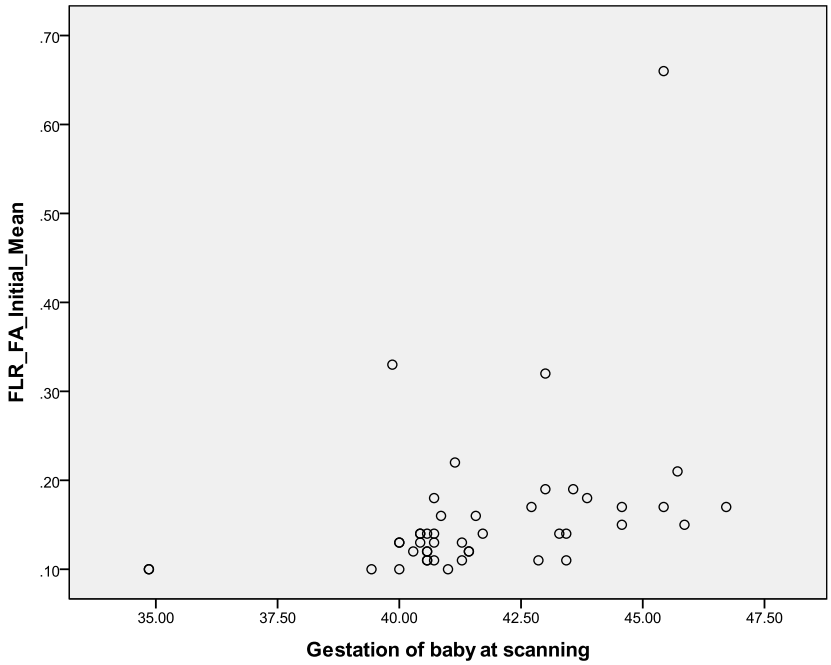

Figure 5.17 Correlation between FA in the right frontal lobe and gestation of baby at scanning (FLR= frontal lobe right).

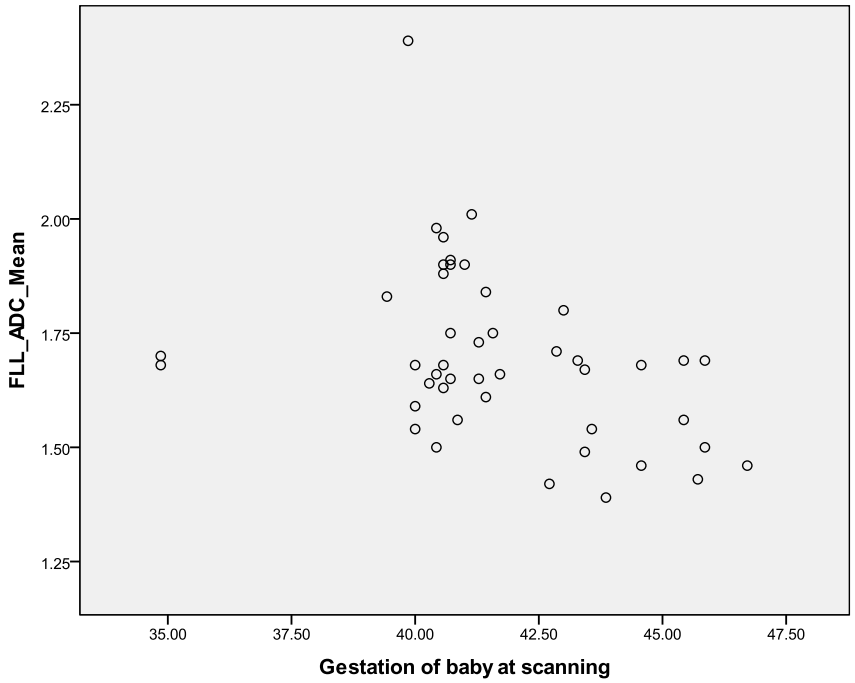

Figure 5.18 Correlation between ADC in the left frontal lobe and gestation of baby at scanning (FLL= frontal lobe left).

Total thyroxine levels (TT4) and free thyroxine levels (FT4) at screening positively correlated with gestational age at birth. TT4 at 36 weeks positively correlated with the infant's birth weight and gestation at birth (Table 5.15 and Figures 5.19- 5.22).

	Birth weight		Gestation	
	Pearson Correlation	P	Pearson Correlation	P
TT4 Screening	.18	.33	**.42**	**.02**
TT4 Day 14	-.14	.45	-.08	.69
TT4 Day 21	-.18	.38	-.16	.44
TT4 Day 28	-.10	.66	-.16	.47
TT4 Week 36	**.58**	**.00**	**.44**	**.02**
FT4 Screening	.20	.23	**.45**	**.00**
FT4 Day 14	-.04	.81	-.06	.73
FT4 Day 21	-.09	.64	-.16	.39
FT4 Day 28	-.22	.23	-.10	.58
FT4 Week 36	.23	.19	.25	.15
TT3 Screening	.19	.56	.17	.59
TT3 Day 14	.00	1	.06	.81
TT3 Day 21	.04	.85	-.06	.81
TT3 Day 28	.15	.62	.33	.26
TT3 Week 36	.37	.11	.15	.52

Table 5.15 Correlations between TT4, FT4, TT3 with weight and gestation at birth (TT4= total thyroxine, FT4= free thyroxine, TT3= total triiodothyronine).

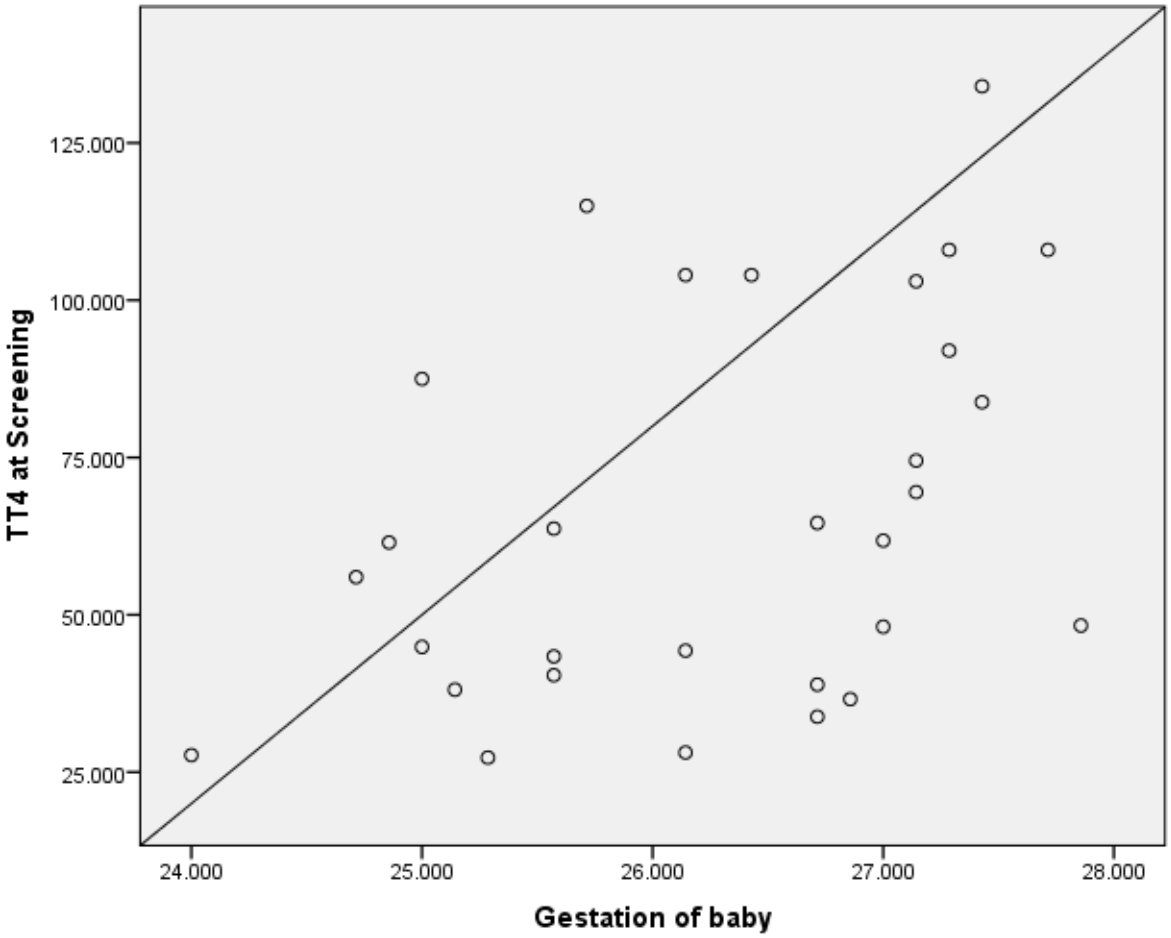

Figure 5.19 Positive Correlation between TT4 at screening and gestational age at birth. (TT4= total thyroxine).

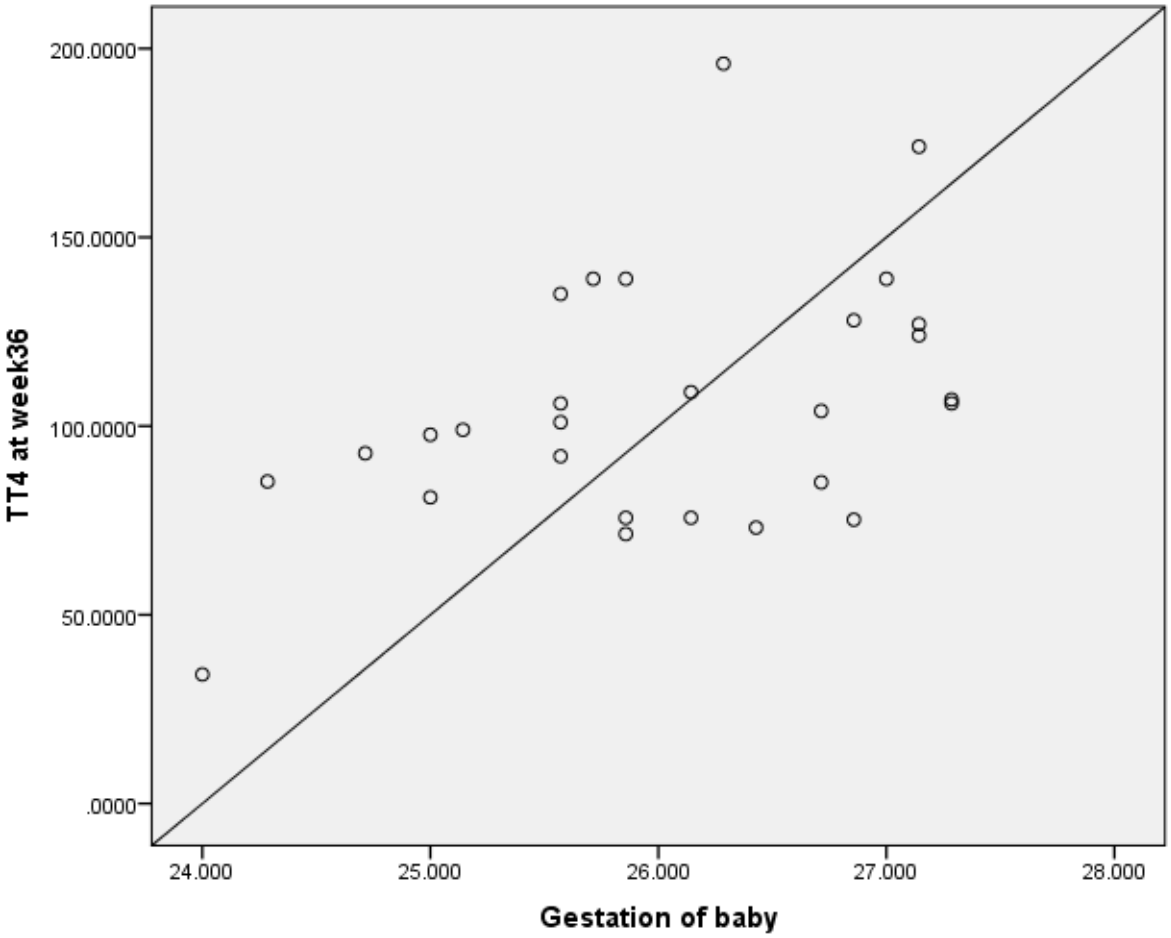

Figure 5.20 Correlation between TT4 levels at week 36 and gestation at birth (TT4= total thyroxine).

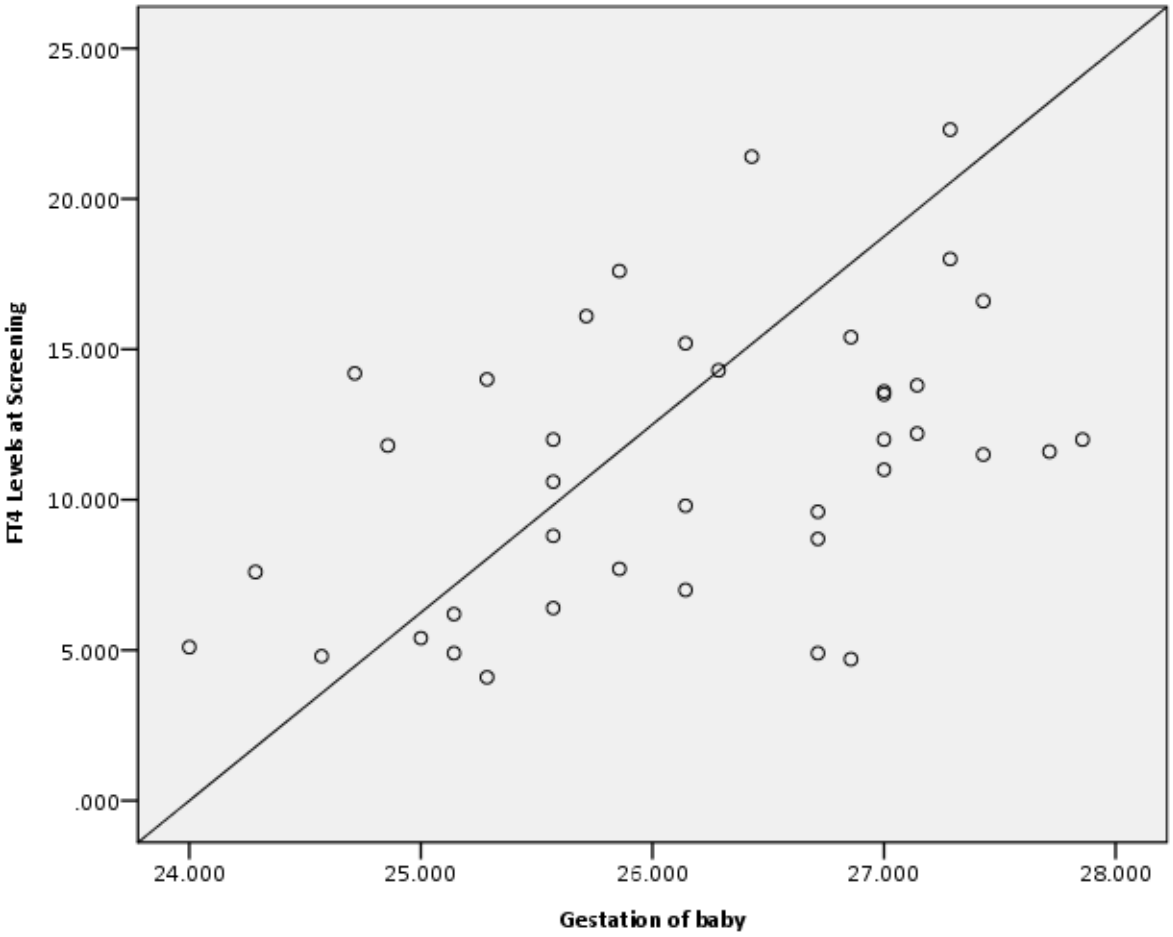

Figure 5.21 Correlation between FT4 levels at screening and gestation at birth (FT4= free thyroxine).

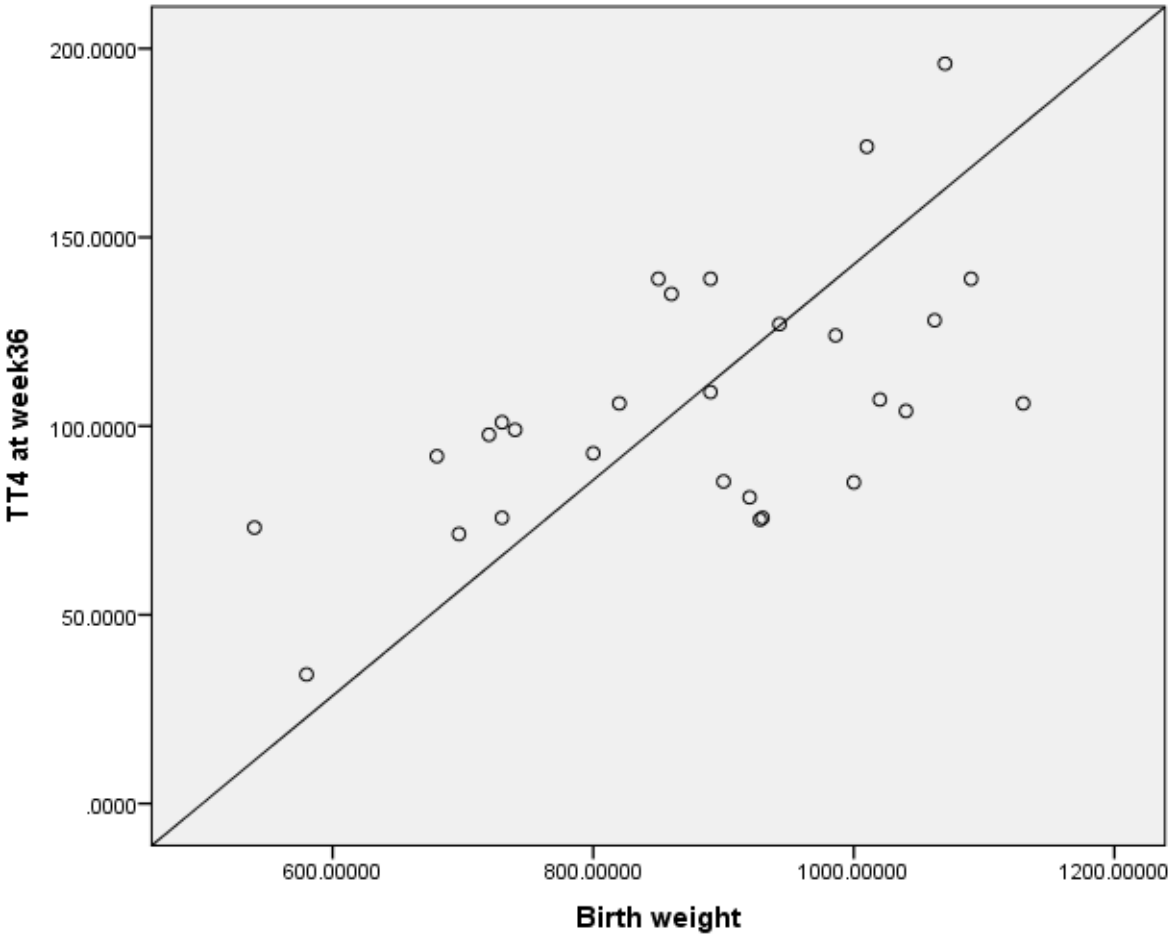

Figure 5.22 Correlation between TT4 at 36 weeks and birth weight (TT4= total thyroxine).

Gender Differences

In the placebo group males had lower FA and higher ADC in comparison to females (Figure 5.23 and 5.24).

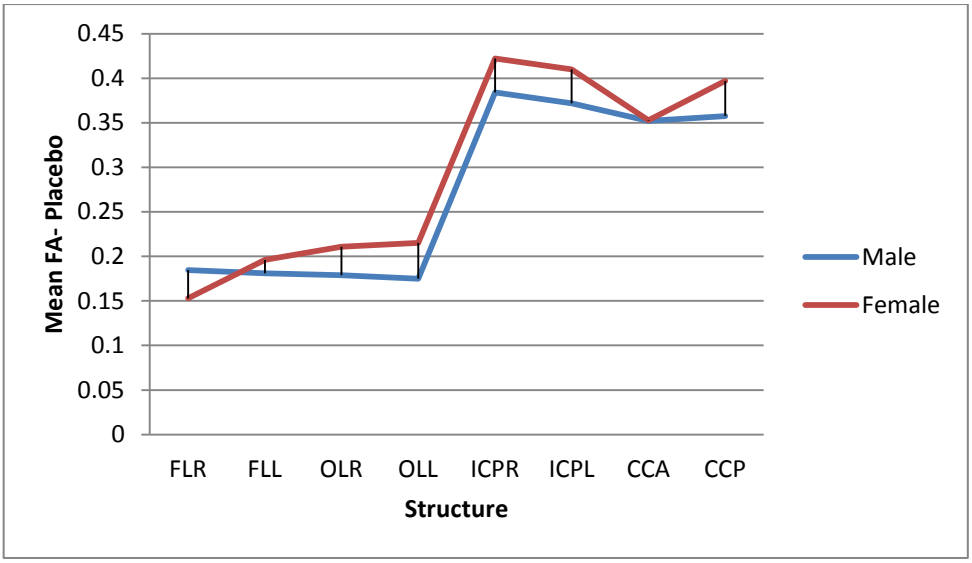

Figure 5.23 Gender differences in mean FA in the Placebo group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

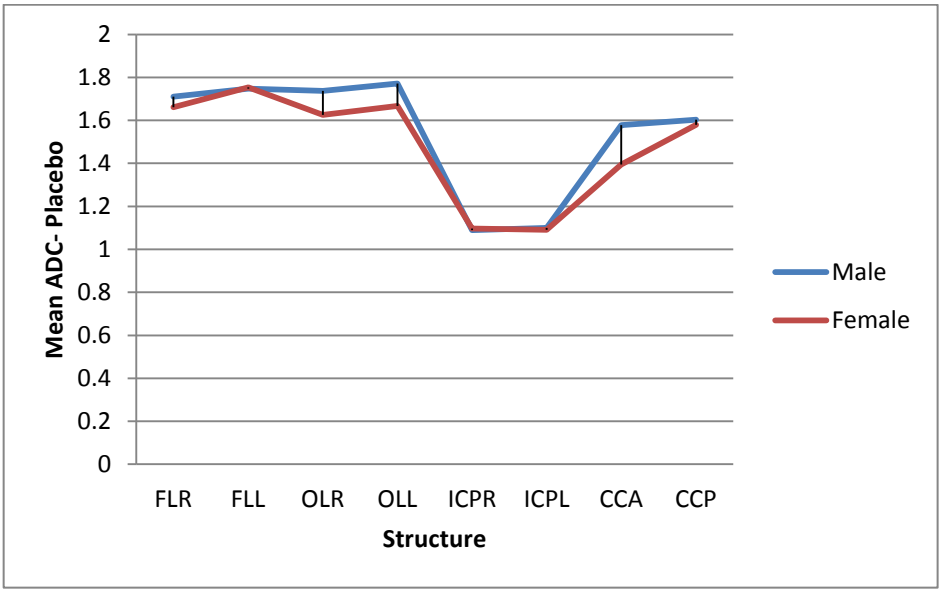

Figure 5.24 Gender differences in mean ADC in the Placebo group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

FA was significantly lower in males in the right and left internal capsule, ADC was significantly higher in males in the anterior corpus callosum (Table 5.16).

Treatment	Structure	Gender	Mean	Std Deviation	F	P
Placebo	FLR - FA	Male	.19	.16	.32	.58
		Female	.15	.07		
	FLL- FA	Male	.18	.10	.07	.79
		Female	.20	.14		
	OLR- FA	Male	.18	.10	.36	.56
		Female	.21	.14		
	OLL- FA	Male	.18	.11	.47	.50
		Female	.22	.15		
	ICPR- FA	**Male**	**.38**	**.04**	**5.25**	**.04**
		Female	**.42**	**.04**		
	ICPL - FA	**Male**	**.37**	**.03**	**6.07**	**.03**
		Female	**.41**	**.04**		
	CCA- FA	Male	.35	.07	.86	.37
		Female	.35	.05		
	CCP- FA	Male	.36	.08	1.32	.27
		Female	.40	.06		
	FLR - ADC	Male	1.71	.20	.28	.60
		Female	1.66	.23		
	FLL- ADC	Male	1.75	.17	.00	.95
		Female	1.75	.28		
	OLR- ADC	Male	1.74	.20	1.14	.30
		Female	1.63	.26		
	OLL- ADC	Male	1.77	.18	1.37	.26
		Female	1.67	.22		
	ICPR- ADC	Male	1.09	.04	1	.76
		Female	1.10	.06		
	ICPL - ADC	Male	1.10	.07	.09	.77
		Female	1.09	.07		
	CCA-ADC	**Male**	**1.58**	**.13**	**.94**	**.01**
		Female	**1.39**	**.11**		
	CCP- ADC	Male	1.60	.23	.06	.82
		Female	1.58	.14		
Thyroxine	FLR - FA	Male	.15	.06	.05	.83
		Female	.15	.02		
	FLL- FA	Male	.16	.10	.05	.83
		Female	.15	.04		
	OLR- FA	Male	.17	.07	.71	.41
		Female	.15	.04		
	OLL- FA	Male	.15	.08	.00	1
		Female	.15	.04		
	ICPR- FA	Male	.40	.02	.11	.75
		Female	.39	.05		
	ICPL - FA	Male	.42	.03	.19	.679
		Female	.41	.06		
	CCA- FA	Male	.34	.05	.01	.93
		Female	.34	.06		
	CCP- FA	Male	.36	.07	2.37	.14
		Female	.41	.08		
	FLR - ADC	Male	1.69	.15	.58	.46
		Female	1.64	.18		
	FLL- ADC	Male	1.67	.13	.31	.58
		Female	1.63	.18		
	OLR- ADC	Male	1.77	.11	1.21	.28

		Female	1.70	.18		
	OLL- ADC	Male	1.78	.11	2.84	.11
		Female	1.66	.22		
	ICPR- ADC	Male	1.11	.06	.19	.67
		Female	1.10	.06		
	ICPL - ADC	Male	1.12	.02	.39	.54
		Female	1.13	.06		
	CCA-ADC	Male	1.55	.17	1.84	.19
		Female	1.46	.16		
	CCP- ADC	Male	1.55	.20	.64	.43
		Female	1.48	.15		

Table 5.16 Gender differences (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

In the thyroxine group the structural means were similar between males and females with no significant differences (Table 5.16, figures 5.25 and 5.26).

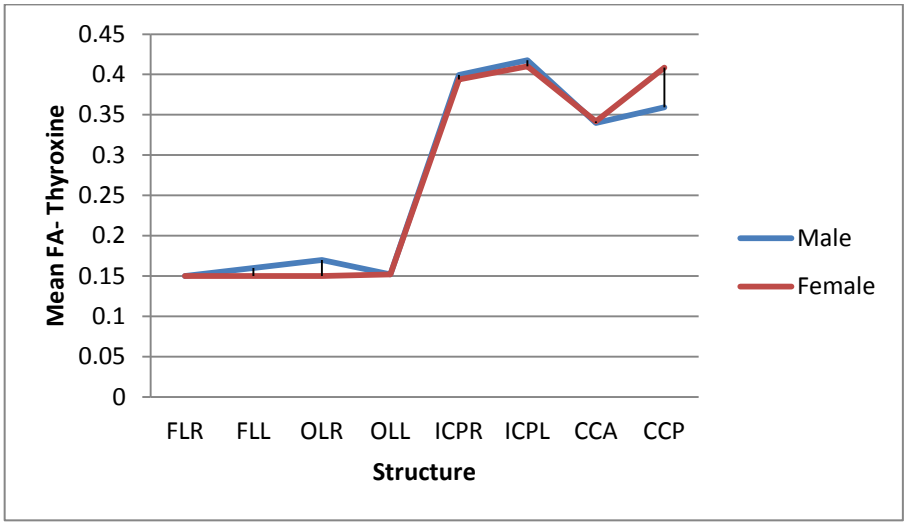

Figure 5.25 Gender differences in mean FA in the thyroxine group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

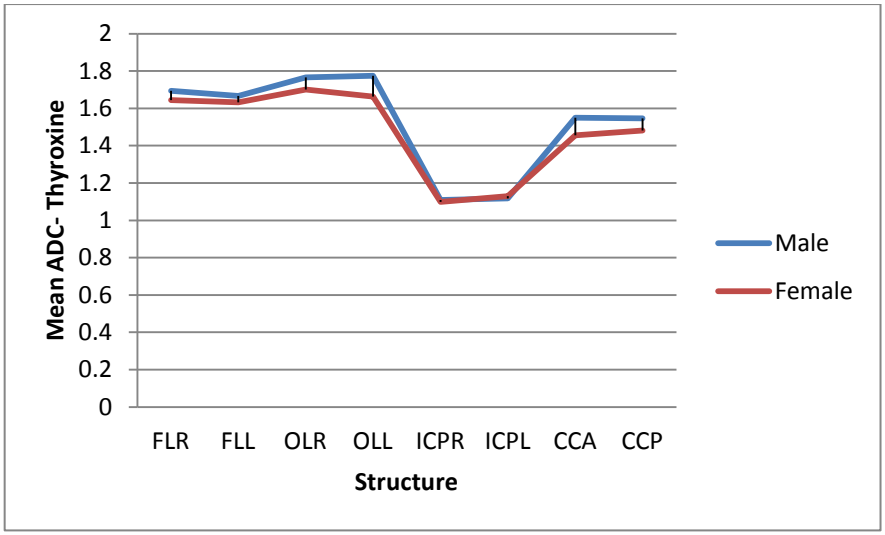

Figure 5.26 Gender differences in mean ADC in the thyroxine group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Smoking

There are considerably more smokers during and before pregnancy in the thyroxine group (Table 5.17).

Treatment Group	No	Smoked during pregnancy?		Smoking before pregnancy?	
Placebo	22	Yes	3	Yes	6
		No	19	No	16
Thyroxine	29	Yes	5	Yes	11
		No	24	No	17

Table 5.17 Frequencies of smokers in the treatment groups.

Differences in DTI measures between the groups demonstrated borderline significantly higher FA in the treatment group in the left posterior limb of the internal capsule and borderline significantly higher ADC in the placebo group in the left frontal lobe (Table 5.5). After exclusion of the smokers there were no significant differences in DTI measures between groups (Table 5.18).

	Structures	Treatment	Means	F	P
FA	FLL	Placebo	.19	2.47	.62
		Thyroxine	.17		
	LPLIC	Placebo	.39	2.67	.12
		Thyroxine	.42		
ADC	FLL	Placebo	1.73	1.56	.22
		Thyroxine	1.63		
	LPLIC	Placebo	1.11	.53	.48
		Thyroxine	1.12		
	CCA	Placebo	1.50	.37	.55
		Thyroxine	1.54		
	CCP	Placebo	1.63	2.47	.13
		Thyroxine	1.53		

Table 5.18 Table of DTI Measures between groups excluding smokers (FLL= left frontal lobe, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Alcohol

A separate analysis was performed investigating the effects of alcohol. Data was divided into two groups, those who drank alcohol before or during pregnancy and those who didn't drink alcohol. The alcohol free group were matched by treatment group and gender (Table 5.19).

Group	No	Gender	Gestational Age (GA)	Birth weight (BW)	Treatment Group	No	GA	BW
Alcohol	13	7 Female 6 Male	26.52	915.15	Placebo	6	26.52	895.33
					Thyroxine	7	26.51	932.14
No Alcohol	13	7 Female 6 Male	25.74	829.92	Placebo	6	26.05	874.83
					Thyroxine	7	25.47	791.43

Table 5.19 Participant Information for Alcohol Sub-analysis.

FA and ADC were generally similar between alcohol groups particularly in the placebo group (Figures 5.27 and 5.28).

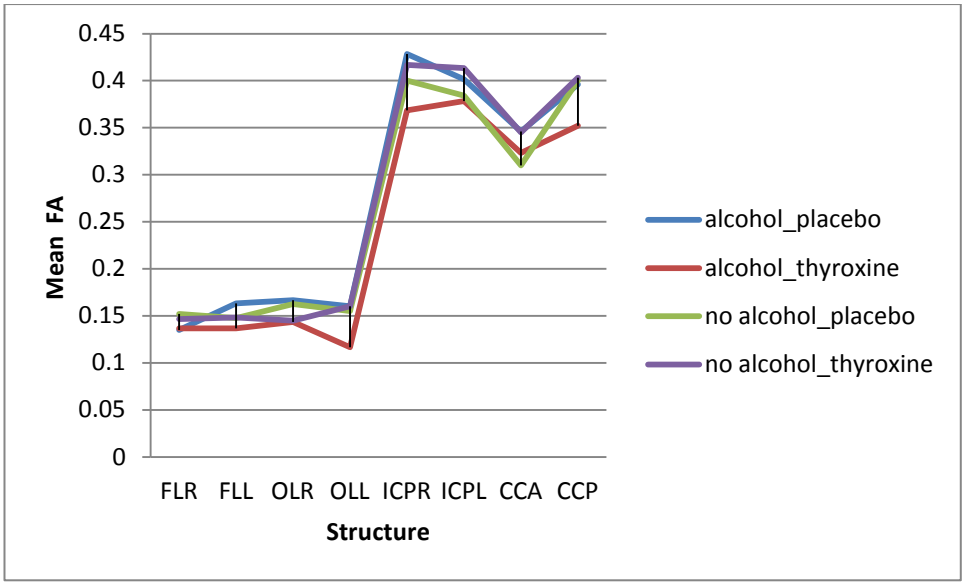

Figure 5.27 Effect of alcohol on mean FA (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

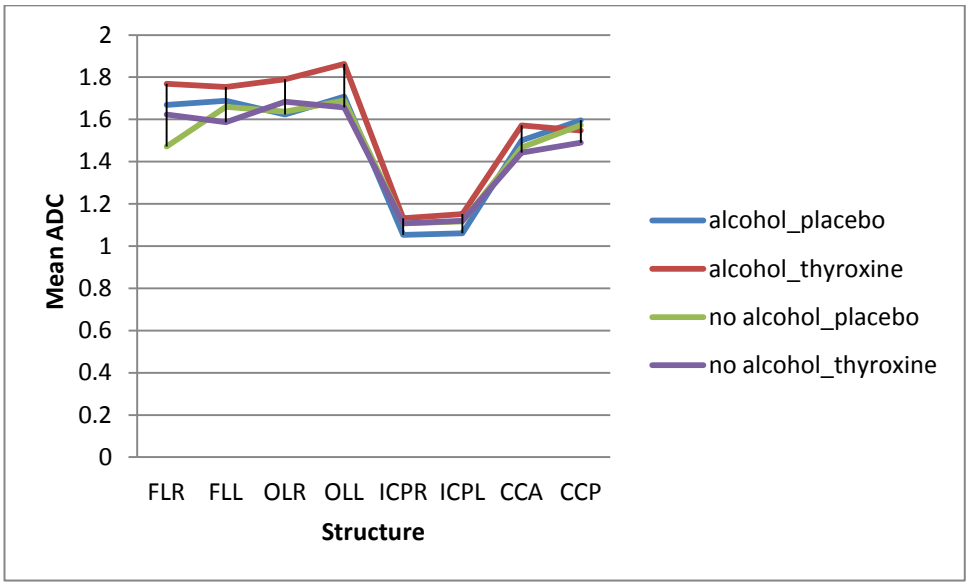

Figure 5.28 Effect of alcohol on mean ADC (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

In the placebo group FA and ADC were higher in the alcohol group in the majority of the structures measured; none of these differences were significant. In the

thryoxine group differences were more pronounced with lower FA and higher ADC in the alcohol group for all structures. FA was significantly lower in the left occipital lobe and right internal capsule, ADC was significantly higher in the left occipital lobe and borderline significant in the left frontal lobe (Table 5.20).

Treatment	Structure	Group	Mean	Std Deviation	F	P
Placebo	FLR - FA	Alcohol	.14	.03	.62	.45
		No Alcohol	.15	.04		
	FLL- FA	Alcohol	.16	.05	.34	.57
		No Alcohol	.15	.02		
	OLR- FA	Alcohol	.17	.05	.02	.88
		No Alcohol	.16	.02		
	OLL- FA	Alcohol	.16	.05	.03	.87
		No Alcohol	.16	.03		
	RPLIC- FA	Alcohol	.43	.04	1.40	.27
		No Alcohol	.40	.04		
	LPLIC - FA	Alcohol	.40	.04	.49	.50
		No Alcohol	.38	.05		
	CCA- FA	Alcohol	.35	.05	.68	.44
		No Alcohol	.31	.08		
	CCP- FA	Alcohol	.40	.06	.01	.92
		No Alcohol	.40	.05		
	FLR - ADC	Alcohol	1.67	.16	2.64	.14
		No Alcohol	1.47	.24		
	FLL- ADC	Alcohol	1.69	.21	.04	.84
		No Alcohol	1.66	.20		
	OLR- ADC	Alcohol	1.62	.24	.01	.92
		No Alcohol	1.64	.17		
	OLL- ADC	Alcohol	1.71	.25	.02	.90
		No Alcohol	1.69	.16		
	RPLIC- ADC	Alcohol	1.05	.06	3.33	.10
		No Alcohol	1.11	.04		
	LPLIC - ADC	Alcohol	1.06	.07	2.20	.17
		No Alcohol	1.12	.06		
	CCA-ADC	Alcohol	1.50	.09	.14	.72
		No Alcohol	1.47	.17		
	CCP- ADC	Alcohol	1.60	.31	.01	.91
		No Alcohol	1.57	.12		
Thyroxine	FLR - FA	Alcohol	.14	.02	.74	.41
		No Alcohol	.15	.02		
	FLL- FA	Alcohol	.14	.02	.60	.46
		No Alcohol	.15	.03		
	OLR- FA	Alcohol	.14	.02	.02	.89
		No Alcohol	.15	.02		
	OLL- FA	**Alcohol**	**.12**	**.02**	**8.37**	**.02**
		No Alcohol	**.16**	**.03**		
	RPLIC- FA	**Alcohol**	**.37**	**.04**	**7.29**	**.02**
		No Alcohol	**.42**	**.02**		

	LPLIC - FA	Alcohol	.38	.04	2.78	.13
		No Alcohol	.41	.03		
	CCA- FA	Alcohol	.32	.042	.91	.36
		No Alcohol	.35	.04		
	CCP- FA	Alcohol	.35	.07	1.31	.28
		No Alcohol	.40	.08		
	FLR - ADC	Alcohol	1.77	.18	2.26	.16
		No Alcohol	1.62	.16		
	FLL- ADC	**Alcohol**	**1.75**	**.19**	**3.81**	**.08**
		No Alcohol	**1.59**	**.09**		
	OLR- ADC	Alcohol	1.79	.10	2	.19
		No Alcohol	1.68	.16		
	OLL- ADC	**Alcohol**	**1.86**	**.15**	**5.91**	**.04**
		No Alcohol	**1.66**	**.15**		
	RPLIC- ADC	Alcohol	1.13	.05	.31	.59
		No Alcohol	1.11	.09		
	LPLIC - ADC	Alcohol	1.15	.06	1.22	.30
		No Alcohol	1.12	.04		
	CCA-ADC	Alcohol	1.57	.20	1.85	.20
		No Alcohol	1.44	.12		
	CCP- ADC	Alcohol	1.55	.12	.53	.49
		No Alcohol	1.49	.14		

Table 5.20 Differences in alcohol groups (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

The IUGR Cohort

Descriptive statistics are shown in Table 5.21 and 5.22 for the IUGR cohort.

Structure		Min	Max	Mean	Std. Deviation
FLR	IUGR	.12	.20	.15	.03
	Control	.09	.23	.15	.04
FLL	IUGR	.12	.20	.15	.03
	Control	.10	.33	.16	.07
OLR	IUGR	.10	.18	.13	.03
	Control	.10	.21	.15	.03
OLL	IUGR	.12	.20	.15	.03
	Control	.08	.20	.14	.04
ICPR	IUGR	.22	.43	.35	.07
	Control	.34	.44	.40	.04
ICPL	IUGR	.21	.44	.37	.08
	Control	.32	.44	.38	.04
CCA	IUGR	.25	.50	.37	.10
	Control	.33	.53	.44	.07
CCP	IUGR	.21	.50	.40	.10
	Control	.40	.54	.46	.05

Table 5.21 FA table of descriptive statistics (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Structure		Min	Max	Mean	Std. Deviation
FLR	IUGR	1.32	1.63	1.51	.16
	Control	1.45	1.96	1.67	.15
FLL	IUGR	1.24	1.66	1.49	.15
	Control	1.44	1.76	1.63	.11
OLR	IUGR	1.23	1.85	1.59	.20
	Control	1.49	1.80	1.69	.11
OLL	IUGR	1.24	1.78	1.56	.20
	Control	1.33	1.92	1.71	.17
ICPR	IUGR	1.00	1.16	1.09	.06
	Control	1.01	1.28	1.09	.09
ICPL	IUGR	1.01	1.18	1.09	.07
	Control	.98	1.15	1.08	.06
CCA	IUGR	1.31	1.67	1.49	.12
	Control	1.35	1.71	1.50	.12
CCP	IUGR	1.29	1.54	1.43	.10
	Control	1.16	1.68	1.43	.15

Table 5.22 ADC table of descriptive statistics (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Comparison of Structures: FA and ADC

FA is higher in the internal capsule and corpus callosum in comparison to the occipital and frontal lobes in both groups (Figure 5.29).

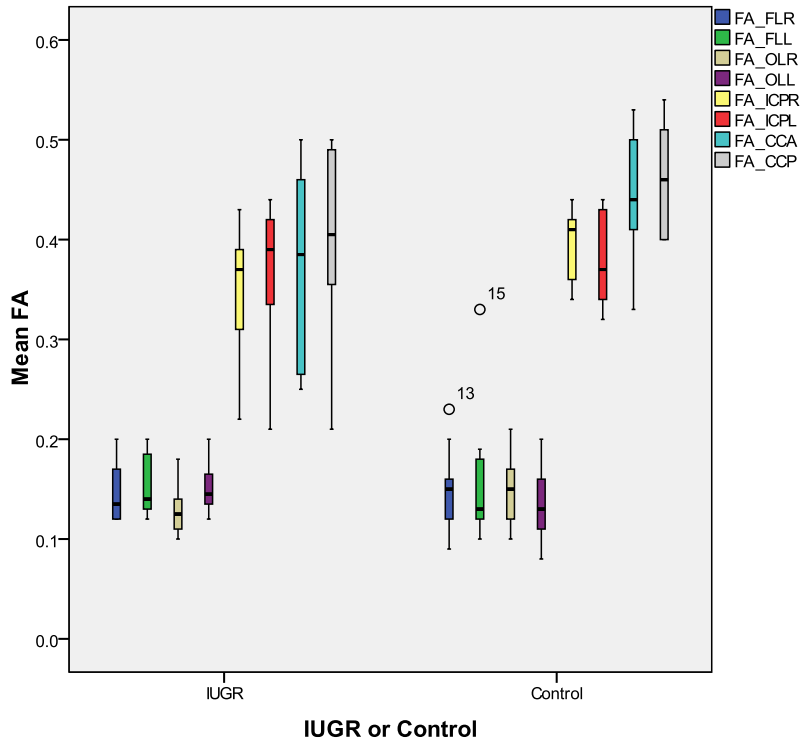

Figure 5.29 Box plot demonstrating FA means (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

FA was lower in the left occipital but higher in the frontal lobe and right occipital lobe in the control group compared to the IUGR group (Table 5.21, figure 5.29). Higher ranges of scores were seen in the IUGR group for measures of FA in the internal capsule and corpus callosum. FA in the internal capsule was lower in the IUGR group compared to the control group. In the anterior corpus callosum FA was lower in the IUGR group. In the posterior corpus callosum FA was higher in the IUGR group. There were no significant differences in FA values between groups (Table 5.23).

ADC is higher in the frontal and occipital lobes compared to the internal capsule and corpus callosum (Figure 5.30).

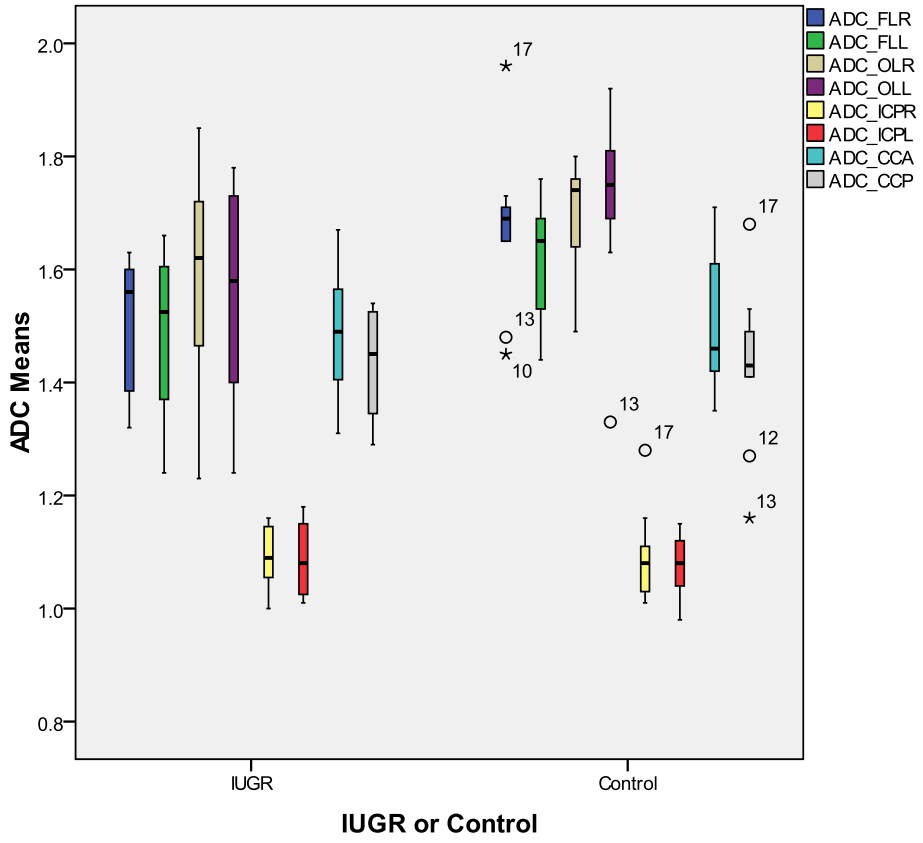

Figure 5.30 Box plot demonstrating ADC means (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

ADC values in the right and left internal capsule were very similar between groups. In the posterior corpus callosum values were slightly lower in the control group whereas in the anterior corpus callosum values were slightly higher. Left and right frontal and occipital lobes had higher ADC values in the control group. ADC values were significantly lower in the IUGR group for the frontal lobes left and right and borderline significantly lower in the left occipital lobe (Table 5.23).

Structure	Group	Mean	Std Deviation	F	P
FLR - FA	IUGR	.15	.03	.07	.80
	Control	.15	.04		
FLL- FA	IUGR	.15	.03	.05	.82
	Control	.16	.07		
OLR- FA	IUGR	.13	.03	1.90	.19
	Control	.15	.03		
OLL- FA	IUGR	.15	.03	.94	.35
	Control	.14	.04		
RPLIC- FA	IUGR	.35	.07	2.76	.12
	Control	.40	.04		
LPLIC - FA	IUGR	.37	.08	.21	.65
	Control	.38	.04		
CCA- FA	IUGR	.37	.10	2.69	.12
	Control	.44	.07		
CCP- FA	IUGR	.40	.10	2.31	.15
	Control	.46	.05		
FLR - ADC	**IUGR**	**1.51**	**.16**	**6.23**	**.03**
	Control	**1.67**	**.15**		
FLL- ADC	**IUGR**	**1.49**	**.15**	**4.69**	**.05**
	Control	**1.63**	**.11**		
OLR- ADC	IUGR	1.59	.20	1.93	.19
	Control	1.69	.11		
OLL- ADC	**IUGR**	**1.56**	**.20**	**3.15**	**.10**
	Control	**1.71**	**.17**		
RPLIC- ADC	IUGR	1.09	.06	.00	.98
	Control	1.09	.09		
LPLIC - ADC	IUGR	1.09	.07	.13	.73
	Control	1.08	.06		
CCA-ADC	IUGR	1.49	.12	.08	.79
	Control	1.50	.12		
CCP- ADC	IUGR	1.43	.10	.01	.91
	Control	1.43	.15		

Table 5.23 A table of significant difference in ADC and FA means (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

Laterality

FA is slightly lower in the right compared to left structures in both the IUGR and control group, differences between laterality were not significant (Table 5.24 and Figure 5.31).

	Right Structures	Left Structures	Wilks' Lambda	P
FA	FLR	FLL	.974	.539
	OLR	OLL	.962	.452
	RPLIC	LPLIC	.997	.849
ADC	FLR	FLL	.926	.290
	OLR	OLL	.997	.831
	RPLIC	LPLIC	.925	.286

Table 5.24 FA and ADC in right vs. left structures (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule).

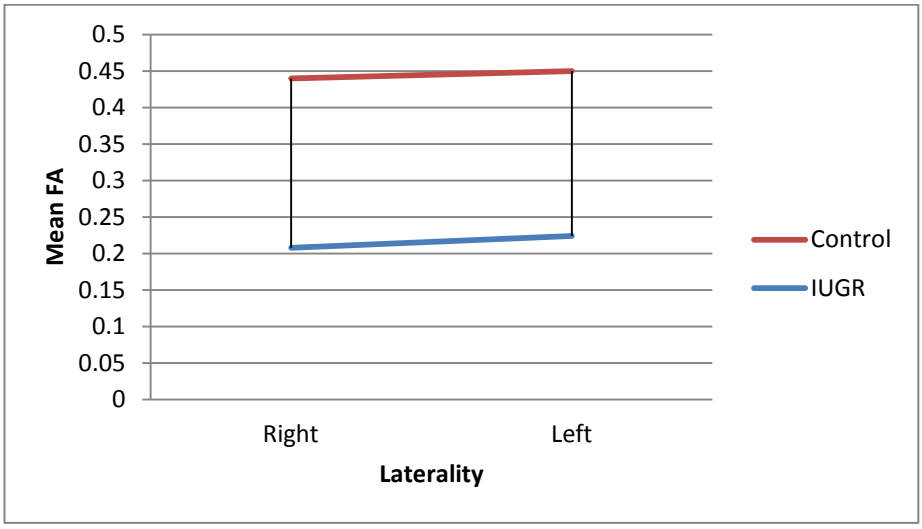

Figure 5.31 Mean FA for the three right and left structures.

There was no significant difference in mean ADC between the laterality of the structures (Table 5.24 and Figure 5.32).

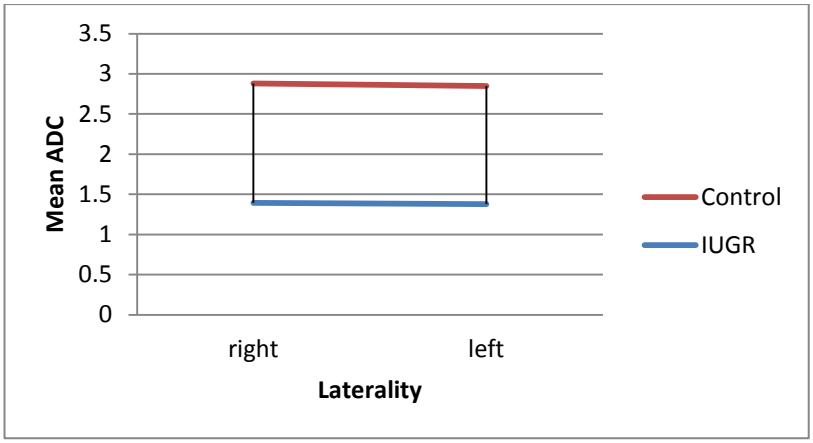

Figure 5.32 Mean ADC for the three right and left structures.

FA and ADC measures were entered into repeated measures ANOVA with region and laterality as within subject factors and treatment group as between subject factor. As there was a 2 way interaction with laterality and group (Table 5.25) additional analysis was performed.

DTI Measure	Interaction		Mean Square	F	P
FA	Region *Group	Linear	.003	.77	.39
		Quadratic	.001	.62	.44
	Laterality*Group	Linear	.003	4.24	.06
	Laterality*Region	Linear	.000	.13	.72
		Quadratic	1.968	.00	.96
	Region*Laterality*Group	Linear	.001	1.06	.32
		Quadratic	.001	.68	.42
ADC	Region *Group	Linear	.106	7.42	.01
		Quadratic	.019	.81	.38
	Laterality*Group	Linear	.000	.02	.90
	Laterality*Region	Linear	.002	.79	.39
		Quadratic	.001	.38	.55
	Region*Laterality*Group	Linear	.000	.12	.73
		Quadratic	.007	1.93	.19

Table 5.25 Repeated measures anova for region and laterality.

Laterality of the structure did not interact with the groups mean FA or ADC with the exception of FA in the occipital lobes (Table 5.26, Figure 5.33).

	Structures	Value	F	P
FA	FLR and FLL	1.00	.00	.96
	OLR and OLL	.62	9.11	.01
	RPLIC and LPLIC	.87	2.24	.16
ADC	FLR and FLL	.98	.24	.63
	OLR and OLL	.92	1.26	.28
	RPLIC andLPLIC	.98	.35	.56

Table 5.26 Interaction with Group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule).

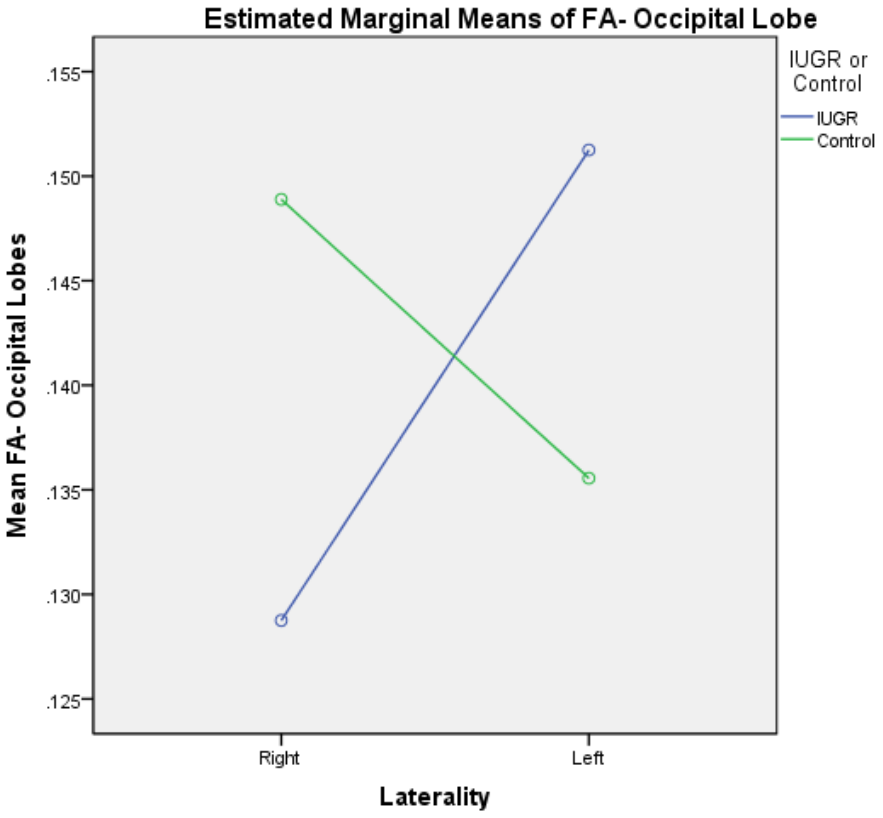

Figure 5.33 Interaction between laterality and group.

Further analysis revealed no significant difference between groups (Table 5.27).

Structure	Group	Mean	Std Deviation	t	P
FA OLR	IUGR	.13	.03	-1.38	.19
	Control	.15	.03		
FA OLL	IUGR	.15	.03	.97	.35
	Control	.14	.04		

Table 5.27 Group Difference (OLR= right occipital lobe, OLL= left occipital lobe).

Correlations

In the IUGR group Pearson's correlations with birth weight and gestational age were mostly positive for FA and negative for ADC, these correlations were not significant. In the control group Pearson's correlations were mostly negative for FA and positive for ADC (Table 5.28).

In the control group there was a borderline significant correlation between FA in the right occipital lobe and birth weight (Figure 5.34).

Similarly there was also a borderline significant correlation in the control group between FA in the right occipital lobe and gestational age (Figure 5.35).

All other correlations in the control group were not significant (Table 5.28).

Gender

In the IUGR group males generally had higher FA than females with the exception of FA in the left internal capsule. ADC was lower in males in the frontal lobes and posterior corpus callosum but higher for all the other structures (occipital lobes, posterior limb of the internal capsule and anterior corpus callosum). There were no significant differences in FA and ADC measures between gender groups, although FA in the left frontal lobe and ADC in the anterior corpus callosum was borderline significantly higher in males. In the control group males generally had lower FA than females with the exception of the left frontal lobe and posterior corpus callosum. Males generally had higher ADC in the control group with the exception of the left frontal lobe. There were no significant differences in FA and ADC measures between gender groups in the control group (Table 5.29, Figures 5.36-5.39).

		Birth weight		Gestation at Birth	
		Pearson Correlation	P	Pearson Correlation	P
FLR FA	IUGR	.28	.51	.01	.82
	Control	-.46	.21	-.45	.22
FLL FA	IUGR	.41	.31	.29	.48
	Control	-.02	.96	.03	.95
OLR FA	IUGR	.27	.52	.12	.79
	Control	**-.65**	**.06**	**-.64**	**.06**
OLL FA	IUGR	.38	.36	.06	.88
	Control	-.48	.19	-.50	.18
RPLIC FA	IUGR	.32	.44	.32	.44
	Control	-.45	.22	-.40	.29
LPLIC FA	IUGR	.46	.25	.61	.11
	Control	-.25	.52	-.07	.85
CCA FA	IUGR	-.06	.88	-.14	.75
	Control	.04	.93	-.10	.79
CCP FA	IUGR	.06	.89	-.06	.90
	Control	-.04	.92	.16	.68
FLR ADC	IUGR	-.28	.51	-.06	.88
	Control	.03	.95	-.02	.96
FLL ADC	IUGR	-.25	.56	-.01	.99
	Control	.35	.36	.21	.60
OLR ADC	IUGR	.03	.94	.10	.81
	Control	.45	.22	.36	.35
OLL ADC	IUGR	-.19	.65	-.02	.96
	Control	.57	.11	.49	.18
RPLIC ADC	IUGR	-.29	.49	-.31	.46
	Control	.04	.93	-.09	.82
LPLIC ADC	IUGR	-.31	.46	-.39	.34
	Control	.34	.37	.20	.61
CCA ADC	IUGR	.03	.95	-.09	.83
	Control	.12	.77	.13	.74
CCP ADC	IUGR	-.30	.47	-.43	.28
	Control	-.01	.98	-.00	.99

Table 5.28 Correlation between birth weight and DTI measures (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLIC= right posterior limb of the

internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

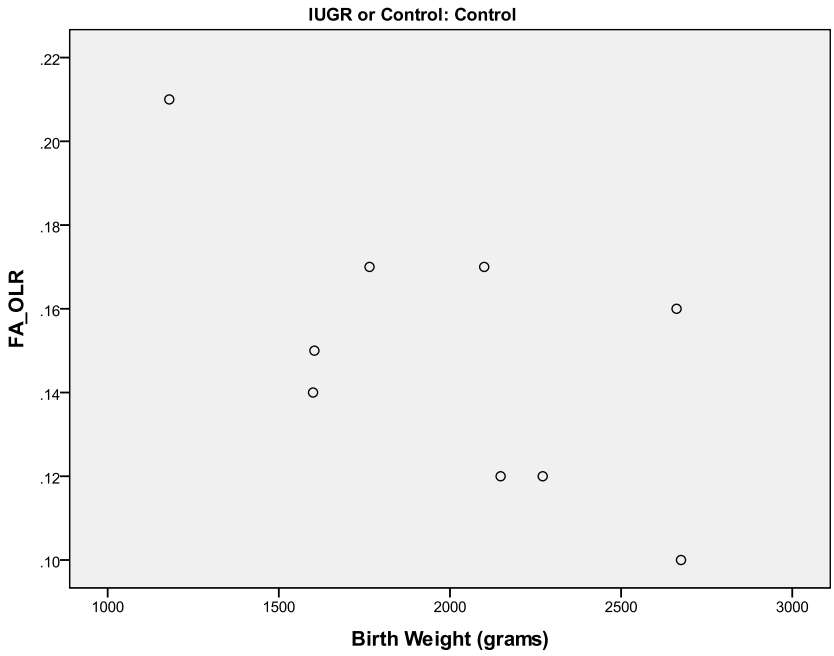

Figure 5.34 Correlation between FA in the OLR and birth weight in the control group (OLR= right occipital lobe).

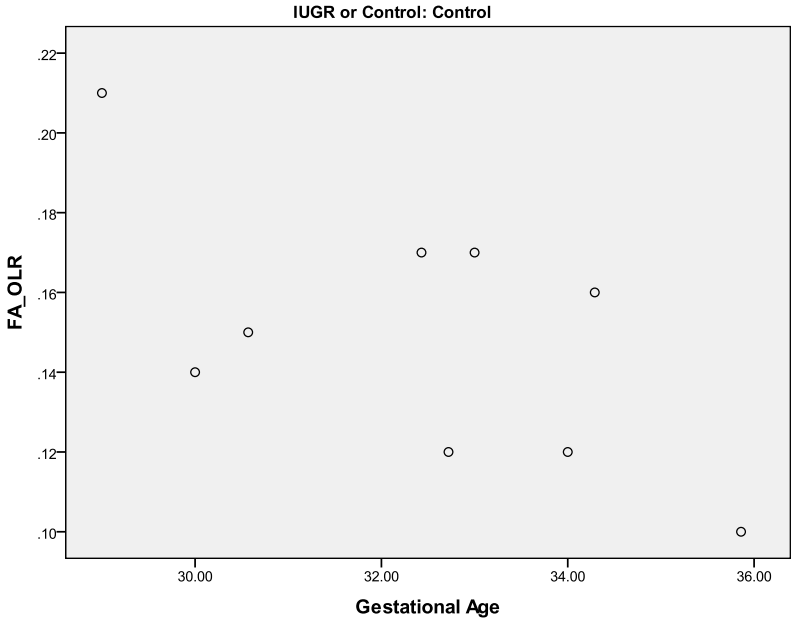

Figure 5.35 Correlation between FA in the OLR and gestation at birth in the control group (OLR= right occipital lobe).

Group	Structure	Gender	Mean	Std Deviation	F	P
IUGR	FLR - FA	Male	.16	.04	1.20	.32
		Female	.13	.02		
	FLL- FA	Male	.17	.03	3.98	.09
		Female	.13	.01		
	OLR- FA	Male	.13	.03	.03	.88
		Female	.13	.03		
	OLL- FA	Male	.16	.03	.39	.55
		Female	.14	.01		
	RLIC- FA	Male	.35	.08	.02	.89
		Female	.34	.08		
	LPLIC- FA	Male	.35	.10	.53	.49
		Female	.39	.01		
	CCA- FA	Male	.39	.09	.34	.58
		Female	.34	.14		
	CCP- FA	Male	.44	.07	2.90	.14
		Female	.33	.11		
	FLR - ADC	Male	1.49	.16	.09	.78
		Female	1.52	.08		
	FLL- ADC	Male	1.46	.18	.40	.55
		Female	1.53	.10		
	OLR- ADC	Male	1.61	.23	.15	.72
		Female	1.55	.15		
	OLL- ADC	Male	1.56	.24	.01	.94
		Female	1.55	.16		
	RPLIC- ADC	Male	1.10	.07	.32	.59
		Female	1.08	.038		
	LPLIC- ADC	Male	1.11	.07	2.11	.20
		Female	1.05	.04		
	CCA-ADC	**Male**	**1.54**	**.10**	**4.99**	**.07**
		Female	**1.39**	**.08**		
	CCP- ADC	Male	1.43	.11	.08	.79
		Female	1.45	.10		
Control	FLR - FA	Male	.14	.04	2.57	.15
		Female	.19	.06		
	FLL- FA	Male	.16	.08	.01	.92
		Female	.16	.04		
	OLR- FA	Male	.14	.03	1.72	.23
		Female	.18	.05		
	OLL- FA	Male	.13	.03	.63	.45
		Female	.16	.06		
	RPLIC- FA	Male	.39	.04	2.38	.17
		Female	.43	.01		
	LPLIC - FA	Male	.37	.04	.72	.42
		Female	.41	.05		
	CCA- FA	Male	.42	.07	2.39	.17
		Female	.50	.00		
	CCP- FA	Male	.46	.057	.29	.61
		Female	.44	.06		
	FLR - ADC	Male	1.70	.15	1.42	.27
		Female	1.57	.12		
	FLL- ADC	Male	1.62	.11	.01	.91
		Female	1.64	.18		
	OLR- ADC	Male	1.71	.11	.63	.45

		Female	1.64	.15		
	OLL- ADC	Male	1.75	.09	1.80	.22
		Female	1.58	.35		
	RPLIC- ADC	Male	1.11	.09	.81	.40
		Female	1.05	.05		
	LPLIC - ADC	Male	1.09	.06	.97	.36
		Female	1.04	.06		
	CCA-ADC	Male	1.52	.13	.97	.36
		Female	1.43	.04		
	CCP- ADC	Male	1.45	.12	.76	.41
		Female	1.35	.26		

Table 5.29 Gender differences (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, RPLPIC= right posterior limb of the internal capsule, LPLIC= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

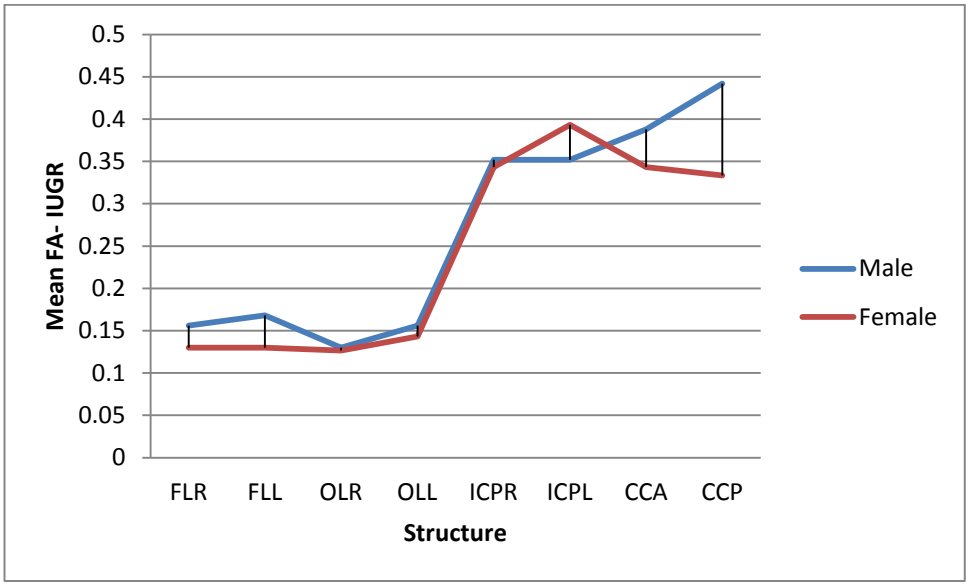

Figure 5.36 Line graph showing mean FA in both genders for the IUGR group (FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

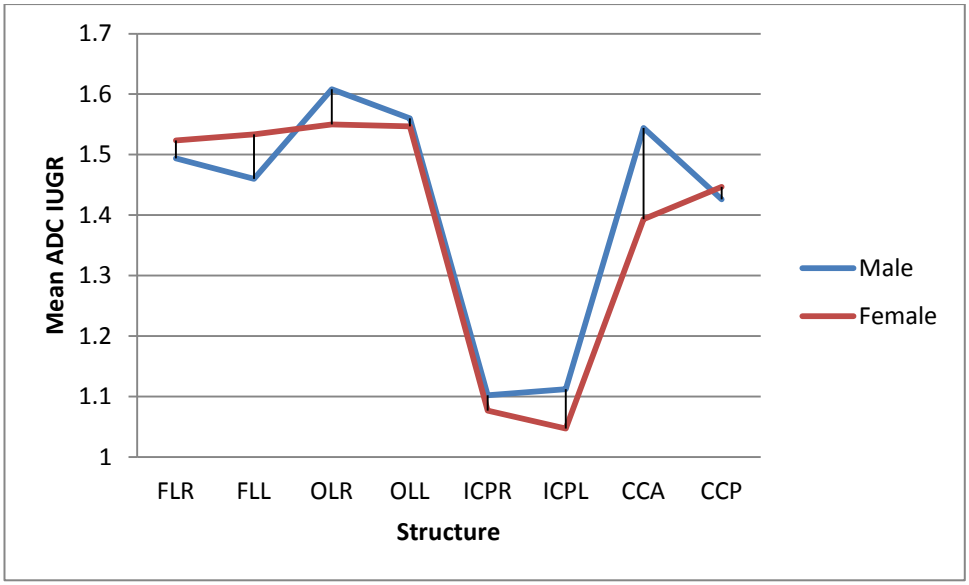

Figure 5.37 Line graph showing mean ADC in both genders for the IUGR group (IUGR= intrauterine growth restriction FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

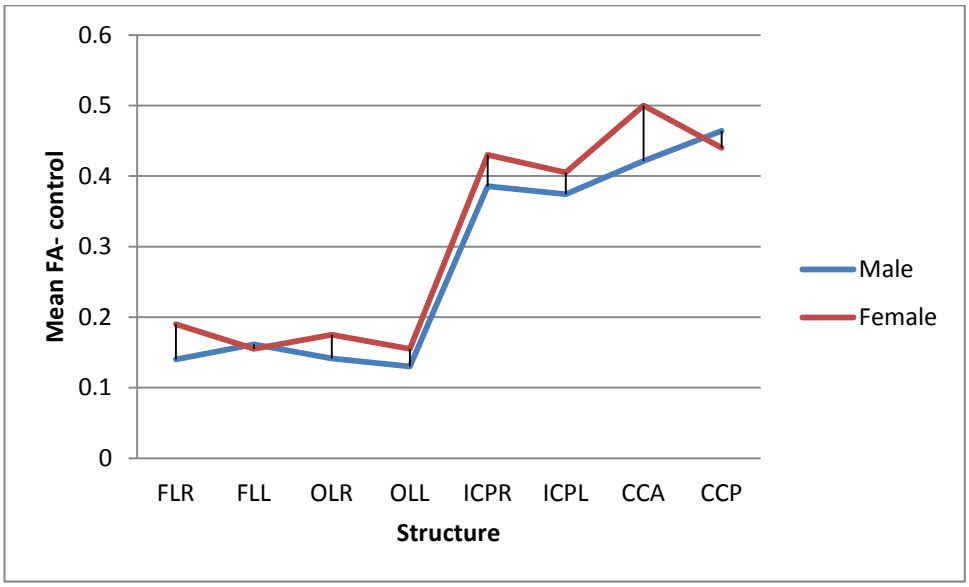

Figure 5.38 A line graph showing FA in both genders for the control group (IUGR= intrauterine growth restriction, FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

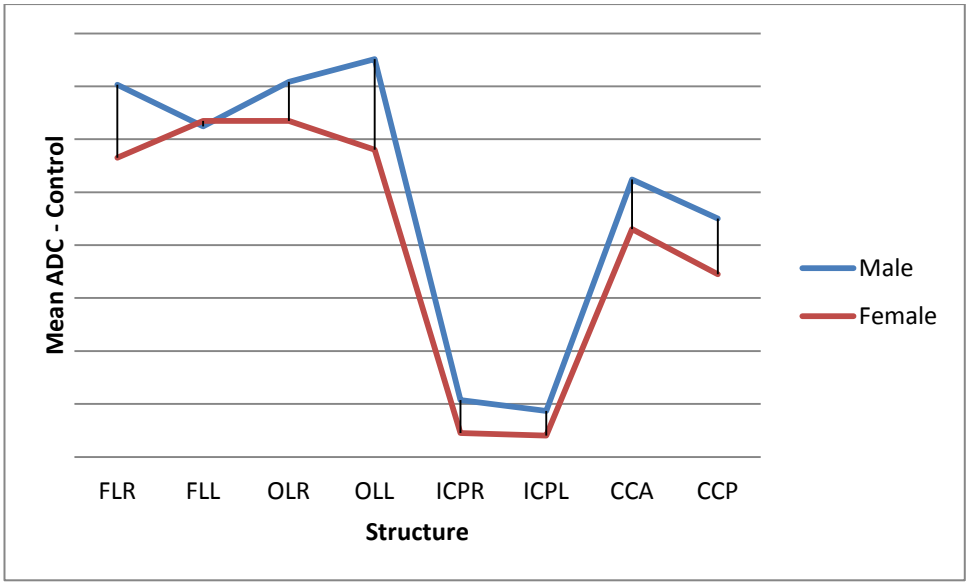

Figure 5.39 A line graph showing ADC in both genders for the control group (IUGR= intrauterine growth restriction, FLR= right frontal lobe, FLL= left frontal lobe, OLR= occipital lobe right, OLL= left occipital lobe, ICPR= right posterior limb of the internal capsule, ICPL= left posterior limb of the internal capsule, CCA= anterior corpus callosum, CCP= posterior corpus callosum).

CHAPTER 6

Stereology Results

Aim of Chapter

This chapter presents the results of the stereology study for:

a) The preterm cohort
b) The IUGR cohort

The Preterm Cohort

Descriptive statistics are shown in Table 6.1 for the preterm cohort. Structural volumes were lower in the placebo group for the dorsal lateral prefrontal cortex, left dorsal medial prefrontal cortex, orbital lateral prefrontal cortex, right and left hemisphere volumes. Structural volumes were higher in the placebo group for the right dorsal medial prefrontal cortex, right and left orbital medial prefrontal cortex (Table 6.1, Figures 6.1 and 6.2).

Structure		Min	Max	Mean	Std.Deviation
RDLPFC	Placebo	87.61	2496.87	1034.28	666.73
	Thyroxine	131.41	11433.05	2106.14	2198.69
LDLPFC	Placebo	131.41	2584.48	1158.39	680.82
	Thyroxine	350.44	18003.77	2561.71	3466.05
RDMPFC	Placebo	10294.13	22603.28	15042.08	3009.57
	Thyroxine	4380.48	23523.18	14823.54	4246.58
LDMPFC	Placebo	10732.18	19449.33	16334.32	2714.85
	Thyroxine	4030.40	22471.86	16449.59	4691.08
ROLPFC	Placebo	.00	525.66	183.87	193.10
	Thyroxine	.00	4088.45	485.16	888.46
LOLPFC	Placebo	.00	506.19	160.08	166.08
	Thyroxine	.00	6619.39	541.23	1328.55
ROMPFC	Placebo	282.45	8760.96	4726.06	1961.29
	Thyroxine	2258.38	6930.89	4293.26	1434.08
LOMPFC	Placebo	2589.35	8254.77	5172.21	1807.14
	Thyroxine	1557.50	8468.93	5092.26	1773.4
RHEMISPHERE	Placebo	58041.36	104766.50	85865.52	13529.71
	Thyroxine	58771.44	121558.30	91946.28	15624.51
LHEMISPHERE	Placebo	60961.68	113527.40	89353.68	14855.22
	Thyroxine	8839.68	120463.20	90547.67	22534.43

Table 6.1 Structural volumes table of descriptive statistics (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

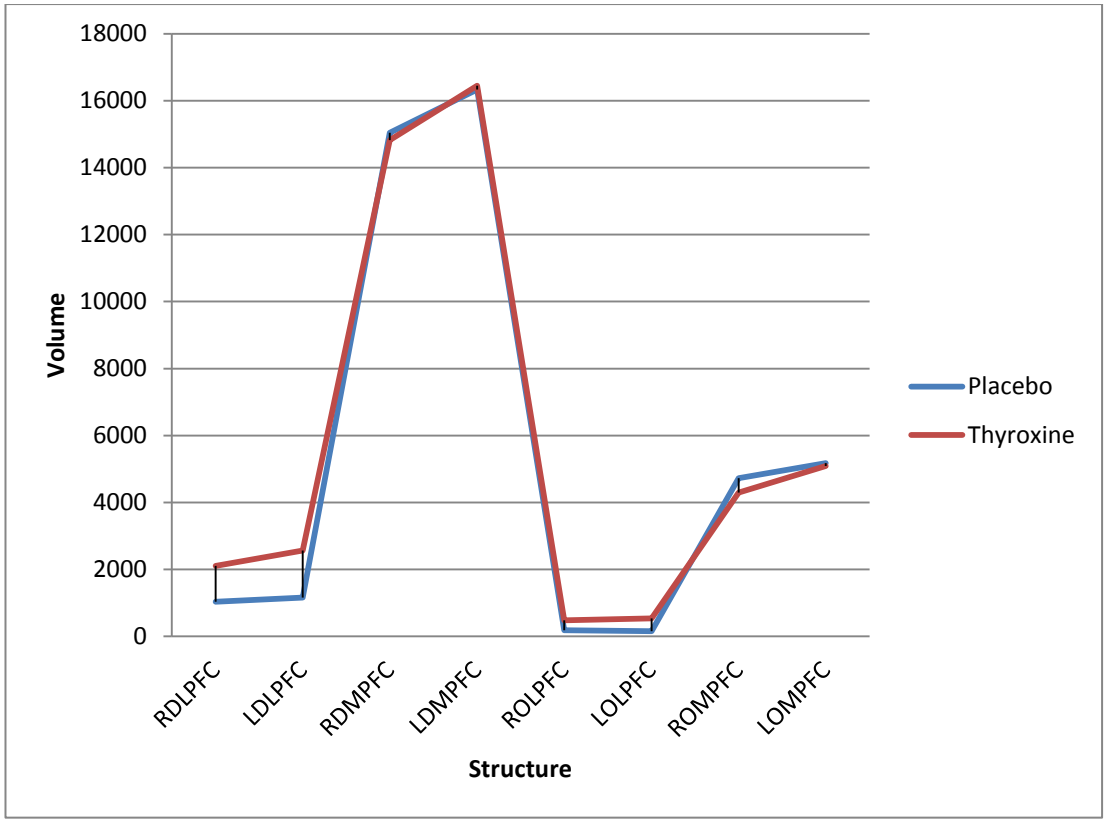

Figure 6.1 A line graph to show mean volume in structures between treatment groups (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

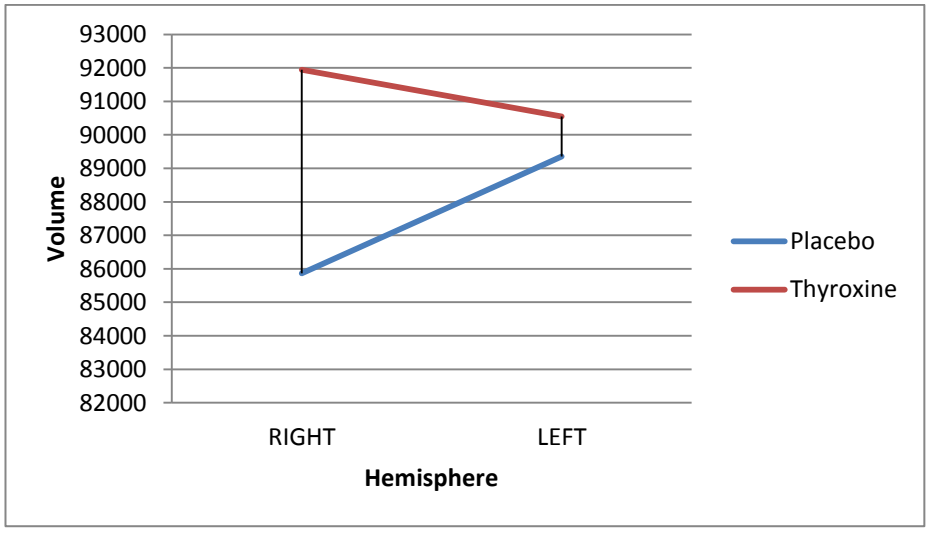

Figure 6.2 Hemisphere volumes between groups.

Laterality

Structural volumes in the right dorsal lateral prefrontal cortex were significantly lower in the placebo group. All other differences between treatment groups for the other structures were non-significant (Table 6.2).

Structure	F	Significance
RDLPFC	**3.99**	**.05**
LDLPFC	2.85	.10
RDMPFC	.04	.85
LDMPFC	.01	.93
ROLPFC	1.99	.17
LOLPFC	1.46	.24
ROMPFC	.70	.41
LOMPFC	.02	.89
Right Hemisphere	1.77	.19
Left Hemisphere	.04	.85

Table 6.2 One-Way ANOVA results of difference between treatment groups (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

Structural volumes were generally lower in the right (Table 6.1 and Figures 6.3 and 6.4).

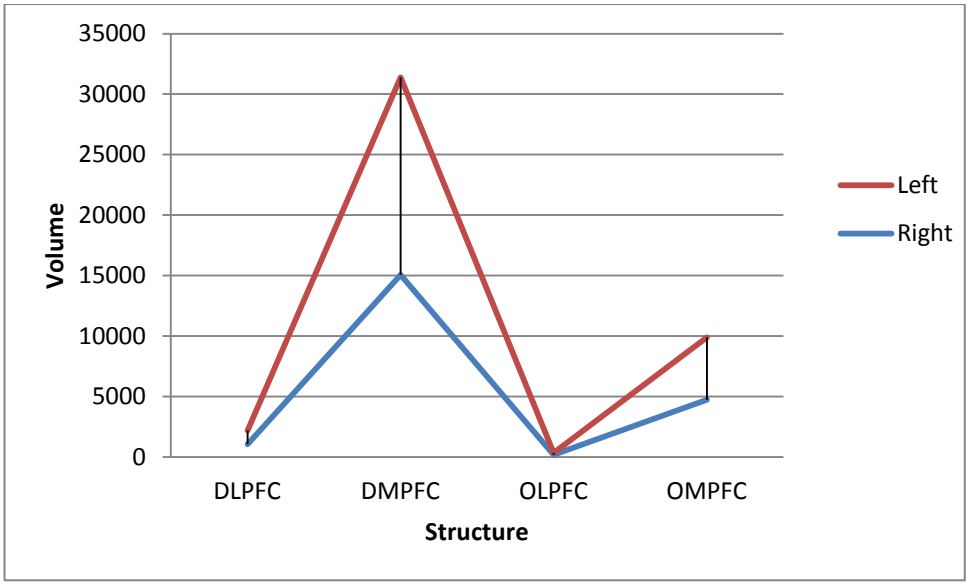

Figure 6.3 Volumes of structures in the placebo group between left and right (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, OMPFC= orbital medial prefrontal cortex).

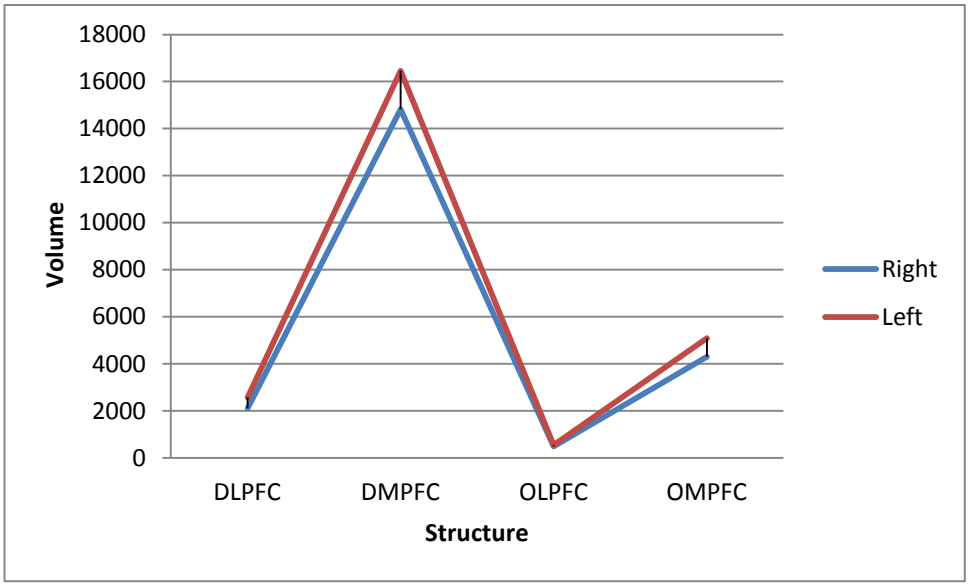

Figure 6.4 Volumes of structures in the thyroxine group between left and right (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex OMPFC= orbital medial prefrontal cortex).

Although in the thyroxine group the left hemisphere had lower volume (Figure 6.5).

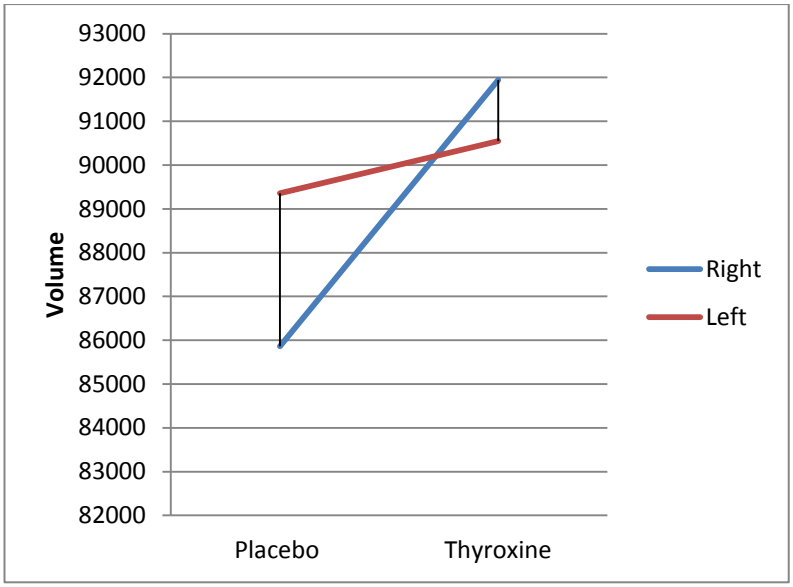

Figure 6.5 Hemisphere volumes between left and right for both groups.

Differences in laterality were significant for the medial structures (Table 6.3).

Structure	Wilks' Lambda	P
DLPFC	.96	.22
DMPFC	.88	.02
OLPFC	1	.84
OMPFC	.78	.00
Hemisphere	.99	.62

Table 6.3 Laterality differences (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, OMPFC= orbital medial prefrontal cortex).

Comparison of Structures

Volumes were significantly higher in the medial cortex compared to the lateral cortex (Table 6.4 and Figure 6.6).

Structures	Means	Wilks' Lambda	Sig
Right DLPFC	1657.45	.12	.00
Right DMPFC	14915.03		
Left DLPFC	1974.27	.14	.00
Left DMPFC	16401.34		
Right OLPFC	359.04	.16	.00
Right OMPFC	4474.43		
Left OLPFC	381.68	.17	.00
Left OMPFC	5125.73		

Table 6.4. Comparison of Lateral and Medial Structures (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex).

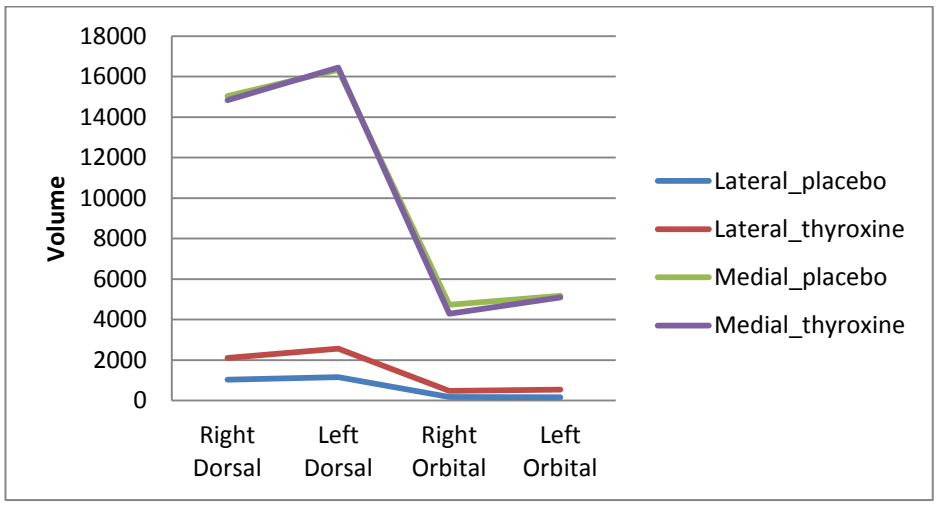

Figure 6.6 Graph demonstrating lateral and medial differences.

Volumes were significantly higher in the dorsal compared to orbital regions (Table 6.5 and Figure 6.7)

Structures	Means	Wilks' Lambda	Sig
Right DLPFC	1657.45	.45	.00
Right OLPFC	359.04		
Left DLPFC	1974.27	.55	.00
Left OLPFC	381.68		
Right DMPFC	14915.03	.10	.00
Right OMPFC	4474.43		
Left DMPFC	16401.34	.09	.00
Left OMPFC	5125.73		

Table 6.5 Comparison of dorsal and orbital Structures (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex).

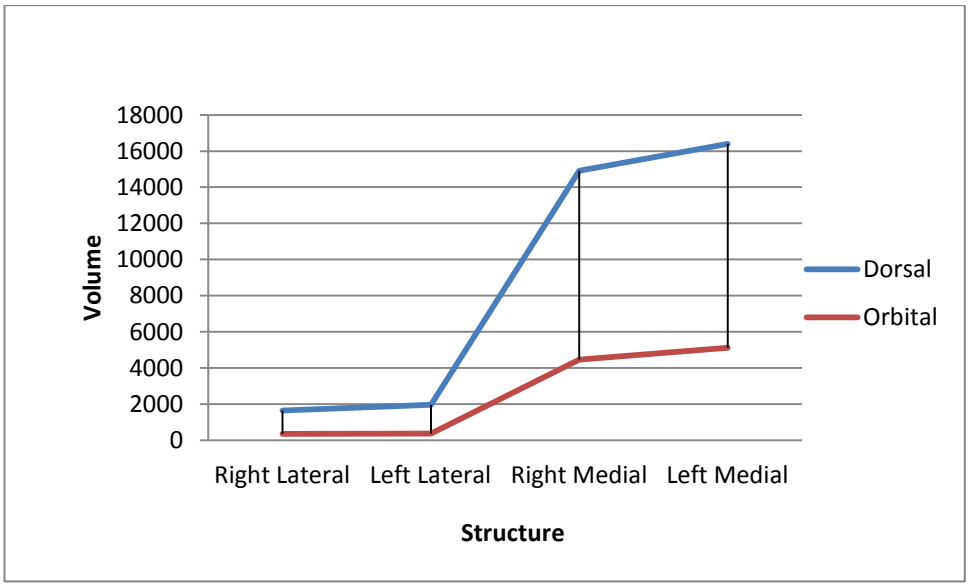

Figure 6.7 Comparison of dorsal and orbital Structures.

Sex Differences

In the placebo group males had lower structural volumes with the exception of the left dorsal medial prefrontal cortex (Figure 6.8 and 6.9).

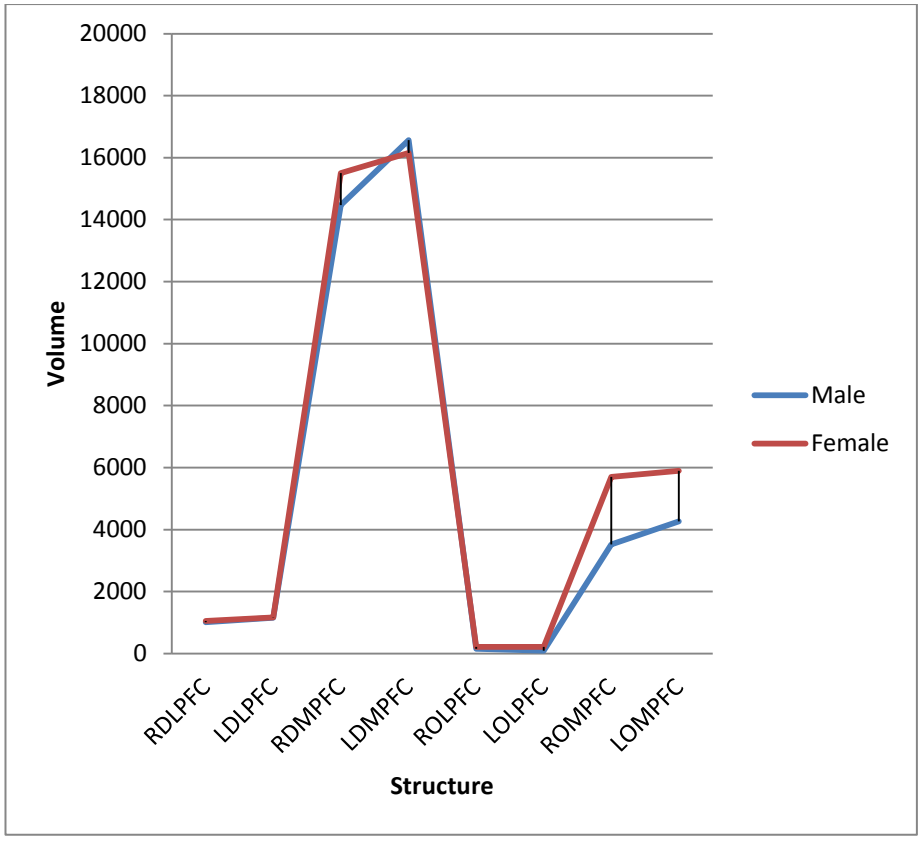

Figure 6.8 Gender differences in the placebo group (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex).

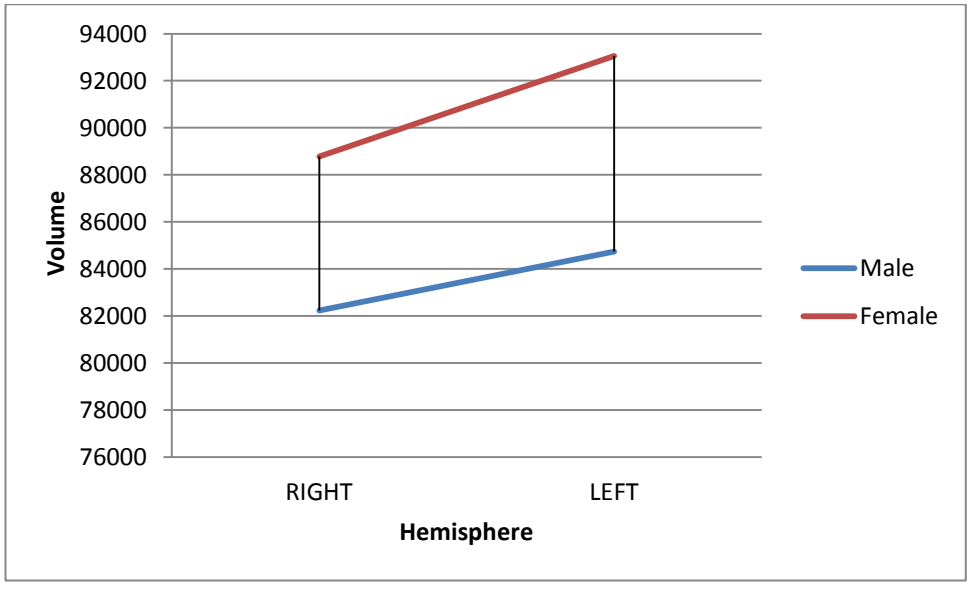

Figure 6.9 Gender differences in hemisphere volumes in the placebo group.

In the thyroxine group females had lower structural volumes with the exception of the left dorsal medial prefrontal cortex (Figure 6.10 and 6.11).

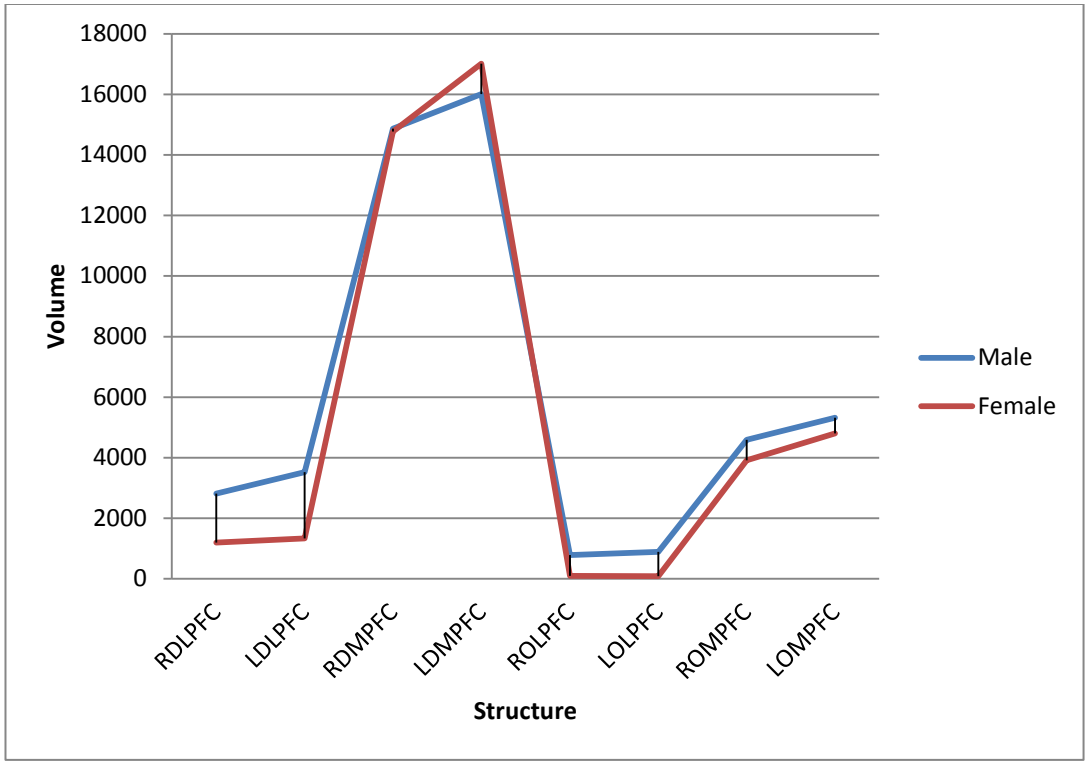

Figure 6.10 Gender Differences in the thyroxine group (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex).

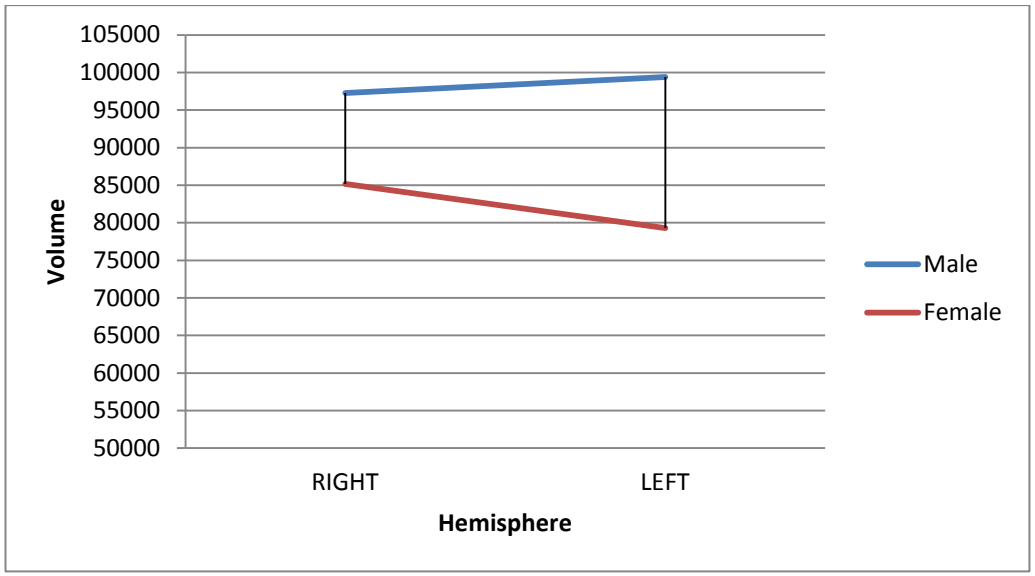

Figure 6.11 Gender differences in the hemisphere volumes in the thyroxine group.

In the placebo group males had significantly lower volume in the orbital medial prefrontal cortex. In the thyroxine group volumes were significantly lower in females in the right orbital lateral and both hemispheres. (Table 6.6).

Treatment	Structure	Gender	Mean	Std Deviation	F	P
Placebo	RDLPFC	Male	1012.99	723.37	.01	.91
		Female	1051.32	657.23		
	LDLPFC	Male	1155.35	476.38	.00	.99
		Female	1160.83	836.06		
	RDMPFC	Male	14472.01	2017.32	.50	.49
		Female	15498.14	3663.8		
	LDMPFC	Male	16563.69	2382.71	.10	.76
		Female	16150.83	3069.53		
	ROLPFC	Male	148.45	168.43	.47	.50
		Female	212.21	215.32		
	LOLPFC	Male	92.48	95.8	2.61	.13
		Female	214.16	194.04		
	ROMPFC	**Male**	**3515.35**	**1459.24**	**7.63**	**.01**
		Female	**5694.63**	**1806.77**		
	LOMPFC	**Male**	**4268.54**	**1031.75**	**4.30**	**.06**
		Female	**5895.15**	**2008.50**		
	RHEMISPHERE	Male	82225.26	16289.78	1.05	.32
		Female	88777.73	10870.87		
	LHEMISPHERE	Male	84734.91	17923.03	1.43	.25
		Female	93048.7	11526.18		
Thyroxine	RDLPFC	Male	2816.02	2668.05	3.69	.07
		Female	1202.64	862.94		
	LDLPFC	Male	3526.29	4407.01	2.63	.12
		Female	1334.06	789.79		
	RDMPFC	Male	14868.60	4994.03	.00	.95
		Female	14766.2	3294.14		
	LDMPFC	Male	16010.68	5763.56	.27	.61
		Female	17008.21	3002.98		
	ROLPFC	**Male**	**789.88**	**1102.56**	**4.25**	**.05**
		Female	**97.35**	**136.84**		
	LOLPFC	Male	895.57	1716.67	2.40	.14
		Female	90.27	74.53		
	ROMPFC	Male	4591.86	1372.26	1.40	2.48
		Female	3913.23	1484.62		
	LOMPFC	Male	5321.94	1482.73	.52	.48
		Female	4799.95	2126.5		
	RHEMISPHERE	**Male**	**97283.16**	**14964.07**	**4.21**	**.05**
		Female	**85153.88**	**14288.97**		
	LHEMISPHERE	**Male**	**99395.17**	**11773.43**	**.59**	**.02**
		Female	**79287.21**	**28097.26**		

Table 6.6 Gender Differences (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

Correlations

Structural volumes measured by stereology did not correlate with birth weight, gestation at scanning or gestation at birth with the exception of the orbital medial prefrontal cortex (Table 6.7).

	Birth weight		Gestation at Birth		Gestation at Scanning	
	Pearson Correlation	P	Pearson Correlation	P	Pearson Correlation	P
R DLPFC	-.06	.70	-.14	.36	.04	.79
L DLPFC	-.07	.65	-.15	.33	.05	.75
R DMPFC	.06	.71	.00	1	-.14	.38
L DMPFC	-.12	.43	-.14	.37	.10	.51
R OLPFC	.10	.55	.04	.81	.13	.42
L OLPFC	.03	.87	.00	.99	.05	.74
R OMPFC	.21	.19	**.43**	**.00**	.04	.81
L OMPFC	**.28**	**.07**	**.45**	**.00**	.08	.62
R Hemisphere	.11	.48	.12	.45	.19	.23
L Hemisphere	.19	.24	.20	.21	.14	.37

Table 6.7 Correlation between stereology measures, birth weight, gestational age and gestation at scanning (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

There was a significant positive correlation with the right and contra lateral orbital medial prefrontal cortex and gestation at birth and a borderline significant correlation between the left orbital medial prefrontal cortex and birth weight (Table 6.7 and figures 6.12-14).

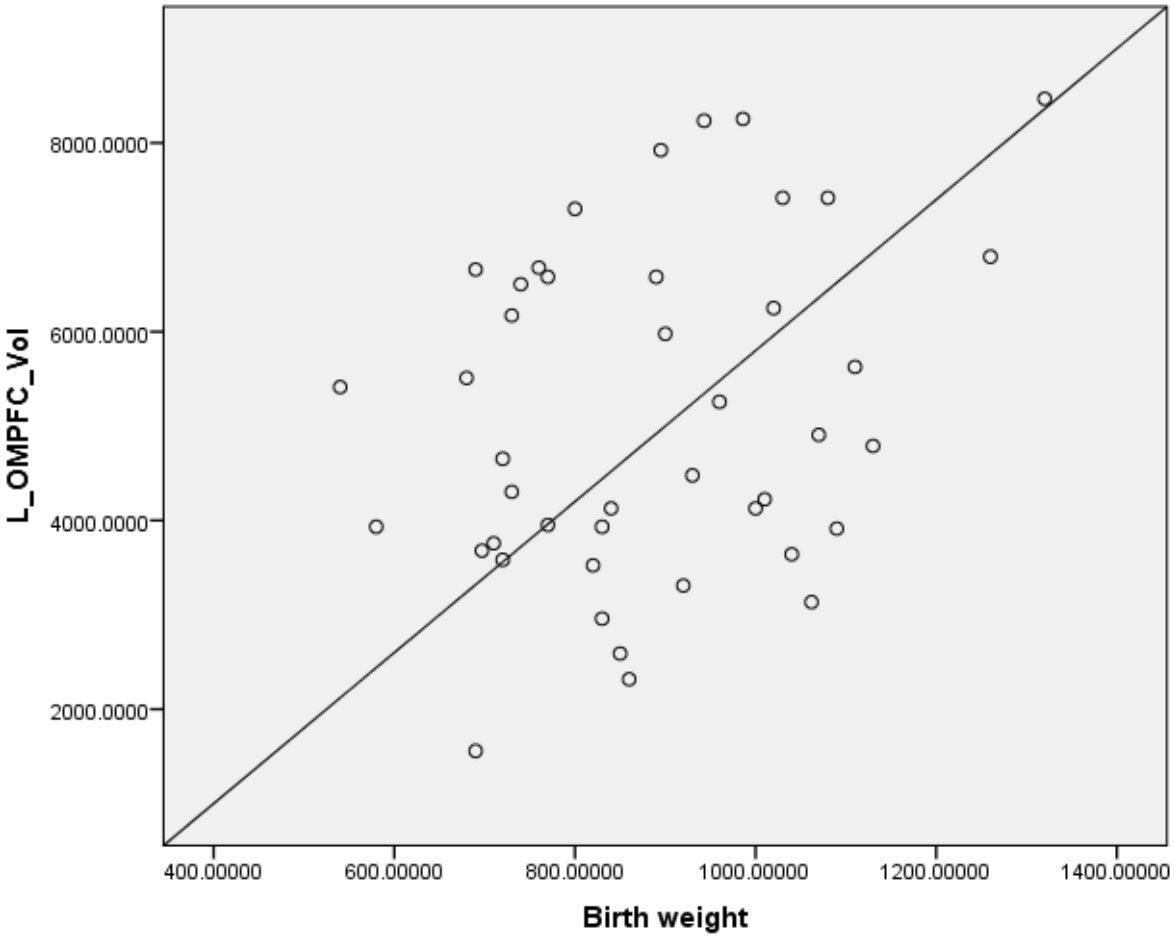

Figure 6.12.Correlation between birth weight and left orbital medial prefrontal cortex (L_OMPFC).

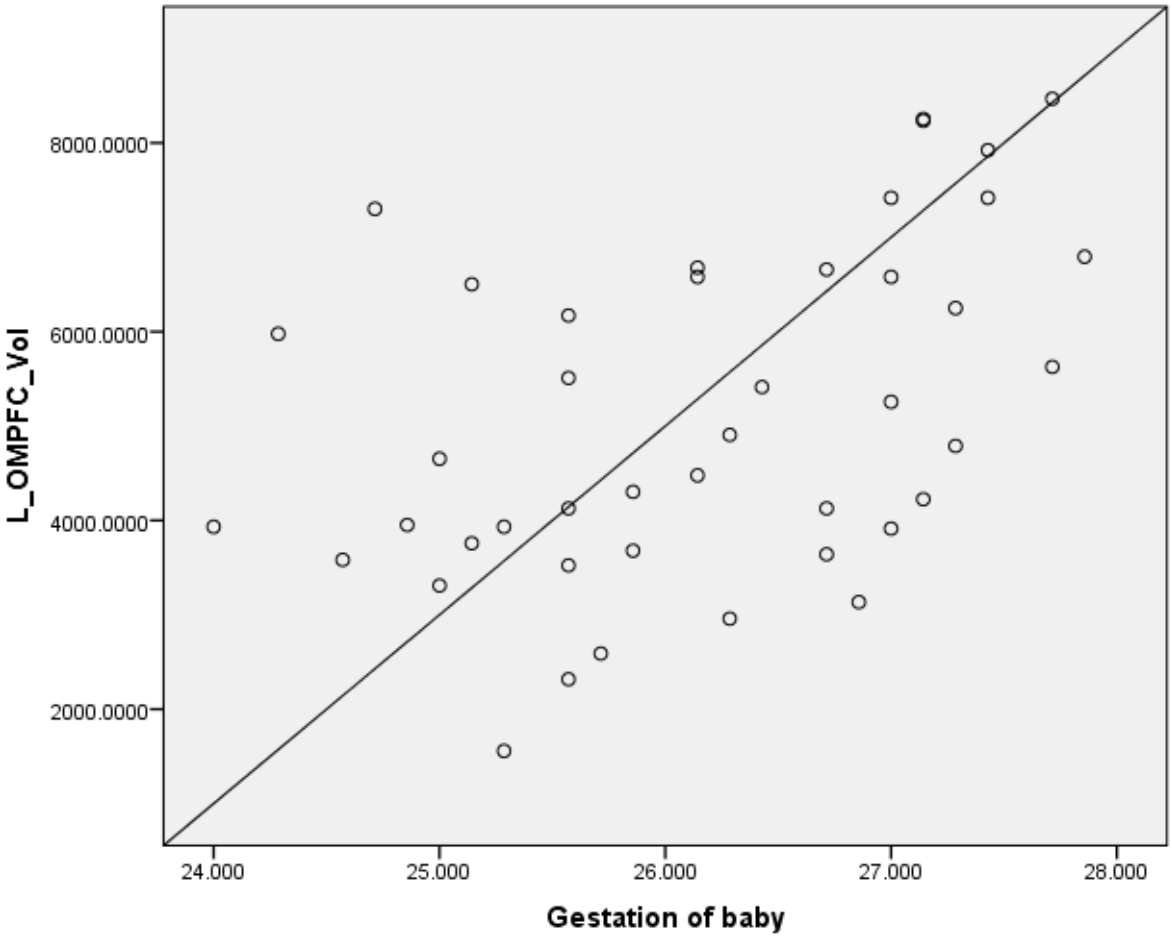

Figure 6.13 Correlation between gestation at birth and left orbital medial prefrontal cortex (L_OMPFC).

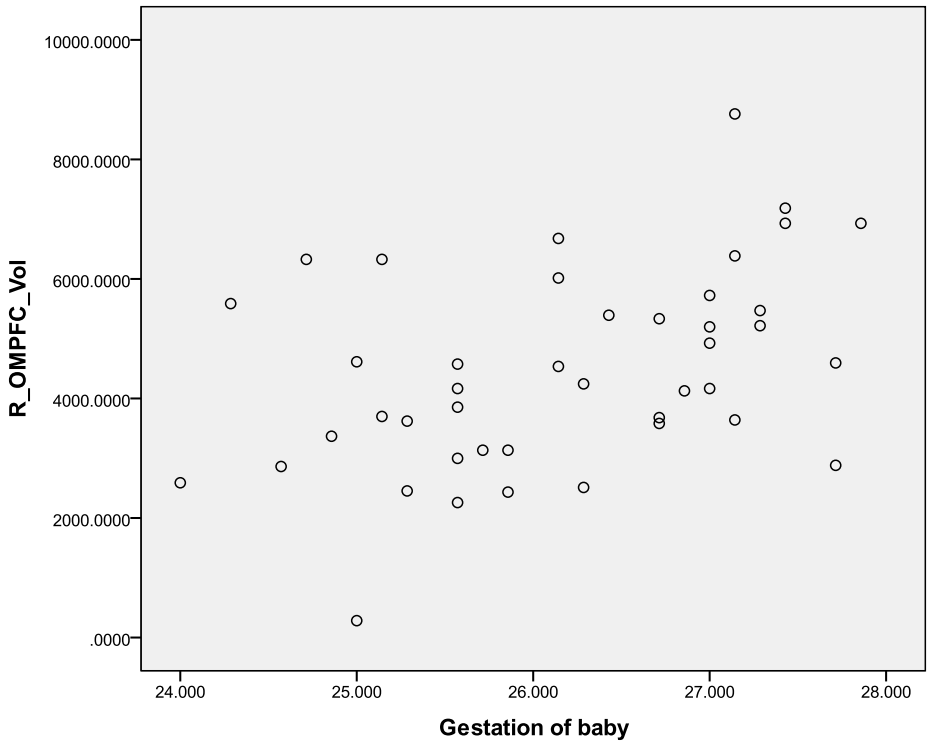

Figure 6.14 Correlation between gestation at birth and right orbital medial prefrontal cortex (R_OMPFC).

Smoking

As there was considerably more smokers in the thyroxine group (Table 5.17) a one-way anova was repeated after excluding smokers. Prior to exclusion of smokers volume in the right dorsal lateral prefrontal cortex was significantly lower in the placebo group (Table 6.2). After exclusion of smokers this was no longer significant; volume in the right hemisphere is lower in the placebo group and this difference became borderline significant after excluding smokers (Table 6.8).

Structures	Treatment	Means	F	P
R_DLPFC	Placebo	1044.58	2.50	.13
	Thyroxine	2302.88		
L_DLPFC	Placebo	1260.23	2.21	.15
	Thyroxine	3125.79		
R_DMPFC	Placebo	14822.87	.42	.52
	Thyroxine	13851.70		
L_DMPFC	Placebo	15773.10	.26	.62
	Thyroxine	16548.83		
R_OLPFC	Placebo	184.21	1.63	.21
	Thyroxine	575.72		
L_OLPFC	Placebo	191.69	1.23	.28
	Thyroxine	728.69		
R_OMPFC	Placebo	4742.16	.39	.54
	Thyroxine	4297.04		
L_OMPFC	Placebo	5156.24	.00	.98
	Thyroxine	5177.31		
R_Hemisphere	Placebo	81797.04	3.96	.06
	Thyroxine	94023.88		
L_Hemisphere	Placebo	84127.69	.32	.58
	Thyroxine	89231.83		

Table 6.8 Table of Stereology Measures between groups excluding smokers (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

Alcohol

A separate analysis was performed investigating the effects of alcohol (Table 6.9). Structural volume was generally similar between groups (Figure 6.15 and 6.16).

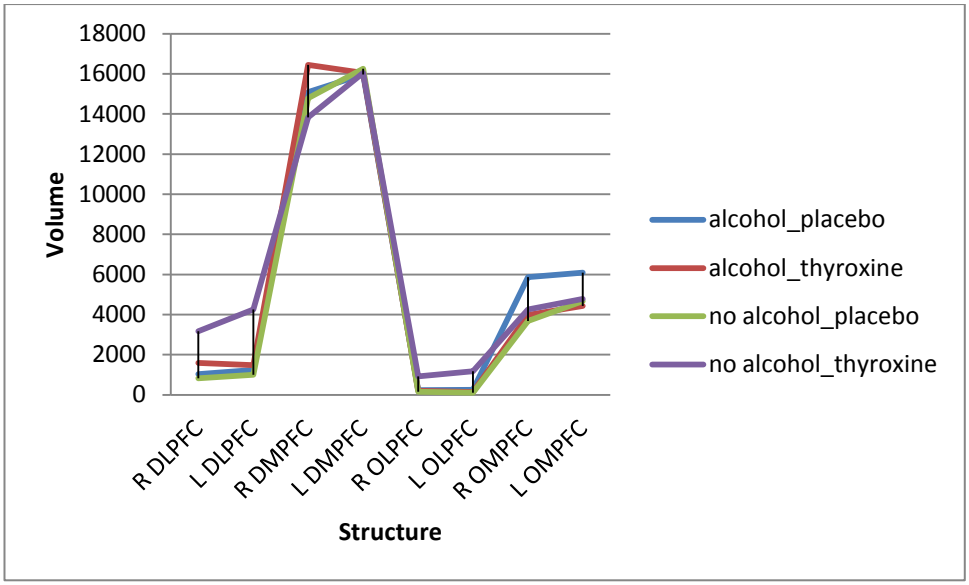

Figure 6.15 Effect of alcohol on volume (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

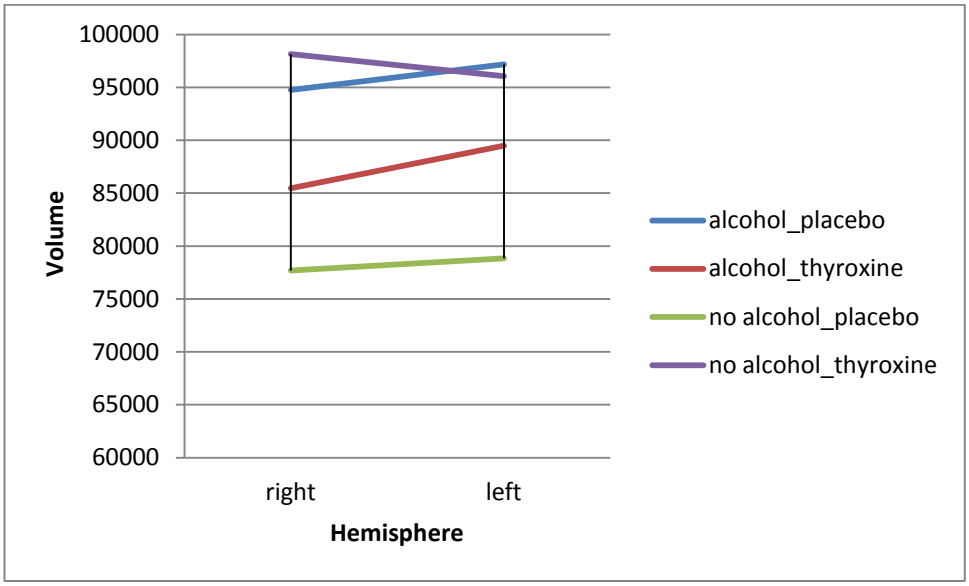

Figure 6.16 Effect of alcohol on hemisphere volume.

In the placebo group volumes were generally higher in the alcohol group (except the left dorsal medial prefrontal cortex). Volumes were significantly higher in the alcohol group in the right and contra lateral hemisphere and borderline significantly higher in the ipsilateral orbital medial prefrontal cortex. In the thyroxine group

volumes were generally lower in the alcohol group with the exception of the right dorsal medial prefrontal cortex. Differences in the thyroxine group were generally non significant (Table 6.9).

Treatment	Structure	Group	Mean	Std Deviation	F	Sig
Placebo	R_DLPFC	Alcohol	1025.03	688.17	.22	.66
		No Alcohol	832.29	622.58		
	L_DLPFC	Alcohol	1235.29	1164.91	.16	.70
		No Alcohol	998.75	594.52		
	R_DMPFC	Alcohol	15095.13	3228.6	.03	.86
		No Alcohol	14779.74	2081.9		
	L_DMPFC	Alcohol	15971.23	3218.77	.02	.88
		No Alcohol	16251.58	2593.75		
	R_OLPFC	Alcohol	237.52	172.71	.53	.49
		No Alcohol	151.86	199.21		
	L_OLPFC	Alcohol	249.2	182.84	2.04	.19
		No Alcohol	109.03	121.27		
	R_OMPFC	**Alcohol**	**5867.9**	**1196.23**	**3.53**	**.10**
		No Alcohol	**3673.79**	**2321.86**		
	L_OMPFC	Alcohol	6093.73	1688.69	1.29	.29
		No Alcohol	4672.51	2229.88		
	R_Hemisphere	**Alcohol**	**94764.38**	**8789.81**	**5.04**	**.06**
		No Alcohol	**77680.51**	**14575.11**		
	L_Hemisphere	**Alcohol**	**97173.64**	**10703.19**	**5.45**	**.05**
		No Alcohol	**78848.64**	**13909.89**		
Thyroxine	R_DLPFC	Alcohol	1595.75	708.98	1.15	.30
		No Alcohol	3172.72	3823.91		
	L_DLPFC	Alcohol	1483.11	1436.78	1.37	.27
		No Alcohol	4255.32	6111.12		
	R_DMPFC	Alcohol	16445.57	4231.94	1.17	.30
		No Alcohol	13836.06	4774.08		
	L_DMPFC	Alcohol	16038.87	6420.66	.00	1
		No Alcohol	16045.07	5520.08		
	R_OLPFC	Alcohol	203.03	344.04	1.58	.23
		No Alcohol	915.04	1460.98		
	L_OLPFC	Alcohol	133.50	137.86	1.28	.28
		No Alcohol	1179.25	2438.45		
	R_OMPFC	Alcohol	3985.54	1562.15	.09	.77
		No Alcohol	4255.32	1776.71		
	L_OMPFC	Alcohol	4430.54	2026.40	.14	.72
		No Alcohol	4789.33	15622.2		
	R_Hemisphere	Alcohol	85471.51	14532.13	2.11	.17
		No Alcohol	98143.61	17961.83		
	L_Hemisphere	Alcohol	89486.95	12058.17	.62	.45
		No Alcohol	96057.66	18554.21		

Table 6.9 Differences in Alcohol groups (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

The IUGR Cohort

Descriptive statistics are shown in Table 6.10 for the IUGR cohort.

Structure		Min	Max	Mean	Std.Deviation
R DLPFC	IUGR	.00	1007.51	413.71	387.56
	Control	262.83	1971.22	890.7	596.21
L DLPFC	IUGR	.00	788.49	403.98	321.73
	Control	306.63	1401.75	764.15	367.88
R DMPFC	IUGR	.00	17215.29	9194.14	6193.79
	Control	11389.25	22778.50	16576.05	3734.45
L DMPFC	IUGR	.00	18310.41	9345.02	6705.25
	Control	9856.08	24618.30	16531.45	4871.55
R OLPFC	IUGR	.00	447.78	97.34	141.06
	Control	.00	564.60	255.26	204.99
L OLPFC	IUGR	.00	428.31	108.16	147.66
	Control	.00	311.50	121.14	117.93
R OMPFC	IUGR	.00	6599.92	3603.89	2397.22
	Control	1538.04	9753.87	5483.71	2340.81
L OMPFC	IUGR	.00	6794.61	3536.83	2293.83
	Control	2238.91	11097.22	5676.24	2646.02
Prefrontal Cortex	IUGR	.00	47416.27	26703.08	17695.92
	Control	32271.97	63911.21	46298.7	11060.25
R Hemisphere	IUGR	.00	141336.19	87587.7	39634.07
	Control	92252.91	136013.90	118864.32	14171.18
L Hemisphere	IUGR	.00	136605.27	86142.14	39276.82
	Control	86339.26	141336.19	117747.3	15970.81
Cerebrum	IUGR	.00	277941.46	173729.84	78791.89
	Control	178592.17	274984.64	236611.62	29731.08

Table 6.10 Structural Volumes Table of Descriptive Statistics (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

Structural volume was higher in the medial compared to lateral structures in both groups and highest in the dorsal medial prefrontal cortex. Volumes were higher in the dorsal compared to orbital regions (Figure 6.17).

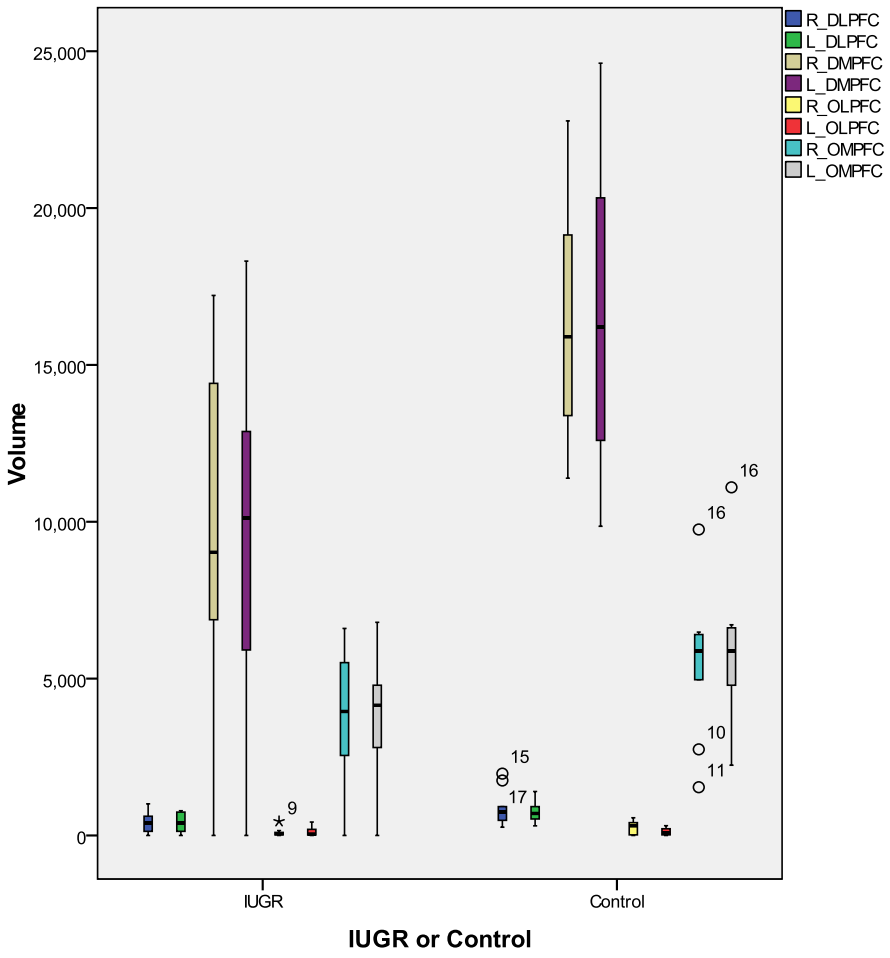

Figure 6.17 Box plot demonstrating Volume in the Dorsal and Orbital structures (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

Total prefrontal cortex, right and contra lateral hemisphere volumes and cerebrum volume were higher in the control group (Figure 6.18).

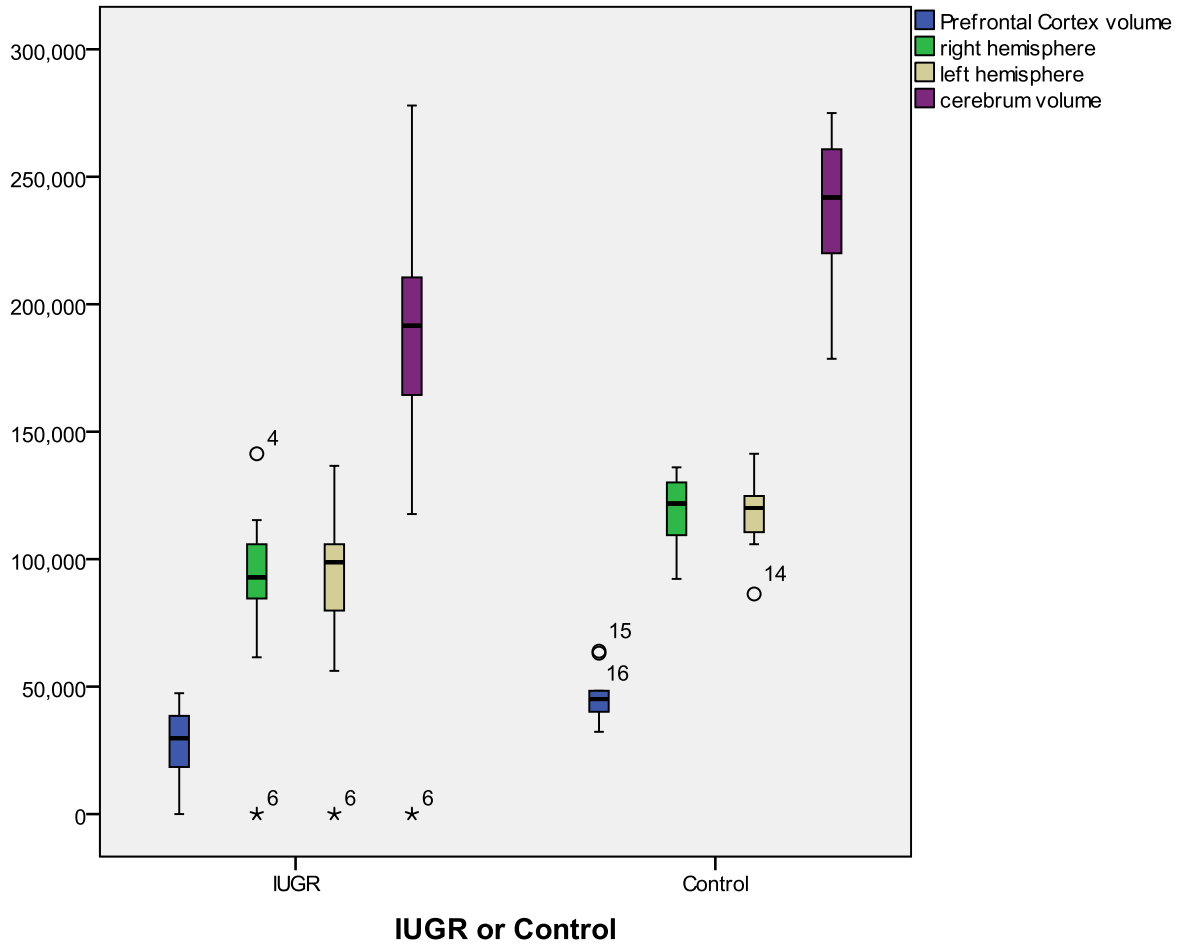

Figure 6.18 Box plot demonstrating volume for the total prefrontal cortex and hemispheres.

Volumes were lower in the IUGR group and differences between groups were generally significant apart from the left orbital lateral and contra lateral orbital medial prefrontal cortex, which did not reach significance (Table 6.11).

Structures		Means	F	Sig
R_DLPFC	IUGR	414	4.05	**.06**
	Control	891		
L_DLPFC	IUGR	404	4.89	**.04**
	Control	764		
R_DMPFC	IUGR	9194	9.38	**.01**
	Control	16576		
L_DMPFC	IUGR	9345	6.77	**.02**
	Control	16532		
R_OLPFC	IUGR	97	3.63	**.08**
	Control	255		
L_OLPFC	IUGR	108	.04	.84
	Control	121		
R_OMPFC	IUGR	3604	2.83	.11
	Control	5484		
L_OMPFC	IUGR	3537	3.36	**.09**
	Control	5676		
Prefrontal Cortex	IUGR	26703	7.94	**.01**
	Control	46299		
R_Hemisphere	IUGR	87588	4.97	**.04**
	Control	118864		
L_Hemisphere	IUGR	86142	5.00	**.04**
	Control	117747		
Cerebrum	IUGR	173730	5.02	**.04**
	Control	236612		

Table 6.11 Table of stereology measures between groups (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

Laterality

Structural and hemisphere volumes were generally very similar between left and contra lateral, except that in the orbital lateral prefrontal cortex there was a

significant difference between left and contra lateral (Table 6.12 and Figures 6.19 - 6.21).

Structure	Wilks' Lambda	Sig
DLPFC	.95	.37
DMPFC	1	.92
OLPFC	.75	**.04**
OMPFC	.99	.68
Hemisphere	.93	.28

Table 6.12 Laterality differences (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex OMPFC= orbital medial prefrontal cortex).

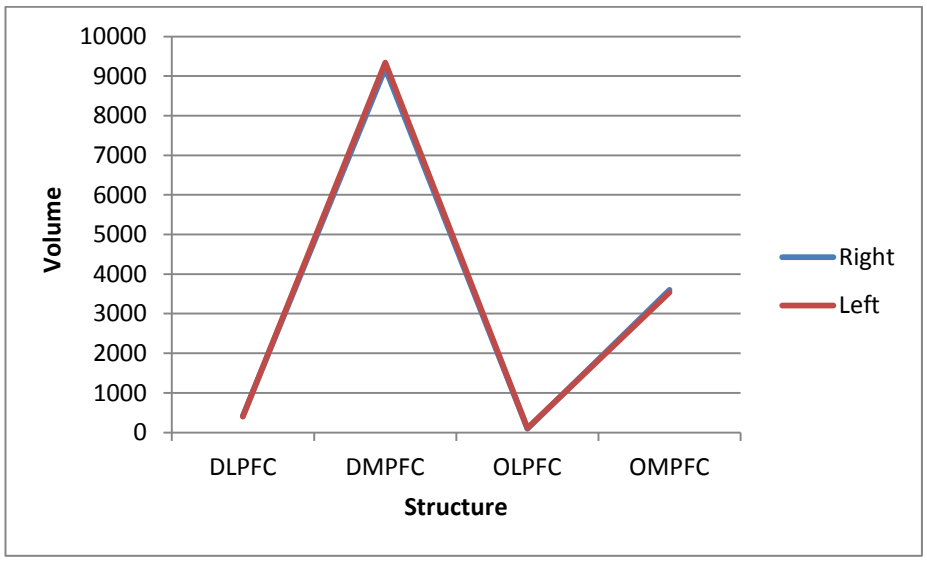

Figure 6.19 Volumes of structures on left and contra lateral in the IUGR group (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex OMPFC= orbital medial prefrontal cortex).

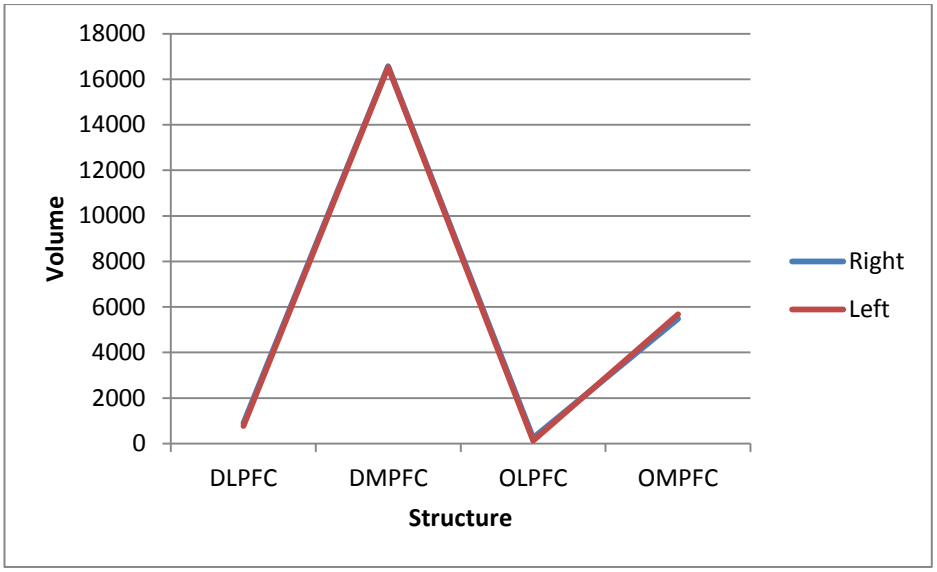

Figure 6.20 Volumes of structures on left and contra lateral in the control group (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex OMPFC= orbital medial prefrontal cortex).

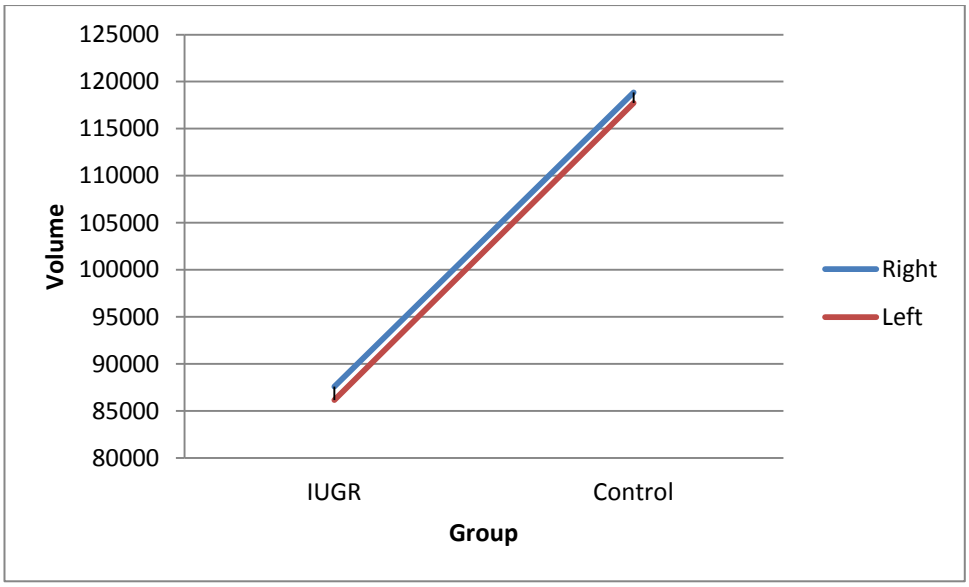

Figure 6.21 Differences between left and contra lateral hemisphere volumes.

Volume was entered into repeated measures ANOVA with structure and laterality as within subject factors and subject group (intrauterine growth restriction or control) as between subject factor. There was a 2 way interaction with structure and subject group (Table 6.13).

Interaction		Mean Square	F	P
Structure *Subject Group	Linear	2.90	4.47	.05
	Quadratic	1.89	4.41	.05
Laterality*Subject Group	Linear	7709.90	.00	.96
Laterality*Structure	Linear	5256424.00	1.18	.29
	Quadratic	4656733.33	1.58	.23
Structure*Laterality*Subject Group	Linear	407496.13	.092	.77
	Quadratic	67739.85	0.23	.88

Table 6.13 Repeated measures ANOVA for region and laterality.

Although overall there was no interaction between laterality and subject group when analysing the structure which had statistically significant differences in laterality (orbital lateral prefrontal cortex) separately there was a significant interaction with subject group (Table 6.14).

Interaction	Mean Square	F	Significance
Laterality	34207.12	5.22	.036
Laterality*Subject Group	47263.49	7.21	.016

Table 6.14 Interaction with laterality and subject group in the orbital lateral prefrontal cortex.

After splitting the dataset and analysing the groups separately there was no longer a difference between left and contra lateral in the orbital lateral prefrontal cortex (Table 6.15).

Structure	Group	Wilks' Lambda	Sig
OLPFC	IUGR	.89	.35
	CONTROL	.95	.54

Table 6.15 Difference between laterality in the different groups (OLPFC= orbital lateral prefrontal cortex).

Comparison of Structures

Volumes were significantly higher in the medial cortex compared to the lateral cortex (Table 6.16 and Figure 6.22).

Structures	Means	Wilks' Lambda	Sig
Right DLPFC	652.20	.13	**.00**
Right DMPFC	12885.10		
Left DLPFC	584.06	.16	**.00**
Left DMPFC	12938.24		
Right OLPFC	176.30	.20	**.00**
Right OMPFC	4543.80		
Left OLPFC	114.65	.21	**.00**
Left OMPFC	4606.54		

Table 6.16 Comparison of lateral and medial structures (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex OMPFC= orbital medial prefrontal cortex).

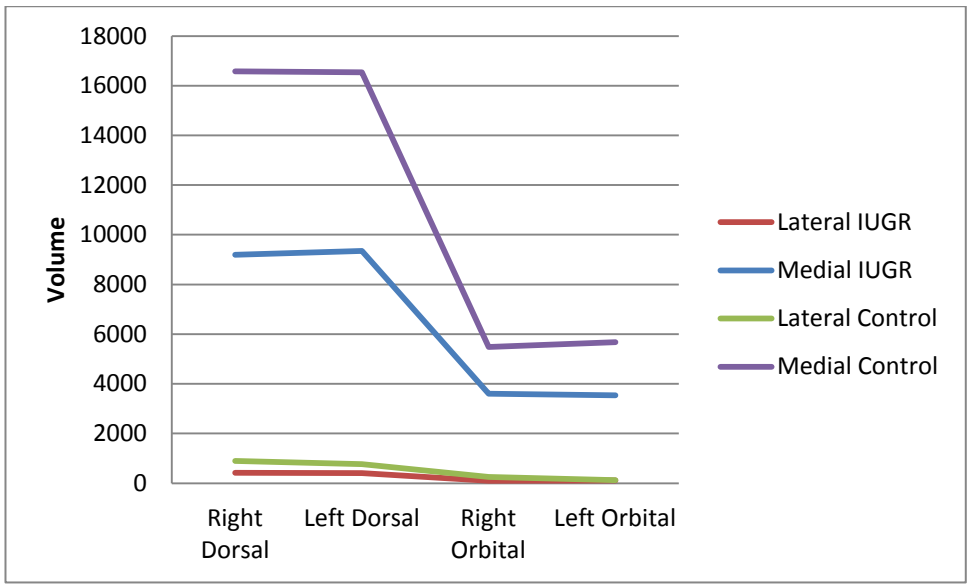

Figure 6.22 Graph demonstrating lateral and medial differences.

A significant interaction with subject group was seen when looking at volumes in the right and contra lateral dorsal prefrontal cortex and close to significant in the left orbital prefrontal cortex (Table 6.17).

Structures	Value	F	Sig
Right DLPFC	.64	8.96	**.01**
Right DMPFC			
Left DLPFC	.71	6.49	**.02**
Left DMPFC			
Right OLPFC	.86	2.56	.13
Right OMPFC			
Left OLPFC	.82	3.43	**.08**
Left OMPFC			

Table 6.17 Interaction with subject group (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, OMPFC= orbital medial prefrontal cortex).

Further analysis splitting for groups showed volumes to be significantly higher in the medial cortex for both the intrauterine growth restriction and control group (Table 6.18).

Structures	IUGR		CONTROL	
	Wilk's_Lamda	Sig	Wilk's_Lamda	Sig
Right DLPFC	.29	.00	.04	.00
Right DMPFC				
Left DLPFC	.31	.00	.08	.00
Left DMPFC				
Left OLPFC	.27	.00	.17	.00
Left OMPFC				

Table 6.18 Comparison of lateral and medial split for group (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, OMPFC= orbital medial prefrontal cortex).

Volumes were significantly higher in dorsal compared to orbital regions (Table 6.19 and Figure 6.23).

Structures	Means	Wilks' Lambda	Sig
Right DLPFC	652.20	.43	.00
Right OLPFC	176.30		
Left DLPFC	584.06	.33	.00
Left OLPFC	114.65		
Right DMPFC	12885.1	.25	.00
Right OMPFC	4543.80		
Left DMPFC	12938.24	.28	.00
Left OMPFC	4606.54		

Table 6.19 Comparison of dorsal and orbital structures (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, OMPFC= orbital medial prefrontal cortex).

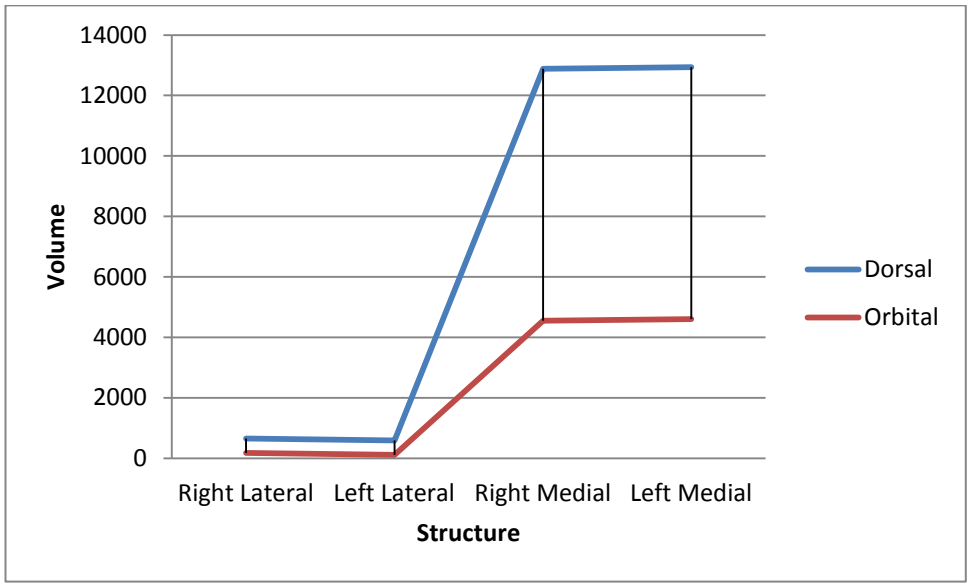

Figure 6.23 Comparing dorsal and orbital regions.

A significant interaction with subject group was seen when looking at volumes in the left dorsal and orbital lateral prefrontal cortex and in both the right and contra lateral dorsal and orbital medial prefrontal cortex (Table 6.20).

Structures	Value	F	Sig
Right DLPFC	.85	2.76	.12
Right OLPFC			
Left DLPFC	.72	6.36	**.02**
Left OLPFC			
Right DMPFC	.68	7.56	**.01**
Right OMPFC			
Left DMPFC	.76	5.08	**.04**
Left OMPFC			

Table 6.20 Interaction with subject group (DLPFC= dorsal lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, OMPFC= orbital medial prefrontal cortex).

Further analysis splitting for groups showed significantly higher volumes in dorsal compared to orbital regions in both the IUGR and Control group (Table 6.21).

Structures	IUGR		CONTROL	
	Wilk's_Lamda	P	Wilk's_Lamda	P
Left DLPFC	.46	.02	.16	.00
Left OLPFC				
Right DMPFC	.32	.00	.12	.00
Right OMPFC				
Left DMPFC	.39	.01	.14	.00
Left OMPFC				

Table 6.21 Comparison of dorsal and orbital split for group (DLPFC= dorsal lateral prefrontal cortex, OLPFC= orbital lateral prefrontal cortex, DMPFC= dorsal medial prefrontal cortex, OMPFC= orbital medial prefrontal cortex)

Sex Differences

In the IUGR group differences between genders were minimal although males had higher volumes in the dorsal medial prefrontal cortex, hemisphere volumes and cerebrum (Table 6.22, figures 6.24 and 6.25).

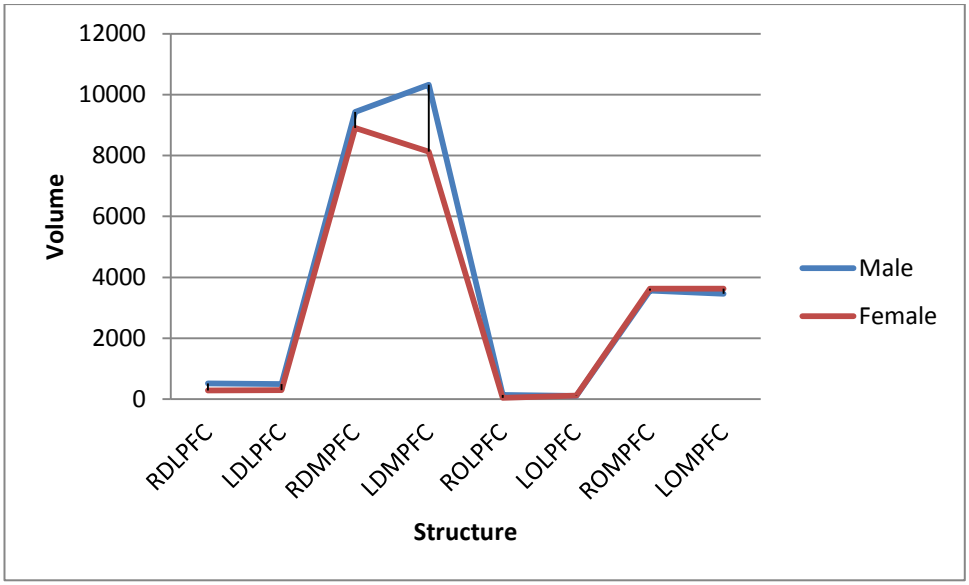

Figure 6.24 Gender differences in the IUGR group.

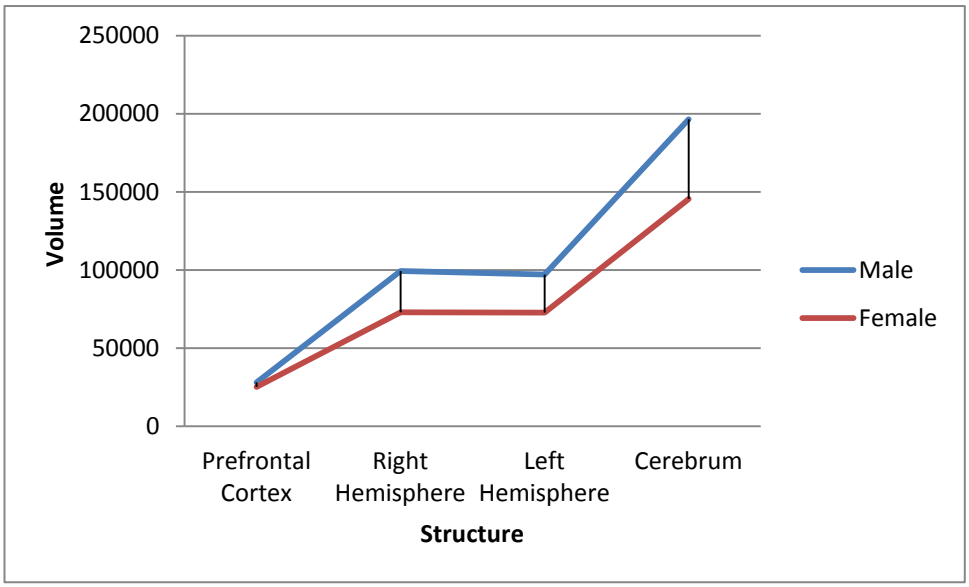

Figure 6.25 Gender differences in the IUGR group in the prefrontal cortex, hemisphere and cerebrum volumes.

These gender differences were not significant. In the control group males generally had higher volumes, which reached significance in the right orbital lateral prefrontal cortex. The right dorsal medial prefrontal cortex was the exception with lower volumes (Table 6.22, Figures 6.26 and 6.27).

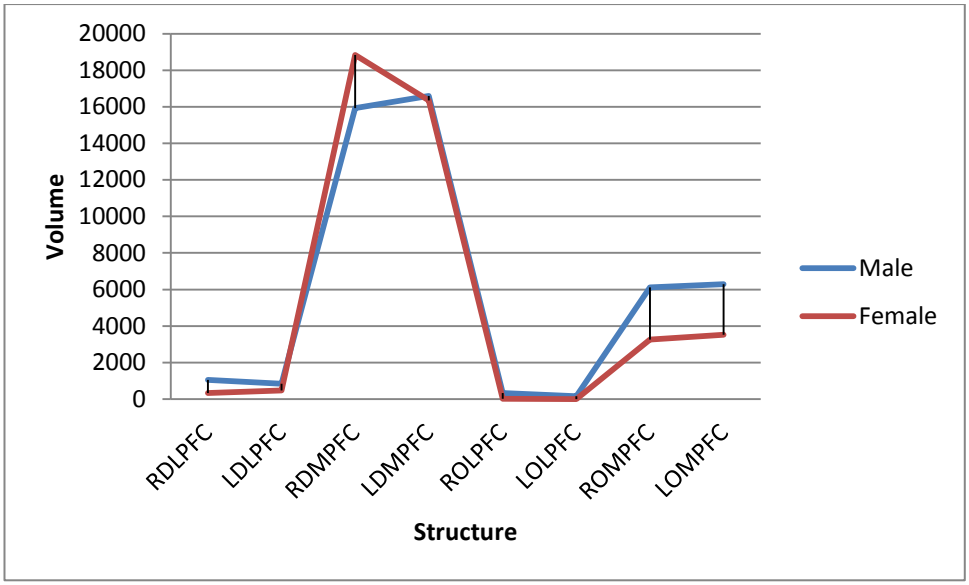

Figure 6.26 Gender differences in the control group. (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere).

Treatment	Structure	Gender	Mean	Std Deviation	F	Sig
IUGR	RDLPFC	Male	516.90	462.97	.78	.41
		Female	284.73	273.56		
	LDLPFC	Male	490.61	391.07	.80	.40
		Female	295.68	209.70		
	RDMPFC	Male	9426.79	6736.29	.01	.91
		Female	8903.33	6449.42		
	LDMPFC	Male	10320.41	7712.92	.22	.66
		Female	8125.79	6083.40		
	ROLPFC	Male	140.18	182.32	1.04	.34
		Female	43.81	43.17		
	LOLPFC	Male	101.24	183.36	.02	.89
		Female	116.82	114.63		
	ROMPFC	Male	3558.90	2376.25	.00	.96
		Female	3630.93	2790.72		
	LOMPFC	Male	3461.55	2154.56	.01	.92
		Female	3630.93	2796.49		
	Prefrontal Cortex	Male	28016.58	19532.31	.06	.82
		Female	25061.21	17885.86		
	RHEMISPHERE	Male	99349.29	30321.51	.99	.35
		Female	72885.71	49440.30		
	LHEMISPHERE	Male	96983.83	29839.03	.84	.39
		Female	72590.03	49858.23		
	Cerebrum	Male	196333.12	59963.05	.92	.37
		Female	145475.74	99222.49		
Control	RDLPFC	Male	1051.31	580.59	2.80	.14
		Female	328.54	92.92		
	LDLPFC	Male	844.81	374.88	1.63	.24

		Female	481.86	185.85		
	RDMPFC	Male	15930.33	3928.48	.93	.37
		Female	18836.07	2416.02		
	LDMPFC	Male	16586.38	5131.29	.00	.95
		Female	16339.19	5637.40		
	ROLPFC	**Male**	**325.41**	**137.67**	**5.99**	**.04**
		Female	**9.74**	**13.77**		
	LOLPFC	Male	155.75	110.70	3.59	.10
		Female	.00	.00		
	ROMPFC	Male	6121.55	2047.34	2.89	.13
		Female	3251.29	2422.90		
	LOMPFC	Male	6293.99	2605.71	1.91	.21
		Female	3514.12	1803.42		
	Prefrontal Cortex	Male	47309.53	12467.33	.24	.64
		Female	42760.79	3720.4		
	RHEMISPHERE	Male	121821.14	14886.27	1.45	.27
		Female	108515.44	1254.48		
	LHEMISPHERE	Male	120469.45	17300.88	.90	.37
		Female	108219.76	3345.27		
	Cerebrum	Male	242290.60	31713.4	1.17	.31
		Female	216735.20	4599.74		

Table 6.22 Gender Differences. (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

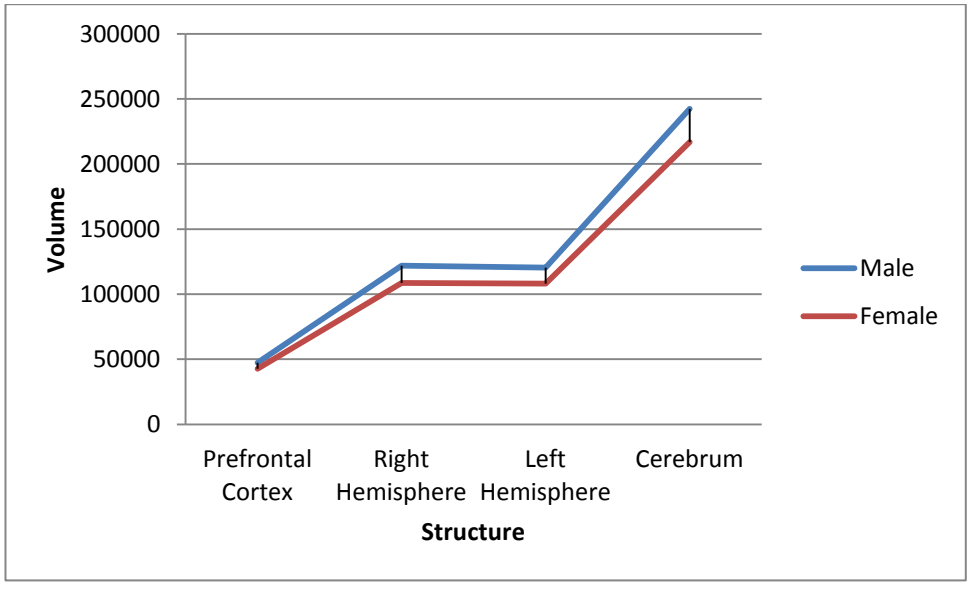

Figure 6.27 Gender differences in the control group for the prefrontal cortex, hemisphere and cerebrum volumes.

Correlations

Birth weight had a positive significant correlation with volume in the majority of the structures although the correlation in the left lateral structures (left dorsal lateral prefrontal cortex and left orbital lateral prefrontal cortex) was not significant (Table 6.23, Figures 6.28-6.37).

	Birth weight		Gestation at Birth	
	Pearson Correlation	Significance	Pearson Correlation	Significance
R DLPFC	**.47**	**.05**	.23	.37
L DLPFC	.39	.11	.12	.62
R DMPFC	**.49**	**.04**	.15	.56
L DMPFC	**.56**	**.02**	.24	.33
R OLPFC	**.49**	**.04**	.27	.28
L OLPFC	.09	.73	.11	.67
R OMPFC	**.54**	**.02**	.40	.10
L OMPFC	**.55**	**.02**	.38	.12
Prefrontal Cortex	**.58**	**.01**	.27	.27
R Hemisphere	**.68**	**.00**	**.42**	**.08**
L Hemisphere	**.67**	**.00**	**.42**	**.08**
Cerebrum	**.68**	**.00**	**.42**	**.08**

Table 6.23 Correlation between stereology measures, birth weight and gestational age. (RDLPFC= right dorsal lateral prefrontal cortex, LDLPFC= left dorsal lateral prefrontal cortex, RDMPFC= right dorsal medial prefrontal cortex, LDMPFC= left dorsal medial prefrontal cortex, ROLPFC= right orbital lateral prefrontal cortex, LOLPFC= left orbital lateral prefrontal cortex, ROMPFC= right orbital medial prefrontal cortex, LOMPFC= left orbital medial prefrontal cortex, RHEMISPHERE = right hemisphere, LHEMISPHERE= left hemisphere).

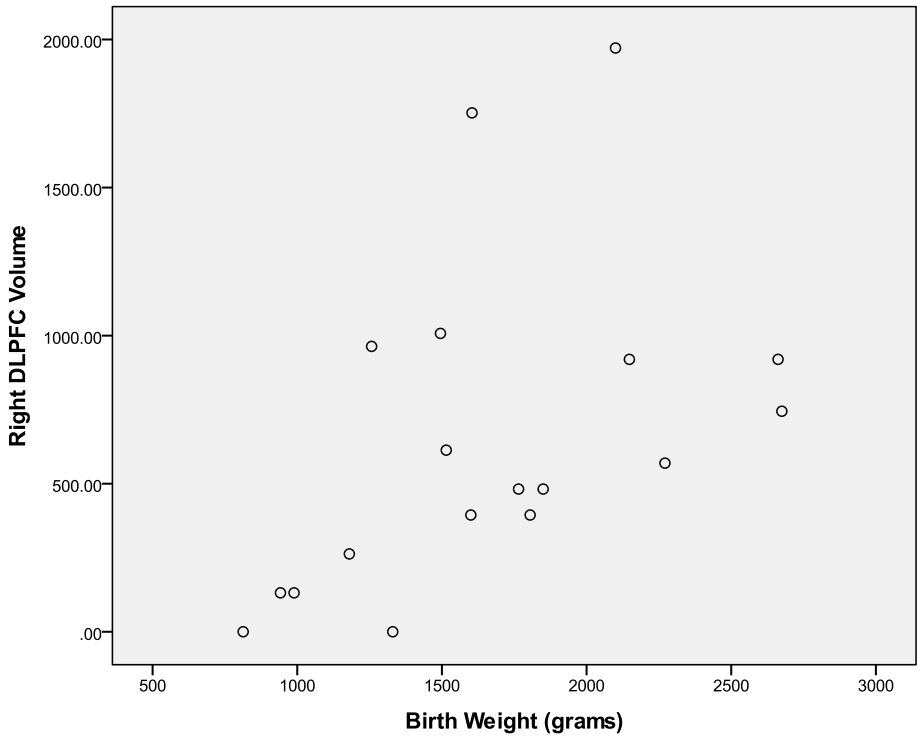

Figure 6.28 Correlation between birth weight and volume in the right DLPFC (DLPFC = dorsal lateral prefrontal cortex).

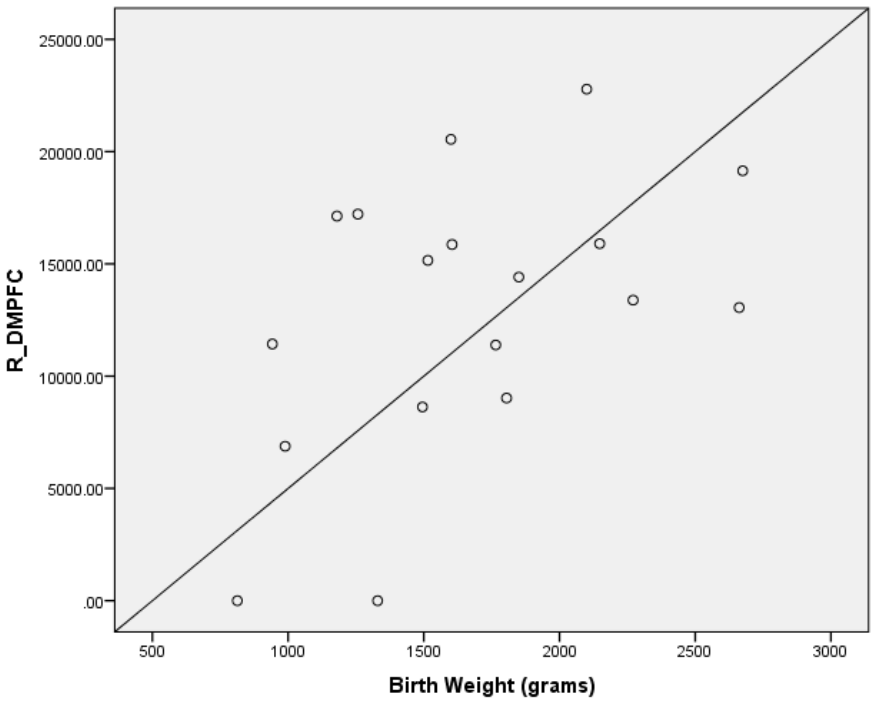

Figure 6.29 Correlation between birth weight and volume in the right DMPFC (R_DMPFC = right dorsal medial prefrontal cortex).

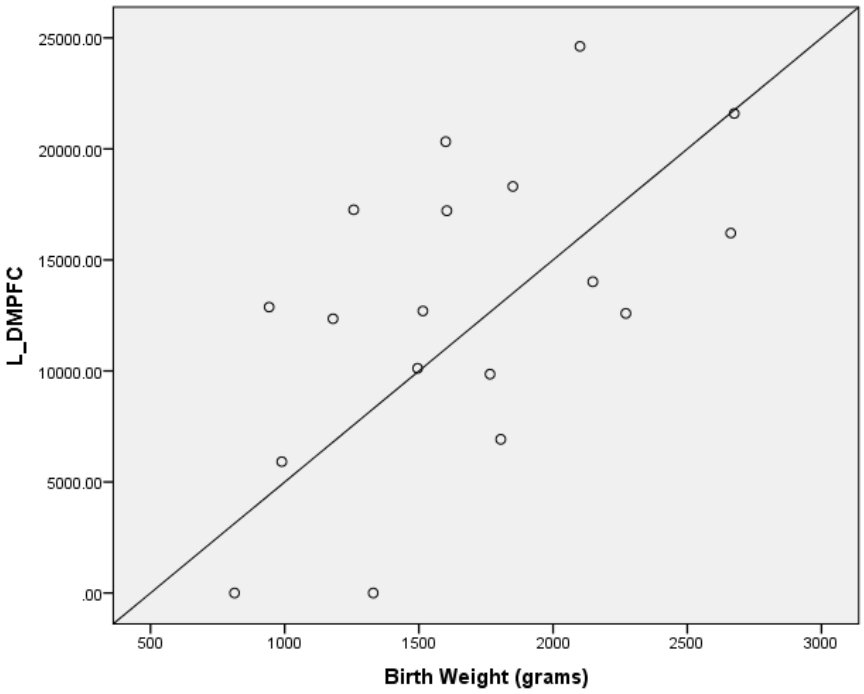

Figure 6.30 Correlation between birth weight and volume in the left DMPFC (L_DMPFC = left dorsal medial prefrontal cortex).

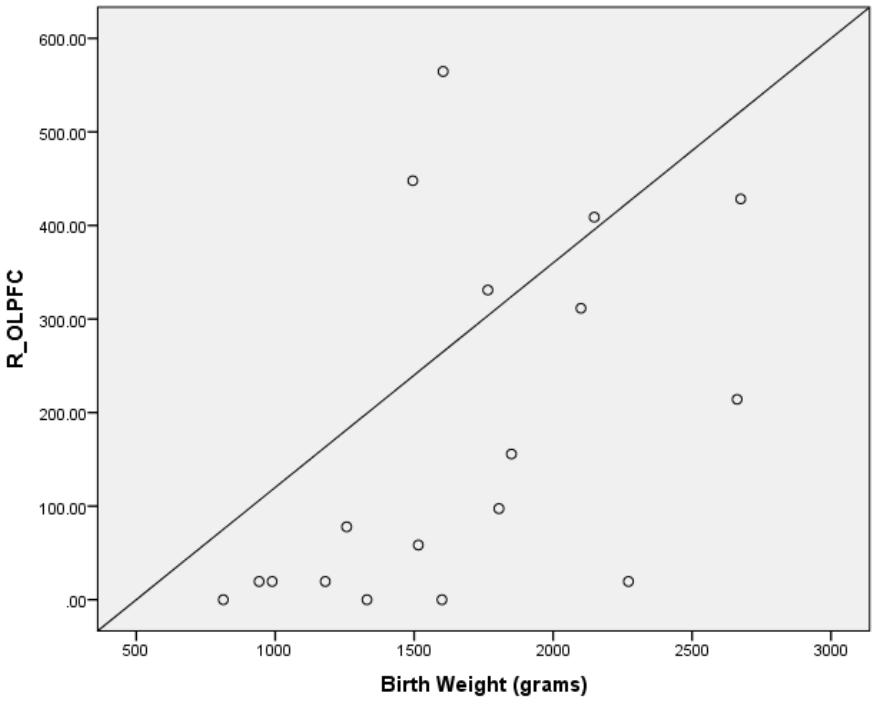

Figure 6.31 Correlation between birth weight and volume in the right OLPFC (R_OLPFC= right orbital lateral prefrontal cortex).

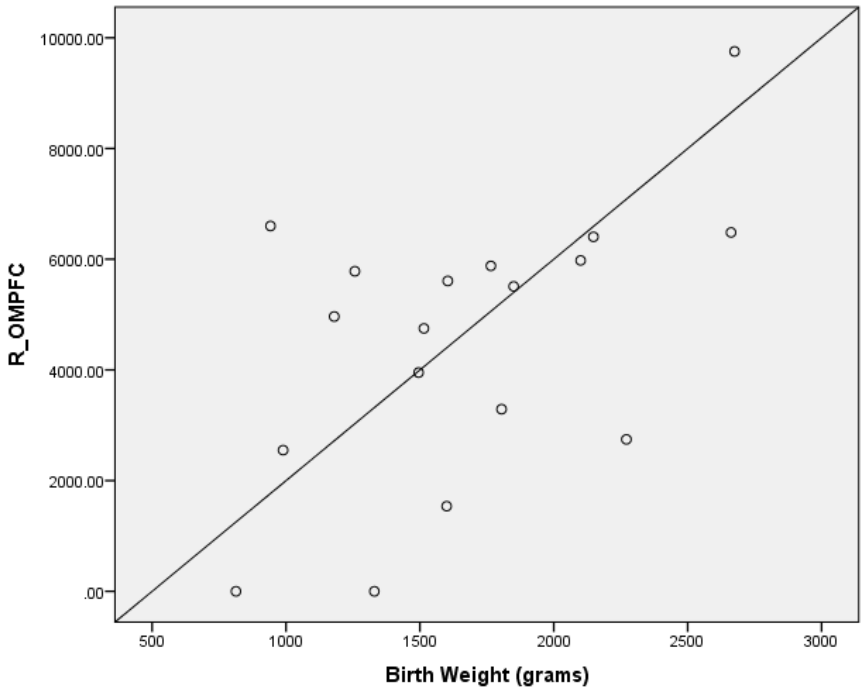

Figure 6.32 Correlation between birth weight and volume in the right OMPFC (R_OMPFC= right orbital medial prefrontal cortex).

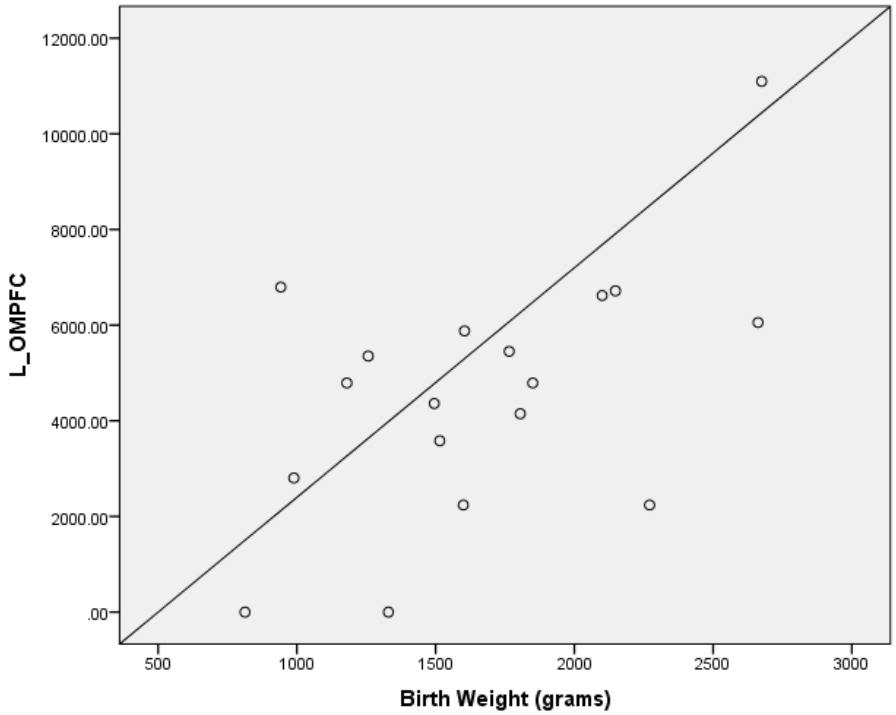

Figure 6.33 Correlation between birth weight and volume in the left OMPFC (L_OMPFC= left orbital medial prefrontal cortex).

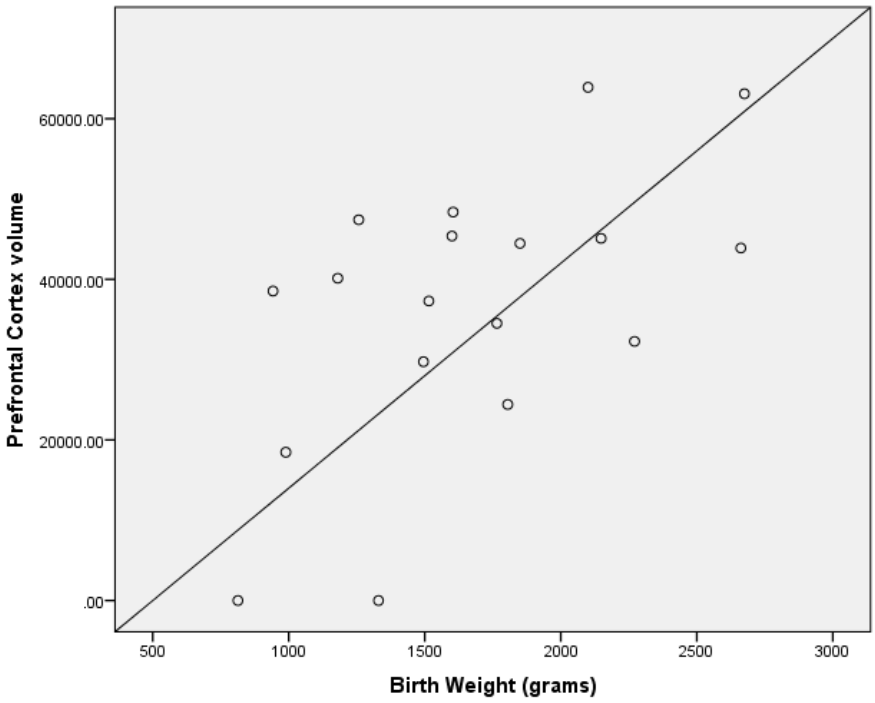

Figure 6.34 Correlation between birth weight and volume in the prefrontal cortex.

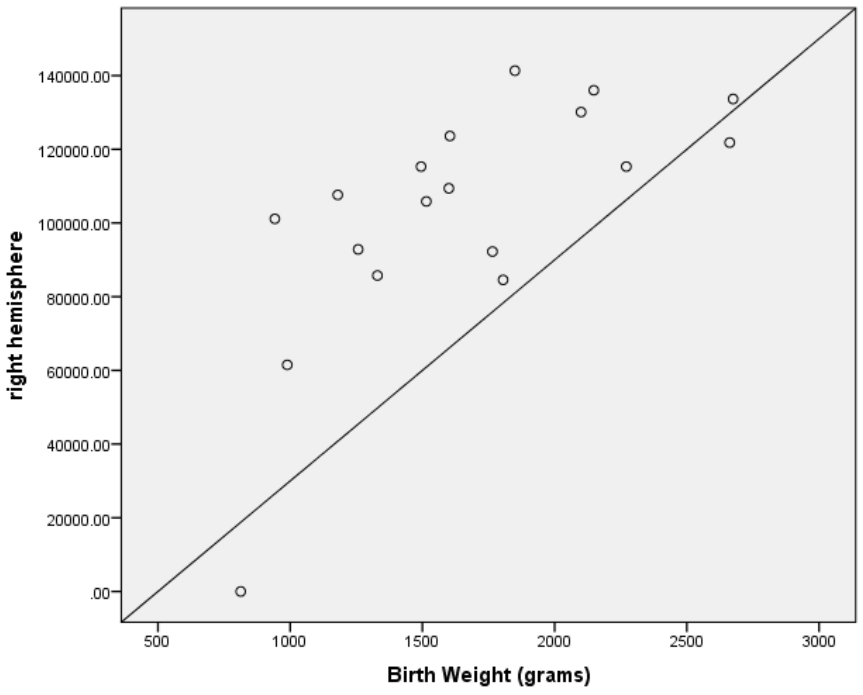

Figure 6.35 Correlation between birth weight and volume in the right hemisphere.

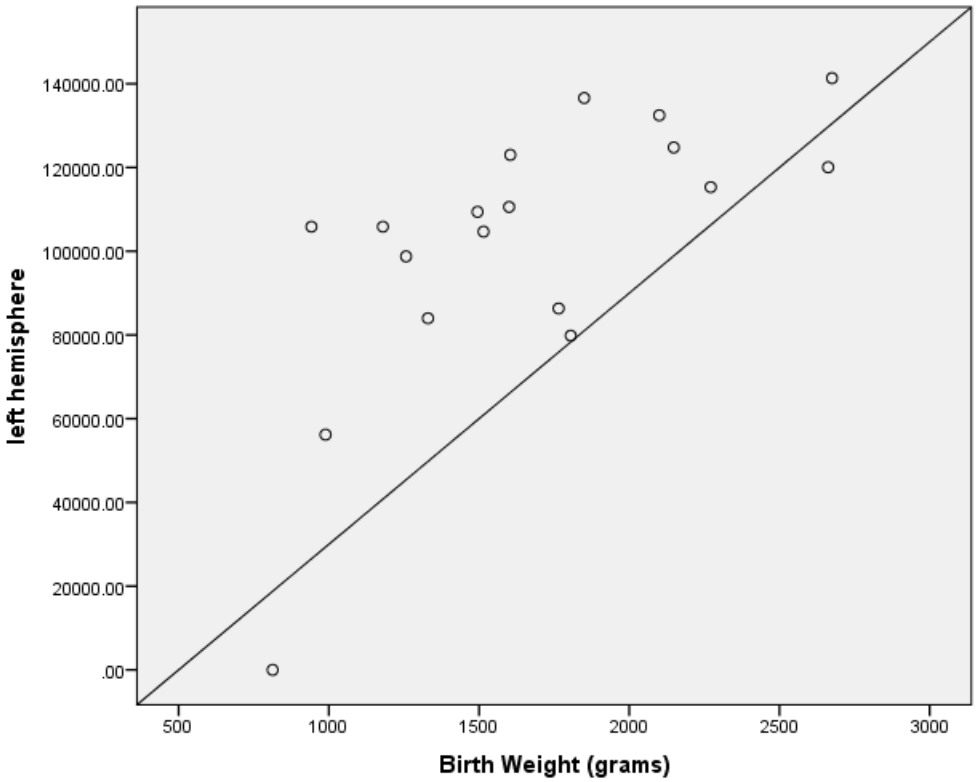

Figure 6.36 Correlation between birth weight and volume in the left hemisphere.

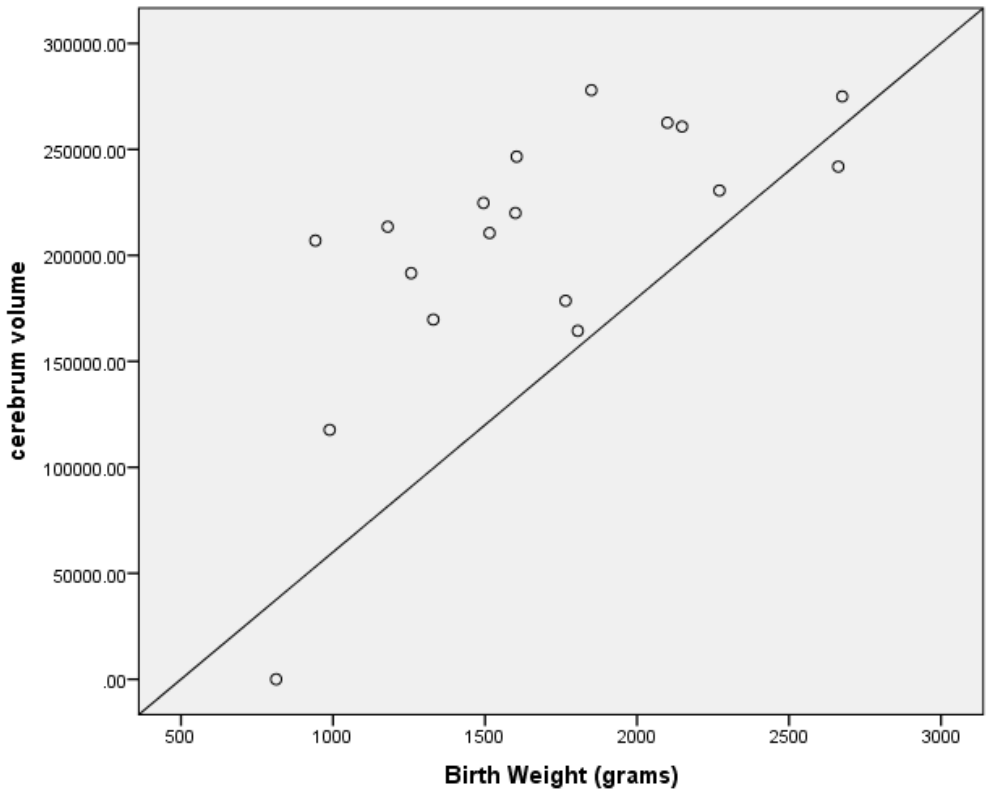

Figure 6.37 Correlation between birth weight and volume in the cerebrum.

Correlations between gestation at birth and volume were positive but generally not significant (Table 6.23). Positive significant correlations were found in the hemisphere volumes and total cerebrum (Table 6.23, Figures 6.38- 6.40).

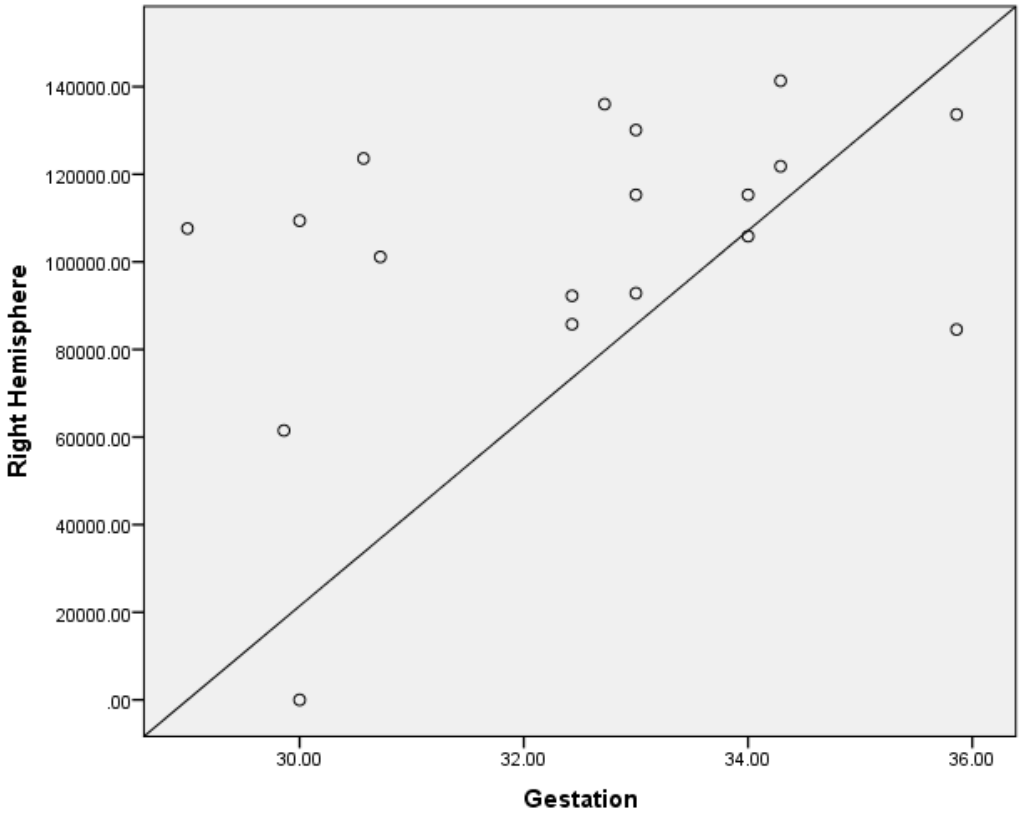

Figure 6.38 Correlation between gestation at birth and volume in the right hemisphere.

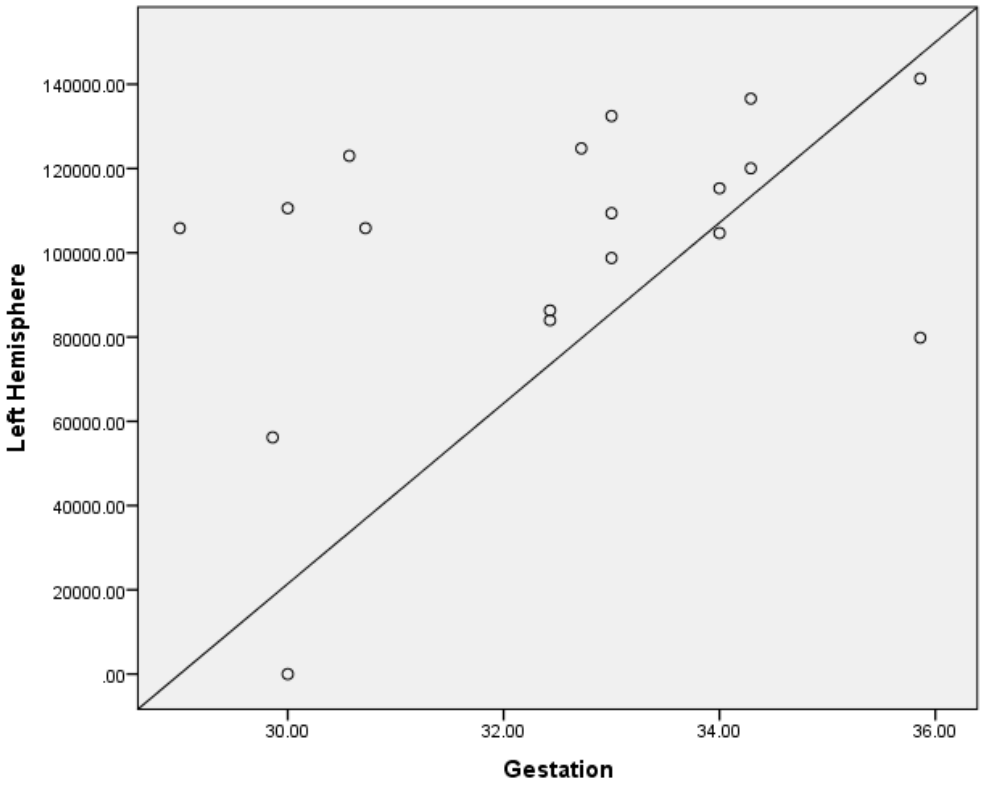

Figure 6.39 Correlation between gestation at birth and volume in the left hemisphere.

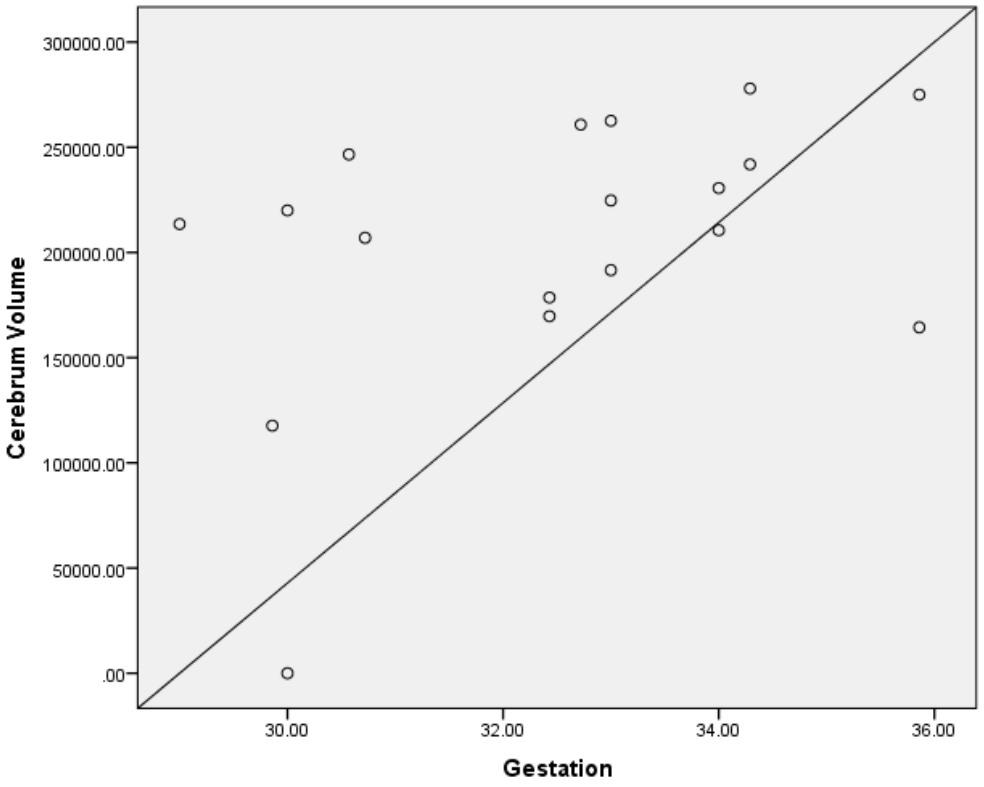

Figure 6.40 Correlations between gestation at birth and volume in the cerebrum.

CHAPTER 7

Discussion

Aim of Chapter

This chapter aims to discuss and interpret the results of this thesis in the light of current research and is split into five sections:

a) Limitations
b) DTI in the preterm cohort
c) DTI in the IUGR cohort
d) Stereology in the preterm cohort
e) Stereology in the IUGR cohort

Limitations

A limitation of this study is the limited age range of participants, preterm infants age ranged from 24-28 weeks, IUGR and the preterm controls ranged from 29-36 weeks. Another limitation is that infants were scanned without sedation increasing the risk of motion artefact.

DTI in Preterm Infants

ADC and anisotropy measures are known to change dramatically during development (Cascio, Gerig & Piven 2007; Drobyshevsky et al. 2005; Dubois et al. 2006; Neil 2002). ADC decreases with age whereas FA increases (Drobyshevsky et al. 2005). ***The hypothesis that higher FA and lower ADC will be found in infants with a higher birth weight and gestational age at birth (Drobyshevsky et al. 2005; Neil et al. 1998) and with which they will significantly correlate is refuted.*** DTI measures did not significantly correlate with birth weight except ADC in the right frontal lobe

which had a significant negative correlation. The only structure whose ADC correlated with gestational age in the right occipital lobe which had a negative relationship. Previous research has found DTI measures to vary with age (Neil 2002) with ADC decreasing and FA increasing (Drobyshevsky et al. 2007). Miller et al (2002) found anisotropy increased with age in all white matter regions in newborns classified as normal. Our research has not generally confirmed this finding although this could be due to small age range (24-27 weeks) available in this cohort. Differences may have been more likely to be observed with a larger age range. Other research has found FA to decrease with decreasing gestational age but no difference in ADC (Tatsuji 2011).

Comparison of Structures

In normal development the internal capsule and corpus callosum generally develop early with the internal capsule myelinating around term age (Cowana & de Vriesb 2005; O'Shea et al. 2005). They have a higher FA and lower ADC in comparison to other slower developing structures (Partridge et al. 2004). ***The hypothesis that higher FA and lower ADC will be found in the posterior part of the internal capsule and corpus callosum measured in both thyroxine and placebo groups is accepted*** with the results demonstrating higher FA and lower ADC in the posterior limb of the internal capsule and corpus callosum compared to the frontal and occipital lobes. Furthermore there was higher FA and lower ADC in the posterior compared to anterior corpus callosum and generally in posterior compared to anterior regions.

The results of DTI in this cohort of preterm infants are similar to the development pattern described above in previous research of normal development. However other research claims that anisotropy in white matter differs between preterm and term infants (Dudink 2008) and higher ADC values and lower relative anisotropy values have been found in the central white matter of preterm infants at term age compared to term infants born at term. Relative anisotropy values were also lower in the posterior limb of the internal capsule in preterm infants (Counsell et al. 2008). Preterm infants have been found to have reduced FA in the corpus callosum and increased FA in the posterior limb of the internal capsule (Padilla et al 2013)

although are study found higher FA in the corpus callosum and posterior limb of the internal capsule compared to frontal and occipital lobes. This study did not have infants born at term to confirm these findings but suggests even if the anisotropy parameters are lower in preterm infants, the pattern of development is still the same with the internal capsule and corpus callosum generally developing earlier.

Dudink et al refer to their sample as preterm infants with normal white matter development and normal developmental outcome (Dudink 2007). Their FA and ADC measures in the posterior limb of the internal capsule was similar to the present data (Table 7.1) although I found slightly higher FA and lower ADC suggesting the posterior limb of the internal capsule in the present cohort to show normal white matter maturation. The higher FA and lower ADC in the present cohort might be explained by the infants in this cohort being at a slightly higher postnatal age at time of scanning; for example scans were performed around 10 days post delivery compared to 4 days post delivery in Dudink et al's cohort.

Structure	Mean FA		Mean ADC	
	Atkinson et al	Dudink et al	Atkinson et al	Dudink et al
PLIC	*0.40*	*0.35*	*1.11*	*1.09*
CCA	*0.34*	*0.42*	*1.49*	*1.24*
CCP	*0.38*	*0.44*	*1.56*	*1.27*

Table 7.1: Comparing our FA and ADC measures with Dudink et al's

As no effect of treatment or laterality was found our FA and ADC measures combine treatment groups and left and right Posterior Internal Capsule to make them more comparable with Dudink et al's measures (Dudink 2007).

Comparing our FA and ADC values in the corpus callosum to Dudink's (Dudink 2007) are not as similar (table 7.1). Our findings show lower FA values and higher ADC values, which may reflect abnormal white matter maturation in the corpus callosum which if true may lead to later minor motor deficits (Abernethy, Cooke & Foulder-Hughes 2004).

Myelination sites begin to myelinate at different times and take different time periods to progress to maturity (Kinney 1988). The posterior limb of the internal capsule begins myelinating between 32 and 35 weeks gestation and is visible on MRI by 36-38 weeks(Cowana & de Vriesb 2005). Myelination within the internal capsule progresses towards the anterior interior capsule and should be complete by

4-5 months of age (Cowana & de Vriesb 2005; Kinney 1988). A lower ADC and higher FA are associated with more extensive myelination (Partridge et al. 2004).

A similar anterior-posterior pattern as described above was found within the corpus callosum. FA was significantly higher in posterior corpus callosum in comparison to the anterior in both the placebo and treated group. ADC values were lower in the anterior compared to posterior corpus callosum but this difference only reached significance in the placebo group. This is similar to previous research findings of myelination in the corpus callosum beginning in the body (between anterior and posterior) and splenium (posterior) before the rostrum (near the anterior or genu) (Kinney 1988) and further ***confirms the hypothesis that the posterior part of the corpus callosum will have higher FA and lower ADC.*** Furthermore statistically significant differences were found between the genu and splenium of the corpus callosum of rats as well as significant differences between anterior and posterior commissures (Sargon et al. 2003). Thus it seems possible that development follows an antero-posterior gradient with earlier development in the posterior part of the brain. This is further supported by previous research finding an increase in the number of myelinated and unmyelinated axons of white matter towards the more posterior regions (Ginus Partadiredja 2003).

There was a general pattern of significantly higher FA and lower ADC in posterior compared to anterior regions. The left posterior limb of the internal capsule had a significantly higher FA and lower ADC than the left frontal lobe and similarly the right posterior limb of the internal capsule had significantly higher FA and lower ADC than the right frontal lobe.

Marin-Padilla (1997) claim there is a progressive increase in the axonic fibres that follow the antero-posterior gradient, meaning the white matter of the frontal regions are more developed than the parieto-occipital at any prenatal age. Therefore we would expect the different structures in the brain will inherently have different FA and ADC measures (Marin-Padilla 1997).

In terms of the development of white matter tracts, the limbic fibres are first to develop, the commissural and projection fibres develop from the core to the

periphery and from anterior to posterior, the association fibres are last to develop (Huang 2006, 2010; Marin-Padilla 1997). As development of white matter tracts follows an antero-posterior gradient, at any prenatal age the frontal regions of the WM are more developed than the parieto-occipital regions (Marin-Padilla 1997).

The last process to occur is the maturation of white matter (Lagercrantz 2002) which forms from the core of the brain to the periphery and from the anterior to posterior regions (Huang et al. 2006). Therefore maturation proceeds from the occipital region anteriorly to the frontal and temporal lobes. This is confirmed by previous research which has found significant differences in number of myelinated and unmyelinated axons in white matter of Frontal compared to occipital regions with numbers increasing from frontal to parietal to occipital although the axons were larger in the frontal compared to occipital regions (Ginus Partadiredja 2003).

Even in the fully developed adult brain FA is expected to be higher and ADC lower in the corpus callosum and internal capsule compared to the frontal and occipital white matter due to the structure being less organised in the frontal and occipital white matter. This same pattern as expected has been shown here with generally significantly higher FA and lower ADC values in the corpus callosum and internal capsule in comparison to the occipital lobe and frontal lobe. Previous research also found higher FA in the corpus callosum and internal capsule in comparison to the other structures they measured (Dudink 2007; Partridge et al. 2004). However whereas Partridge et al found FA to be highest in the corpus callosum, this study found FA to be higher in the posterior limb of the internal capsule and found the difference to generally be significant.

A high FA is usually equated with a low ADC and represents extensive myelination (Partridge et al. 2004) and an increase in the complexity of white matter (Dudink 2008; Huppi et al. 1998). However despite significantly higher FA values in the posterior corpus callosum in both groups ADC values were also higher in the posterior corpus callosum whereas with extensive myelination you would expect to see lower ADC values. These differences in anterior-posterior ADC values only reached significance in the placebo group, so the treated group may possibly have

more extensive myelination and increased complexity of white matter. Furthermore abnormal neurodevelopment in preterm infants has been related to reduced development in the posterior corpus callosum (Counsell et al. 2008; Rose et al. 2009) and DTI measures of the corpus callosum in comparison to the other structures measured suggests this cohort may not have abnormal development particularly those in the treatment group.

This study has found the corpus callosum to have a significantly higher FA and lower ADC than most of the other structures (frontal lobes and occipital lobes) which can be equated to extensive myelination in the corpus callosum. Previous research has also found FA to be high in the corpus callosum particularly the splenium (aka posterior) and genu (aka anterior) (Partridge et al. 2004) which are measured in this study. Therefore at around term age FA in the corpus callosum generally seems to be quite high in comparison to other structures which can be associated with extensive myelination, despite this the corpus callosum is a structure found to be smaller in preterm infants in later childhood; aged 8 (Peterson et al. 2000) and 14 (Nosarti et al. 2004).

Thus it may be assumed the other parts of the brain found to have lower FA at term age in preterm infants (frontal lobes and occipital lobes) would have further diminished size later on in life than the corpus callosum. Although this may not be the case because of the complex process of human development where different parts of the brain develop at different rates (den Ouden 1996). Kinney et al found differences in the myelination sequences of different white matter sites during the first two years of postnatal life and that myelination not only begins at different times but also progresses to maturation over different time intervals (Kinney 1988).

The cerebellum (Allin et al. 2001; Peterson et al. 2000), basal ganglia, amygdala (Peterson et al. 2000) and hippocampus (Lodygensky et al. 2005; Nosarti et al. 2002a; Peterson et al. 2000) have been found to have smaller volumes suggesting the corpus callosum is not the only structure influenced by preterm birth even continuing into later childhood and adolescence. Furthermore Nagy found altered diffusion characteristics of preterm infants to remain prevalent once the infants

reached 11 years of age in the posterior limb of the internal capsule and the corpus callosum (NAGY 2003). The question arises about the effect of preterm birth on the other structures investigated in this study but not seen in other research later in childhood including the frontal lobe and occipital lobe. This is a suggestion for future research and a subject for exploration in later articles from this cohort when developmental follow up is available.

Another possible reason for the finding of high FA values in the corpus callosum of preterm infants at term, yet small size later on in childhood, is that although high FA is associated with extensive myelination it has also been found prior to myelination and could be related to changes in the development of fibre tracts in central white matter such as the 'early wrapping of axons by oligodendroglial processes' (Barrett 2010; Huppi et al. 1998; Snell 2010). This has been confirmed by previous research finding high anisotropy values in the corpus callosum of preterm infants even though it is mostly unmyelinated (Partridge et al. 2004). Furthermore both the unmyelinated corpus callosum at 28 weeks gestation and the posterior limb of the internal capsule which at 28 weeks gestation is in the process of myelination demonstrated anisotropy indicating that anisotropy can be caused by both unmyelinated and myelinated fibres (Huppi et al. 1998).

Asymmetry

In healthy individuals brain structures are generally found to be symmetrical (Filipek et al. 1994; Snook, Plewes & Beaulieu 2007), however asymmetries have been found in the occipital and frontal lobes. The left occipital lobe is often wider than the contra lateral and the contra lateral frontal lobe usually extends further than the left (Le May & Kido 1978) i.e. the left occipital lobe is larger than the right and the right frontal lobe is larger than the left (Petty 1999). This has been confirmed by Weinberger et al who also found the right frontal lobe and contra lateral occipital lobe to be larger in the majority of brains measured. These asymmetries were observed in foetuses as young as 20 weeks gestation indicating that this asymmetry is an early manifestation of human brain development (Weinberger et al. 1982). ***The hypothesis that generally there will be no asymmetries present in the structures***

measured is refuted. Although asymmetry may be found in the occipital and frontal lobes and dorsal lateral prefrontal cortex is accepted. Significant differences between the right and contra lateral sides of a structure has been found in FA in the posterior limb of the internal capsule in both the placebo and thyroxine group, occipital lobe in the thyroxine group and frontal lobe in the placebo group. ADC measures demonstrated less asymmetry with only significant differences in the left occipital lobe although the ADC differences between right and contra lateral in the posterior limb of the internal capsule were close to significance.

The second part of the hypothesis that the right frontal lobe and left occipital lobe will have higher FA and lower ADC is refuted. In both the placebo and thyroxine groups FA was higher in the left compared to contra lateral frontal lobes and reached significance in the placebo group,although ADC findings of the frontal lobes was similar to the hypothesis in the placebo group with lower ADC in the right frontal lobe. However this finding was not significant and not true in the thyroxine group where lower ADC was seen in the left frontal lobe. Opposite to the hypothesis, FA was significantly higher in the right occipital lobe in the thyroxine group although the ADC measure was similar to the hypothesis with lower ADC in the left Occipital lobe in the thyroxine group although this finding was not significant. Similar to the hypothesis, FA was higher in the occipital lobe in the placebo group but this finding was not significant and not associated with lower ADC as ADC was significantly higher in the left occipital lobe.

I hypothesised higher FA and lower ADC in the right frontal lobe and left occipital lobe based on findings of larger right frontal and contra lateral occipital lobe (Le May & Kido 1978; Petty 1999; Weinberger et al. 1982). ***The hypothesis was refuted*** as FA was higher in the left frontal lobe (both treatment groups) and contra lateral occipital lobe (thyroxine group) and when there were any similarities to the hypothesis the findings were not generally significant. FA is the same between left and contra lateral occipital lobe in the placebo group and higher in the contra lateral in the thyroxine group. ADC was significantly higher in the left occipital lobe in the placebo group. In the thyroxine group ADC was similar to the hypothesis with lower ADC in the left although this was not significant. Thus although previous

research has found larger right and left frontal lobes, a larger structure does not necessarily mean higher FA and lower ADC.

Laterality

In the placebo group FA was higher in the right and contra lateral occipital lobe but significantly higher in the right in the thyroxine group suggesting extensive myelination in the right compared to contra lateral occipital lobe in the thyroxine group. Reductions in FA can indicate reduced myelination, axonal damage or decreased fibre coherence (Counsell et al. 2008) so the right occipital lobe may have reduced myelination, decreased fibre coherence or axonal damage in comparison to the left occipital lobe in preterm infants who have received levothyroxine.

FA measures show extensive myelination in the right occipital lobe in the thyroxine group and ADC measures show the same in the placebo group. The significantly higher FA in left compared to contra lateral frontal lobe in the placebo group suggests extensive myelination in the left frontal lobe in the placebo group.

FA was found to be significantly higher in the left posterior limb of the internal capsule compared to the contra lateral in the treatment group but lower in the control group receiving placebo. ADC was lower in the right posterior limb of the internal capsule in both the treated and non-treated group. Abnormal motor outcome can be predicted around 36-38 weeks gestation by the absence of myelin in the posterior limb of the internal capsule (Counsell et al. 2002; Rose et al. 2009) and Evensen et al (2011) found lower FA values in the major central, anterior and posterior tracts to be related to lower scores on the Movement Assessment Battery for Children-2 and on the High-Mobility-Tool (Evensen et al. 2011).

Differences in FA and ADC measures between the laterality of a structure have been observed in this study and previous research (Rose et al. 2009; Szeszko 2003). Previous studies have shown the importance of these laterality differences to be significant for later development. FA has been found to be higher in the left hemisphere of woman healthy volunteers which correlated with better verbal comprehension and long term recall of words (Szeszko 2003). Rose has shown that

in normal development of preterm infants the left side has lower FA whereas infants with abnormal neurodevelopment had higher FA in the left although this finding was only within the posterior limb of the internal capsule (Rose et al. 2009) and has not been replicated so should be taken with caution, particularly as other researchers have found asymmetry to be a sign of abnormality . Further analysis of laterality showed a significant laterality by treatment effect in the frontal lobe and posterior limb of the internal capsule, meaning the administration of thyroxine affects these structures on different sides of the brain differently, however the effect of treatment was non-significant although with even further analysis the left internal capsule was found to have significantly higher FA in the treated group. This suggests a possible improvement in development of the internal capsule in treated infants as FA increases with age (Drobyshevsky et al. 2005) and therefore development.

Thyroxine

Thyroid hormones have an important role in the developing brain (Bernal & Nunez 1995; Morreale de Escobar 2001; Ng et al. 2008; PORTERFIELD & HENDRICH 1993; Williams, Visser & Hume 2006) and contribute to the regulation of the myelination process (Ng et al. 2008). Low levels of this hormone have been associated with poor neurodevelopmental outcome (Caroline Delahunty 2001; den Ouden 1996; Ishaik et al. 2000; Morreale de Escobar 2001; Ng et al. 2008; Osborn 2009; Reuss et al. 1996; Siu 2002; Van Wassenaer 2002; Williams, Visser & Hume 2006) and have been found to be common in the first few weeks of life in preterm and low birth weight infants (Siu 2002). It has been thought by raising these low levels to norm with levothyroxine administration will minimise any poor neurodevelopmental outcome. Researchers of congenital hypothyroidism (CH: lack of thyroid hormone) have argued that levothyroxine treatment is needed to minimise neurocognitive impairment. When the dose of levothyroxine is tailored according to severity, FT4 i.e. free thyroxine (Lafranchi 2008) and TSH i.e. thyrotropin levels have been found to quickly normalise resulting in an improvement in IQ aged 4 (Salerno et al. 2002). Total thyroxine (T4) levels have also been found to be higher in infants treated with thyroxine (Siu 2002).

However other previous research has generally found no positive effect of levothyroxine supplementation (Oden 2002; Osborn 2001; Smit et al. 1998; Smith 2000; van Wassenaer 1997; van Wassenaer et al. 1997a; Vanhole et al. 1997) and has concluded further research is needed (Briet et al. 2001). Sub analysis in van Wassenaer et al's study found a possible benefit of levothyroxine in infants born under 27 weeks gestation (van Wassenaer 1997; van Wassenaer et al. 1997a) and another found benefit under 29 weeks with infants born at 25/26 weeks gestation benefiting especially (Briet et al. 2001),although the results from Van Wassenaer's articles (van Wassenaer 1997; van Wassenaer et al. 1997a) should be treated with caution (Osborn 2001). This study found a significant interaction when investigating anterior vs. posterior structures and laterality of structures with treatment in the frontal lobes and posterior limb of the internal capsule suggesting a group difference between infants treated with levothyroxine and those receiving placebo.

Thus the hypothesis that supplementation with levothyroxine will be associated with the DTI measures FA and ADC is confirmed. Further analysis revealed FA to be borderline significantly higher in thyroxine group in the posterior limb of the internal capsule and ADC to be borderline significantly higher in the placebo group in the left frontal lobe. There was no significant difference between treatment groups in FA of the left frontal lobe, ADC of the left posterior limb of the internal capsule, anterior and posterior corpus callosum. ***The hypothesis that within the thyroxine group there will be higher FA and lower ADC is refuted as generally the findings were not significant.***

In this study total T4 (TT4) and FT4 levels were higher in the thyroxine group until week 36 and free T3 (FT3) levels post screening were lower in the thyroxine group. Previous research has found higher TT4 levels in infants treated with levothyroxine although by week 3 levels were close to those in the placebo group (Vanhole et al. 1997) and FT4 levels to be significantly higher in a thyroxine compared to placebo group (van Wassenaer et al. 1997a). At screening there was no significant difference between groups. At day 14 and 21 TT4 and FT4 were significantly higher in the thyroxine group. At Day 28 FT4 was significantly higher in the thyroxine group but by week 36 there were no significant differences between groups thus the

hypothesis that FT4 levels will have a temporary increase following thyroxine supplementation in comparison to the placebo group but that levels will stabilise between groups over time is confirmed. Supplementation with thyroxine causes a temporary increase in thyroxine levels although by week 36 those who received placebo have also increased and are similar to the levels of the thyroxine group. Similarly in previous research TT4 and FT4 levels at screening were similar and then rose in the treated group with FT4 remaining higher in the treatment group until week 8 and TT4 is considerably higher in the treatment group until week 3, after which it remains higher but with minimal difference between groups (van Wassenaer et al. 1997a; Vanhole et al. 1997).

In the thyroxine group split for high and low levels of TT4, FT4 and TT3 at screening, there was no significant differences between levels at day 14, 21, 28 and week 36. In the placebo group split for high and low levels of TT4, FT4 and TT3 at screening, there was no difference between levels at day 14 and 28 but at day 21 and week 36 TT4 and FT4 levels were significantly lower in the low level group. As there is no difference in the treatment group between those that had low or high levels at screening but TT4 and FT4 levels are significantly lower in the low group in the placebo group suggests a need for thyroxine in those with low levels at screening.

Comparing the pattern of increases in TT4, FT4 and TT3 (see figure 7.1) between our results and those of previous research by Van Wassenaer demonstrated an increase in TT4 levels from after day 14 to day 28. Van Wassenaer found this increase to begin as early as 7 days but this research has no data from day 7 although it is quite possible there was a gradual increase prior to day 14 also (van Wassenaer 1997).

Van Wassenaer found lower levels of FT4 in infants born under 28 weeks gestation continually (van Wassenaer 1997) and this study found FT4 levels to be rising since screening at day 14 and day 21 but by day 38 levels had lowered slightly. Both studies show an increase in T3 levels over time from day 7 until week eight.

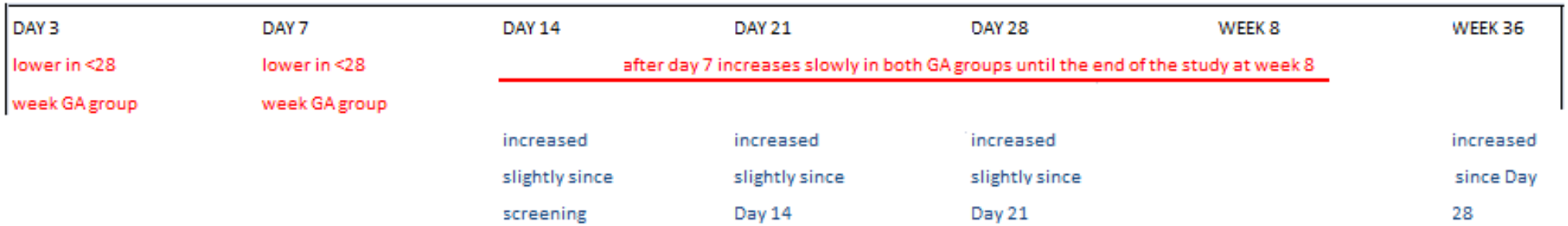

Figure 7.1 Comparison of FT4, TT4 and TT3 levels in van Wassenaer (1997) and this study overtime. Red = van Wassenaer, blue= the present cohort.

TT4 and FT4 levels at screening significantly correlated in a positive direction with gestational age and at 36 weeks TT4 levels significantly positively correlated with gestational age and birth weight ***confirming the hypothesis that T4 levels will positively correlate with gestational age and birth weight.*** The older the premature infants were at birth the higher TT4 and FT4 levels they had at screening. The higher the infants birth weight and the older they were at birth the higher TT4 levels were at 36 weeks. Similarly previous research has found younger infants (23-28 weeks gestation) to have significantly lower T4 and T3 values compared to older infants (29-31 weeks gestation) (Mercado et al. 1988). The age range at birth in this cohort was 24- 27 weeks so this research has shown small differences in gestational age impact levels of thyroxine in the blood significantly.

Furthermore other research found the percent of infants with low levels of thyroxine (three standard deviations below the mean) to increase with decreasing gestational age (den Ouden 1996). These low levels of thyroxine have been found to affect later development with neurologic dysfunction age five and school failure age nine significantly related to lower levels of thyroxine. (den Ouden 1996). Another study found low thyroxine values were associated with a delay in psychomotor development, infants with lower levels of thyroxine had a significantly increased risk of developmental delay (Meijer 1992). Also severe hypothyroxinemia (clinically defined low levels of thyroxine 3 standard deviations below the mean) in infants demonstrated an increased risk for cerebral palsy (Reuss et al. 1996). This cohort will be followed up throughout development as part of the TIPIT project (Ng et al. 2008).

Negative Effects of Administering Levothyroxine

Negative effects of levothyroxine supplementation have included refractory hypotension (Okada et al. 2011) in the short term and behavioural problems longitudinally (Briet et al. 1999). In one study 44% of infants who received levothyroxine treatment developed refractory hypotension and the occurrence of refractory hypotension was associated with prolonged mechanical ventilation and increased hospitalisation. Although those who developed refractory hypotension had significantly lower free T4 (FT4) values prior to

treatment (Okada et al. 2011). Behavioural problems aged 2 have been found in infants born under 27 weeks gestation who received treatment with levothyroxine although the authors concluded these problems were not related to treatment (Briet et al. 1999). Furthermore a review article found evidence of behavioural problems to be weak although this specifically looked at high starting doses (Hrytsiuk et al. 2002).

Gender

Previous research has generally found sex differences in the development of the brain after premature birth, with males seeming to be more vulnerable to the effects of prematurity (Kesler et al. 2008; NuÑEz & McCarthy 2003; Rose et al. 2009). DTI measures in this study demonstrated sex differences in the placebo group but not the thyroxine group. FA was significantly lower in males in the right and left posterior limb of the internal capsule and ADC was significantly higher in the anterior corpus callosum ***supporting the hypothesis that males will have lower FA and higher ADC than females.*** Thyroxine treatment may be of particular benefit in males as the lower FA and higher ADC in males observed in the placebo group was not replicated in the treatment group. Previous research has also found lower FA in males particularly in the left frontal lobe (Szeszko 2003). FA in the placebo group of our study was lower in males in the left frontal lobe but not significantly.

Smoking

After excluding those mothers who smoked during and prior to pregnancy there was no significant difference in DTI measures between the infants treated with thyroxine and those receiving placebo. Smoking may have influenced DTI measures in the thyroxine group resulting in higher FA in the left posterior limb of the internal capsule and lower ADC in the left frontal lobe,although with the low numbers of participants this finding needs to be taken with caution.

Alcohol

The effects of alcohol were also explored with a small sample of infants from the full cohort (table 5.19). In the placebo alcohol group there was higher FA and ADC but this difference

was not significant. In the thyroxine alcohol group there was lower FA and higher ADC, FA was significantly lower in the left occipital lobe and contra lateral posterior limb of the internal capsule and ADC was significantly higher in the left occipital lobe and borderline significant in the left frontal lobe. The effects of alcohol on DTI measures seemed to be exaggerated in the thyroxine group. The low number of participants in each group limits the strength of the results. Alcohol decreases the activity of the central nervous system (Rajendram 2005). In the thyroxine group our results add to this finding, showing reduced FA and higher ADC.

DTI in IUGR Infants

The preterm infants in the IUGR cohort also demonstrated higher FA and lower ADC in the posterior limb of the internal capsule and corpus callosum compared to the frontal and occipital lobes suggesting the overall developmental program remains intact despite the lower values in preterm compared to term infants in previous research (Counsell et al. 2008; Dudink 2008; Partridge et al. 2004). ***The hypothesis that the posterior part of the internal capsule and corpus callosum will have higher FA and lower ADC than the other structures is further confirmed in the IUGR cohort.***

There was generally lower FA in the IUGR infants (lower in frontal lobes, right occipital lobe, posterior limb of the internal capsule and corpus callosum) although in the left occipital lobe FA was lower in the control group suggesting that in the majority of structures there are less barriers to diffusion in the IUGR group. Thus there may reduced myelination, axonal damage or decreased fibre coherence (Counsell et al. 2008) in the IUGR compared to controls. However a low FA is usually equated with a high ADC and in this cohort ADC was generally lower in the majority of structures (frontal, occipital lobes and corpus callosum). ADC values were similar between groups in the internal capsule. This did not support previous research which found higher ADC in IUGR infants at birth and term although our findings of lower FA values in the corpus callosum are similar to the findings of lower relative anisotropy in this structure (Zimine 2002). ***The hypothesis that lower FA will be found in the IUGR group is***

confirmed although the hypothesised higher ADC was not observed in this cohort of IUGR infants.

Similar to our results lower FA has been found in IUGR group of a rabbit model of IUGR, although their findings reached significance whereas in our cohort of IUGR infants the differences were not significant. Their ADC findings are different to ours as they found non significantly higher ADC in the IUGR group (Eixarch 2012) but matched other previous research (Zimine 2002).

Padilla et al (2014) found FA in the splenium of the corpus callosum to be decreased in IUGR infants where as we found FA to be higher in the posterior corpus callosum and to be decreased in the anterior corpus callosum. They also found IUGR infants to have increased FA in the fronto-occipital white matter tracts. Similarly we found FA to be higher in the left occipital lobe in the IUGR group although we found FA to be lower in the IUGR group in the left and contra lateral frontal lobe as well as the right occipital lobe. In our research FA in the posterior limb of the internal capsule was lower in the IUGR compared to the control group. In the anterior corpus callosum FA was lower in the IUGR group where as Padilla et al (2014) found IUGR infants to have higher FA in the anterior regions involving commissural fibres (genu, the body and forceps minor of the CC); projection fibres (anterior, superior and posterior corona radiate, the left anterior limb of the internal capsule) and association tracts (right frontal course of the uncinate fasciculum and inferior fronto-occipital fasciculum and left external capsule including the superior longitudinal fasciculum).

Difference between FA values in groups was not significant but ADC values were significantly lower in the IUGR group in the frontal lobes and close to significance in the left occipital lobe. It seems there may be more barriers in the form of structure development in the frontal lobes and left occipital lobe of the IUGR group. Previous research also found differences in the structure of IUGR infants' brain at 12 months corrected age compared to appropriate for gestational age preterm infants and argued that their findings suggests that IUGR cause a deviation in the development of the brain (Padilla et al. 2011).

Laterality

FA was lower and ADC generally higher in the right structures compared to contra lateral although these differences were not significant. ***This confirms the hypothesis that generally there will be no asymmetries present in the structures measured although asymmetry may be found in the occipital and frontal lobes.*** Indeed with further analysis structural asymmetry was found in the occipital lobe. There was a significant interaction between laterality and group in the occipital lobe and further analysis revealed significantly higher FA in the left compared to contra lateral occipital lobe in the IUGR group but no significant difference in the control group. ***The second part of the hypothesis that the right frontal lobe and left occipital lobe will have higher FA and lower ADC is refuted*** as although there is a finding of significantly higher FA in the left compared to contra lateral occipital lobe this was not associated with the lower ADC, furthermore FA and ADC differences between right and contra lateral frontal lobe were not significant.

Previous research has also found lower FA in right occipital lobe (Sarina et al. 2011) but this was in healthy adults where as our infants were all born preterm and the finding was only present in those with IUGR. It is strange that the IUGR cohort should be similar to healthy adults as IUGR infants are said to be of higher vulnerability (Esteban et al. 2010). Differences between the present study and their's were observed in the frontal lobes where they found higher FA in the right frontal lobe and we found lower FA (Sarina et al. 2011) although our finding of lower FA is not significant. They argue their findings of greater FA and less perpendicular diffusion in the left occipital tracts may be due to greater myelin in these axons compared to the right, however in our study this was found in the IUGR but not control infants. Furthermore previous research refutes their idea of greater myelin in the axons of the left occipital tracts by finding no difference in the number of myelinated and unmyelinated axons in white matter between left and right hemisphere in any region (Ginus Partadiredja 2003).

A further study looking at both male and female healthy adults found higher FA in the left hemisphere in 83% of the subjects (Kang, Herron & Woods 2011). Our finding supports this

with FA generally being lower in right structures, suggesting hemispheric asymmetries begin early in development. FA in grey matter showed no significant differences between the whole hemisphere however when looking at the different cortical lobes differences were found with higher FA in the right frontal lobe and higher FA in the left temporal, parietal and occipital lobes (Kang, Herron & Woods 2011). These FA findings in the frontal lobe match those of another study mentioned earlier on healthy males but are the opposite of our findings where FA was higher in the left frontal lobe. Similarly their findings match with lower FA in the parietal lobes in both studies. However their results differ in the occipital lobes. Sarina et al found higher FA in the left which matched our findings in the IUGR group whereas Kang et al found lower FA in the left which matched the findings in our controls (Kang, Herron & Woods 2011; Sarina et al. 2011).

FA was higher in the left posterior limb of the internal capsule in the IUGR group but higher in the right in the control group, which based on previous research would suggest abnormal development in the IUGR group and normal development in the control group (Rose et al. 2009) IUGR infants are said to be of higher vulnerability so abnormal development is to be expected(Esteban et al. 2010). Although as mentioned previously Rose et al's results should be taken with caution as there results have not been replicated and other researchers argue asymmetry is a sign of abnormality (Filipek et al. 1994; Snook, Plewes & Beaulieu 2007).

Correlations

In the IUGR FA positively correlated with birth weight and gestational age at birth whereas ADC negatively correlated. The older the infants were at birth and the higher the birth weight the higher the FA values and the lower the ADC values although the differences were not significant. In the control group the opposite was seen with FA negatively correlating with birth weight and gestation at birth, whereas ADC positively correlated. The older the infants at birth and the higher their birth weight the lower the FA values and the higher the ADC values. In the control group these differences almost reached significance, with FA in the right occipital lobe and birth weight and FA in the right occipital lobe and gestational age at birth. ***In the control group the hypothesis that DTI measures will correlate with***

gestational age with FA positively correlating and ADC negative correlating is confirmed., Only the first part of the hypothesis that DTI measures will correlate is true for the control group. The findings in the control infants are not similar to previous research which has found FA to increase with increasing gestational age (Drobyshevsky et al. 2007; Tatsuji 2011) and ADC to decrease (Drobyshevsky et al. 2007) but this is similar to our findings in the IUGR group. Possibly higher birth weight and gestational age at birth is a protective factor for infants born with IUGR.

Gender

Differences in DTI values between male and female infants were observed in this study and the gender affect on DTI values was different between groups. In the IUGR group higher FA was observed in males except in the left posterior limb of the internal capsule, lower ADC in the frontal lobes and posterior corpus callosum and higher ADC in the occipital lobes, posterior limb of the internal capsule and the anterior corpus callosum. These findings were not significant although the higher ADC in males in the anterior corpus callosum was close to significance. In the control group males had lower FA in all structures except the left frontal lobe and posterior corpus callosum and higher ADC in all structures except the left frontal lobe. These findings were not significant***. In the control group the hypothesis that males will have lower FA and higher ADC is confirmed but in the IUGR group only higher ADC in the occipital lobes, posterior limb of the internal capsule and corpus callosum support the hypothesis.***

Previous research has found significant gender differences in FA, with FA being higher in males in regions surrounding the temporal/ parietal/ occipital junction (Kang, Herron & Woods 2011) and in the corpus callosum (Westerhausen et al. 2004). This is similar to our finding in the IUGR group in the posterior limb of the internal capsule but the opposite of the findings in the control group. Higher FA in males was also observed in our cohort of normal preterm infants. It has been demonstrated in previous research that the higher FA in the corpus callosum of males may be due to increased brain size in males as demonstrated when brain size as a covariate removed the gender differences (Westerhausen et al. 2004).

ADC values have been found to be significantly higher in women less than 60 years of age compared to men in right frontal, bilateral thalamus and temporal but no difference between males and females over 60 years (Naganawa et al. 2003). Our results show a similar result of ADC measures in preterm infants and infants born with IUGR with ADC values in the frontal lobes to be lower in males except for the right frontal lobe in the control group.

Stereology in the Preterm Cohort

In the preterm cohort lower volumes were found in the placebo group in the dorsal lateral prefrontal cortex, orbital lateral prefrontal cortex, left dorsal medial prefrontal cortex, right and left hemisphere volumes compared to the thyroxine group. ***The hypothesis that supplementation with levothyroxine will be associated with volume of brain structures and the hypothesis that within the thyroxine group there will be larger hemisphere and brain structure volumes than the placebo group is confirmed.*** There were higher volumes in the right dorsal medial prefrontal cortex and orbital medial prefrontal cortex. In the right dorsal lateral prefrontal cortex the lower volumes in the placebo group were significant and in the contra lateral the difference between groups were close to significance. No other significant differences between groups in volumes were observed. In previous research there is evidence of smaller structural volumes in preterm infants in the cerebellum (Allin et al. 2001). IUGR infants have been shown to have a reduction in structural volume including intracranial volume, cortical gray matter , total brain volume (Tolsa et al. 2004), the hippocampus (Lodygensky et al. 2008; Padilla et al. 2014), insular, temporal lobe (Padilla et al. 2011; Padilla et al. 2014; Sanz-Cortes et al. 2010b), absolute gray matter, the amygdala, the right perirolandic area, right frontal lobe, left parietal lobe, basal ganglia, thalami, angular gyrus and right frontal lobe (Padilla et al. 2014). Padilla et al (2011) also found reduced gray matter in the temporal, parietal, frontal and insular regions and reduced white matter in the cerebellum but increased white matter in temporal, frontal, parietal, occipital and insular regions.

Higher dorsal and orbital volumes have been associated with better neurocognitive performance (R.E. Gur. 2000) and generally there were higher dorsal and orbital volumes in the thyroxine group for the majority of regions (right and left dorsal lateral, right and left orbital lateral and left dorsal medial). Thus thyroxine treatment may have a positive effect on the volume of dorsal and orbital structures which may later result in improved neurocognitive performance.

This research found significantly higher volumes in the medial compared to lateral cortex and dorsal compared to orbital regions. This is the same as previous research in healthy adults and patients which found dorsal regions to be larger (Howard 2003).

Asymmetry

Some previous research has found asymmetry in the volumes of structures such as larger right dorsal lateral regions in healthy controls (R.E. Gur. 2000) where as other previous research has found no significant difference between the weight of the right and contra lateral hemispheres (Ginus Partadiredja 2003).

Asymmetry in this research demonstrated lower volumes in the right hemisphere except in the thyroxine group the contra lateral hemisphere had lower volumes, although differences between right and contra lateral were only significant for the medial structures and thus ***the hypothesis that there will be no asymmetries present in the structures measured is accepted. However the second part of the hypothesis that there will be asymmetry in the volume of the dorsal lateral prefrontal cortex is refuted,*** as although there were differences between left and contra lateral dorsal lateral prefrontal cortex these differences were not significant. Furthermore the differences observed between left and contra lateral dorsal lateral prefrontal cortex do not match the differences found in previous research, Kang et al found the fibre tracts connecting the dorsal lateral prefrontal cortex to have greater FA in the right hemisphere (Kang, Herron & Woods 2011) similar to Gur's finding of larger dorsal lateral regions on the ipsilateral (R.E. Gur. 2000), whereas we found lower volume in the ipsilateral hemisphere. Furthermore Howard found the right dorsal lateral prefrontal cortex to be larger than contra lateral (Howard 2003), whereas our results demonstrate a larger

left dorsal lateral prefrontal cortex. The differences between these findings and previous research could be because the present cohort is of preterm infants whereas previous studies were of healthy adults and patients (Howard 2003; Kang, Herron & Woods 2011; R.E. Gur. 2000).

The hemisphere volume in the thyroxine group was similar to previous research in young adult normal subjects, where volume was lower in the left compared to contra lateral hemisphere (Kang, Herron & Woods 2011). Thus thyroxine supplementation may normalise laterality in the hemispheres volumes, and infants born preterm may have different brain lateralisation to infants born at term.

Gender

Males seem to be more vulnerable to the effects of preterm birth and male gender has been reported as a risk factor for poor neonatal outcome, poor neurological outcome, poor respiratory outcome (Peacock et al. 2012) and even mortality (Kusuda et al. 2012). Similarly Britt et al 2011 found boys to have significantly lower Apgar score and a longer ventilation period (Britt J. M. 2011).

Previous research has found sex differences in brain volume; male brains being larger in volume overall and in all brain regions (Filipek et al. 1994; Shah et al. 2006; Thompson et al. 2007). This research has found sex differences in the volumes of various regions of the prefrontal cortex. In the placebo group males had lower volumes except in the left dorsal medial prefrontal cortex. These differences reached significance in the orbital medial prefrontal cortex, and ***accept the hypothesis that males will have lower structural volumes than females***. In the thyroxine group females had lower volumes except the left dorsal medial prefrontal cortex. These differences reached significance in the right orbital lateral prefrontal cortex and hemisphere volumes. The lower volume in the right dorsal lateral prefrontal cortex in females was close to significance. ***In the thyroxine group the hypothesis is refuted*** with males having higher structural volumes than females. Similar to the finding of lower left dorsal medial prefrontal cortex volume in the placebo group, Shah et al (2006) found females to have lower dorsal prefrontal region volumes. Results in the thyroxine

group differed to the findings in the placebo group and Shah et al's (2006) research with higher volumes in the dorsal medial prefrontal cortex.

Correlations

The only structural volume found to correlate with birth weight and gestational age was the orbital medial prefrontal cortex. No structures volume correlated with gestation at scanning. There was a significant positive correlation with the right and left orbital medial prefrontal cortex and gestation at birth and a borderline significant correlation between the left orbital medial prefrontal cortex and birth weight. Previous research has generally been mixed, Peterson et al (2003) found regional volumes not to correlate with gestation at birth where as Peterson et al (2000) found volume in the right sensori motor to positively correlate with gestational age and Nosarti et al (2002) found the more premature the subject the smaller the white matter volume although no other brain regions varied according to gestational age or birth weight. Similarly Peterson et al (2000) found birth weight was not significantly associated with regional volumes where as Ivanovic et al (2004) found brain volume to significantly positively correlate with birth weight. Padilla et al (2013) found FA to positively correlate with birth weight and gestational age where as in this research DTI measures in preterm infants did not correlate with birth weight or gestational age.

Smoking

After excluding those mothers who smoked during and prior to pregnancy, volumes were generally lower in the placebo group. In the right dorsal lateral prefrontal cortex the difference was no longer significant and the lower volume in the placebo group in the ipsilateral hemisphere became borderline significant.

Alcohol

The effects of alcohol on structural volumes were different between the two treatment groups. In the placebo group volumes were higher in the alcohol group except in the left dorsal medial prefrontal cortex and significantly higher in the right and contra lateral

hemisphere volumes. Higher volumes in the placebo-alcohol group were close to significance in the right orbital medial prefrontal cortex. In the thyroxine group structural volumes were generally lower in the alcohol group except for the right dorsal medial prefrontal cortex. Differences in the thyroxine group were not significant.

Stereology in the IUGR Cohort

Structural volumes were significantly higher in the medial compared to lateral cortex and dorsal compared to orbital regions. As this finding is based on the whole cohort I tested for interaction with group. When comparing medial and lateral there was a significant interaction in the dorsal regions and close to significant interaction in the left orbital region and when comparing dorsal and orbital regions there was significant interaction in both the medial regions and the left lateral region. This interaction with group suggests there is a difference between the group's structural volumes. Further analysis showed structural volumes to be significantly higher in the medial compared to lateral cortex and dorsal compared to orbital regions in both the IUGR and control group. This adds to and confirms previous research in healthy adults and patients which found dorsal regions to be larger than orbital regions (Howard 2003). Highest volumes were observed in the dorsal medial prefrontal cortex. Total prefrontal cortex, right and left hemisphere and cerebrum volumes were higher in the control group suggest IUGR to affect volume. Furthermore volumes were significantly lower in the IUGR group except for the left orbital lateral prefrontal cortex and right orbital medial prefrontal cortex. ***Thus the hypothesis that IUGR infants will have smaller brain structure volumes than controls is accepted.*** This supports previous findings of significantly lower brain volumes in IUGR infants (Esteban et al. 2010; Padilla et al. 2011; Sanz-Cortes et al. 2010a; Thompson et al. 2007; Tolsa et al. 2004).

Laterality

Volumes were similar between the left and contra lateral of structures including hemisphere volumes although there was a significant difference between left and contra lateral orbital lateral prefrontal cortex. There was also a significant interaction with group. In the IUGR group volume was higher in the left orbital lateral prefrontal cortex whereas in the control

group volumes were higher in the contra lateral orbital lateral prefrontal cortex. After analysing laterality in the groups separately there was no significant difference between right and contra lateral orbital lateral prefrontal cortex. ***Thus the hypothesis that there may be no asymmetries present in the structures measured is accepted but the second part exclaiming there will be asymmetry in the volume of the dorsal lateral prefrontal cortex is refuted.*** These findings support previous research that found all structures to be symmetric or nearly symmetric in volume (Filipek et al. 1994; Ginus Partadiredja 2003) but not other research which found asymmetries in the dorsal lateral prefrontal cortex (Gur Re & et al. 2000; Howard 2003; Lancefield et al. 2006; Petty 1999).

Gender

In the intrauterine growth restriction group there was minimal gender differences. Males had higher volumes in the dorsal medial prefrontal cortex, right and contra lateral hemisphere and the cerebrum but these differences were not significant. In the control group males generally had higher volumes except in the right dorsal medial prefrontal cortex. Volumes were significantly higher in males in the right orbital lateral prefrontal cortices. The general higher finding of volumes in males ***refutes the hypothesis that males will have*** *lower* ***hemisphere and brain structure volumes*** and is similar to the findings of Filipek et al (1994), Thompson et al (2007) and van Soelen et al (2010) who found male brains to be larger in volume and males to have larger volumes of all brain regions. However the differences were generally not significant and other previous research has found no sex difference in prefrontal cortex (Gur Re & et al. 2000) and regional volumes (Eckerman, Sturm & Gross 1985).

Correlations

Birth weight and gestational age positively correlated with brain structures volume. The correlation for birth weight was significant except for the left dorsal lateral prefrontal cortex and the left orbital lateral prefrontal cortex. The correlation with gestational age at birth was only significant in the hemisphere volumes and the cerebrum volume. ***This confirms the hypothesis that smaller brain structural volumes will be found in infants with lower birth***

weight and gestational age at birth and with which they will significantly correlate. Other previous research has found GA to be associated with cerebrum volume (Inge et al. 2010; van Soelen et al. 2010), white matter volume (Nosarti et al. 2002b) and volume in the right sensori motor cortex positively correlated with gestational age (Peterson Bs & et al. 2000). Although unlike our results Nosarti et al (2002b) found total cerebral volume not to vary according to gestational age or birth weight. Other structures they found not to correlate include grey matter volume and right and left hippocampus volume. Similarly other previous research has found birth weight not to correlate with structural volumes (Peterson et al. 2003; Peterson Bs & et al. 2000; van Soelen et al. 2010). However the positive correlation between birth weight and brain volume found in this thesis has been previously found to be significant in other previous research (Ivanovic et al. 2004; Ivanovic et al. 2000).

CHAPTER 8

Conclusions and Future Research

Aim of Chapter

This chapter aims to summarise the findings of this research in the context of previous research and suggests possible future research.

Conclusions

Birth weight and gestational age: Similar to previous research ADC and anisotropy measures varied with age and birth weight in the IUGR and control infants. Furthermore brain volumes positively correlated with gestational age and birth weight. There is a possible protective factor of higher birth weight and gestational age in infants born with intrauterine growth restriction. These correlations were not replicated in the preterm cohort, FA and ADC measures did not vary with gestational age or birth weight and neither did structural volumes. This supports previous research findings of no association between birth weight, gestational age and structural volumes in preterm infants. However they concluded their ADC measures did not vary with age due to the narrow range (37-44 weeks) (Counsell et al. 2003) and the same can be said for the present study as our age range is even narrower (24-28 weeks). Also other previous research has found ADC values to decrease with increasing age (Huppi et al. 1998). The influences of birth weight and gestational age are more pronounced in preterm infants born with intrauterine growth restriction.

Anterior vs. Posterior: Higher FA and lower ADC was found in the posterior part of the internal capsule and corpus callosum in preterm infants receiving placebo, preterm infants receiving thyroxine, IUGR infants and control infants. This supports previous research of brain development occurring in posterior regions prior to anterior regions. This finding is further supported by a general pattern of significantly higher FA and lower ADC in posterior compared to anterior regions. The pattern of development in the posterior prior to anterior regions is similar to the pattern of normal development suggesting the overall developmental program remains intact despite the lower values in preterm compared to term infants in previous research.

Asymmetry: Some asymmetries have been found in FA and ADC measures in preterm infants, IUGR and control infants although differences were not significant in IUGR and control infants. These measures significantly interacted with treatment and laterality and IUGR and control in some of the structures. Thus the administration of thyroxine affects these structures on different sides of the brain differently as does IUGR. Previous research has argued asymmetry to be a sign of abnormality, thus there may be some abnormality in these infants. Structural volumes were lower in the right hemisphere in the placebo group but lower in the contra lateral hemisphere in the thyroxine group, although these findings were generally not significant. These findings are different to previous research, although the thyroxine group was similar to previous research in young adult normal subjects. Thus thyroxine supplementation may normalise laterality in the hemisphere volume and infants born preterm may have different lateralisation to infants born at term. In IUGR and control infants volumes were similar between right and contra lateral structures, supporting previous research that found all structures to be symmetric or nearly symmetrical in volume.

Thyroxine administration: Higher FA and lower ADC was found in the thyroxine group although the differences between treatment groups were generally not significant thus thyroxine does not have a significant positive effect on DTI measures. Larger brain structural volumes were observed in the thyroxine compared to placebo group thus thyroxine treatment may have a positive effect on structural volumes. Supplementation with

thyroxine causes a temporary increase in thyroxine levels although by week 36 those who received placebo have also increased and are similar to the levels of the thyroxine group. This is similar to the findings of previous research. There was no difference in the treatment group between those that had low or high levels at screening but TT4 and FT4 levels are significantly lower in the low group in the placebo group suggesting a need for thyroxine in those with low levels at screening.

Sex differences: Generally males are thought to be of particular vulnerability to prematurity and this research confirms this in the placebo group where FA was significantly lower. Thyroxine treatment may be of particular benefit in males as the lower FA and higher ADC in males observed in the placebo group was not seen in the treatment group. Sex differences were also observed in IUGR and control infants. The gender affect on DTI values was different in the different groups but overall in the IUGR and control infants' sex differences were not significant. Previous research has found males to have larger brain volumes; this was confirmed in the thyroxine treated group, IUGR infants and control infants but not the placebo group. Thyroxine treatment may normalise the sex difference observed in preterm infants.

Medial compared to lateral and dorsal compared to orbital regions: Volumes were significantly higher in medial compared to lateral regions in all infants studied. Higher volumes were observed in orbital compared to dorsal regions in preterm infants and dorsal compared to orbital regions in the IUGR and control infants. Previous research in healthy adults is similar to the findings in IUGR and control infants with larger dorsal regions.

IUGR vs. Control: Lower FA and lower structural volumes were found in IUGR infants compared to controls although the expected higher ADC associated with lower FA was not found. Previous research has also found lower FA in IUGR infants. Infants born with IUGR are of higher vulnerability than appropriate for gestational age preterm infants and demonstrate lower FA and structural volumes.

Correlations: DTI measures did not generally correlate with birth weight, gestational age or gestation at scanning in the preterm or the IUGR cohort. This is unlike previous research

which generally found FA to increase and ADC to decrease with increasing gestational age. TT4 and FT4 at screening positively correlated with gestational age and TT4 at 36 weeks positively correlated with birth weight and gestation at birth. Stereology did not generally correlate with birth weight, gestational age or gestation at scanning in the preterm cohort. In the IUGR stereology generally correlated with birth weight and correlated with gestational age in the right and left hemisphere and the cerebrum.

Future research

The corpus callosum has been found to be smaller in preterm infants in later childhood despite the higher FA and lower ADC seen at term age. The question arises about the other structures which have lower FA and higher ADC than corpus callosum at term age (frontal and occipital lobes), are they further diminished in size later on in comparison to the corpus callosum or due to the complex process of human brain development are they less affected in later childhood. Future research could explore the effects of preterm birth on the frontal and occipital lobes in comparison to the corpus callosum in later childhood, adolescence and adult hood.

Research into the asymmetry of structures in preterm infants and IUGR infants is generally mixed. Functional asymmetries have been well demonstrated, future research is needed to clarify whether these functional asymmetries represent structural asymmetries.

Future research could look at the effects of administering levothyroxine to specifically those infants who have low levels of thyroxine in their blood (hypothyroxinemia) and male preterm infants.

Future research will be conducted on the cohorts used in this thesis. The preterm infants will be followed up as part of the TIPIT study and the IUGR infants will also be followed up by colleagues.

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