The long-term effect of neonatal nutrition on preterm brain structure and function

Submitted to University College London, for the degree of Doctorate of Philosophy in Neuroimaging
Declaration

I, Winok Lapidaire, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature: [Signature] Date: 04/10/18
Abstract

**Background:** Preterm birth has been associated with altered brain structure and cognitive impairment. Neonatal nutrition has been shown to play an important role in brain development in preterm infants, but there is a paucity of study on the long-term effects.

**Methods:** A total of 926 preterm infants (<37 weeks gestation, birthweight <1850g) were randomised to receive a nutrient enriched preterm formula (PTF), or the standard diet: term formula (TF) or banked donor breast milk (BBM), either as their sole diet or as a supplement to maternal breast milk (MBM). Of those in the original cohort, 768 subsequently completed IQ tests during childhood. For this adult follow-up, 72 preterm born participants from the trial and 72 newly recruited term born controls underwent MRI scans and completed cognitive tests and questionnaires.

**Results:** Preterm born adults had lower grey matter volumes and lower fibre coherence compared to term born controls. Preterm subjects who performed suboptimally on cognitive tests exhibited widespread changes in diffusion parameters. Increased proportion of human milk in the diet was associated with reduced neonatal infection/NEC. Adults who had suffered neonatal infection/NEC demonstrated reduced cognitive performance and changes in diffusion parameters. A high nutrient neonatal diet and increased neonatal weight gain were associated
with higher childhood IQ scores, particularly in children born <30 weeks of gestational age, but this was not significant in adulthood.

**Discussion:** The effects of preterm birth on cognitive outcome and brain structure persists into adulthood. While a large proportion of people born preterm perform in the normal range, there are some who perform suboptimally and exhibit widespread changes in the white matter microstructure. Human milk in the neonatal diet has a positive effect on the risk of infection/NEC and outcomes. The benefits of a high nutrient diet and subsequent increased neonatal weight gain on cognition and brain structure may be limited to low birthweight/low GA infants and could not be established in adulthood.
Impact statement

This study confirms that while preterm birth can result in long-term alterations in brain structure and reduced cognitive ability, a large proportion of people born preterm perform in the normal range. Neonatal infection/necrotising enterocolitis, rather than gestational age or birthweight, is a key predictor of suboptimal performance in adulthood. Preventing neonatal infection and necrotising enterocolitis is key for normal brain development and cognitive outcomes in adulthood and should therefore be prioritised in neonatal care.

Human milk, whether this is donor milk or maternal milk, can play an important role in preventing necrotising enterocolitis and infection. A short (on average 4 weeks) nutritional intervention has a significant impact on childhood and adolescent IQ scores and this study suggests that the effect of neonatal nutrition does not diminish over time.

The knowledge that the benefit of a high nutrient diet only persists for low GA infants brings us one step closer to developing personalised nutrition for preterm infants, optimising benefit and minimising harm. Weight gain is not related to brain structure or cognitive outcome. Current feeding recommendations are still based on the assumption that increased weight gain results in better brain outcomes. Given the
potential adverse cardiovascular and metabolic consequences of increased neonatal weight gain, setting weight gain targets might do infants more harm than good in the long term.

In addition to providing information that can inform clinical practice, this nutrition intervention trial also contributes unique information about the effects of early nutrition and neonatal factors on brain development of preterm infants. MRI biomarkers of preterm birth itself and suboptimal performance were identified. This study demonstrated the application of a new method to characterise white matter microstructure: Spherical Mean Technique (SMT). The fractional anisotropy (FA), radial diffusivity (RD), and intra-neurite volume fraction derived with this model demonstrated increased sensitivity to white matter microstructure alterations compared to the regular tensor model and has the potential to function as biomarkers of suboptimal cognitive performance in people born preterm.

This study also identified widespread differences in fibre coherence between preterm and control subjects, which provides new information about the development of the brain network in people born preterm. This has not yet been widely recognised or reported due to the ubiquitous use of the regular tensor model as opposed to more advanced techniques.
Key findings

Prematurity hypothesis: The effects of premature birth on the brain and cognition persist into adulthood.

- Adults born preterm exhibit widespread decreases in grey matter volumes and fibre coherence as well as reductions in FA in some white matter tracts compared to term born controls.
- The changes in fibre coherence are associated with poorer performance on cognitive test scores.
- There is a subset of people born preterm who perform suboptimally, while others perform in the normal range.
- In addition to some grey matter volume reductions, those who perform suboptimally exhibit signs of widespread reductions in white matter integrity and quantity compared to preterm subjects who perform in the normal range and term born controls.

Human milk hypothesis: Neonatal human milk has a beneficial impact on cognitive and brain structure outcomes in people born preterm.

- A higher proportion of human milk in the neonatal diet is associated with increased visuospatial memory and left white matter volume in males.
• A higher proportion of milk in the neonatal diet is associated with a reduced risk of neonatal infection/necrotising enterocolitis.

• Neonatal infection/necrotising enterocolitis is associated with lower childhood IQ scores as well as differences in white matter microstructure suggesting lower white matter integrity and density.

• These changes in white matter microstructure were associated with lower cognitive test scores.

Nutrient content hypothesis: *A neonatal diet high in nutrients has a beneficial impact on cognitive and brain structure outcomes in people born preterm.*

• A neonatal diet high in nutrients is associated with increased neonatal weight gain.

• A high nutrient neonatal diet and increased neonatal weight gain are associated with higher childhood IQ scores, particularly in children born <30 weeks of gestational age.

• The relationship between a high nutrient diet and neonatal weight gain was not found in adulthood.
This is the first study to:

- Investigate the effects of a high nutrient diet and neonatal weight gain in adults born preterm.

- Investigate the effects of neonatal nutrition in the same cohort in infancy, childhood, and adulthood.

- Demonstrate lower white matter fibre coherence in adults born preterm compared to adults born at term.

- Show a relationship between higher proportion of human milk in the neonatal diet and cognitive outcome and left white matter volume in male adults born preterm.

- Demonstrate that intra-neurite volume fraction, SMT FA and SMT RD could be biomarkers of suboptimal performance in a sample without clinical disease.
Acknowledgments

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In addition to my supervisors, the feedback on my written work from Hanne Stotesbury, Fenella Kirkham, Alissa Kleinnijenhuis, and Adam Lewandowski has been invaluable. My wonderful sister Fides is responsible for the unique title page and lay-out of this thesis.

I am incredibly grateful to all 144 participants who took part in this study. Some travelled for over a hundred miles, arranged annual leave and/or child care to be able to attend the study visit. The radiographers Tina Banks, Jessica Cooper, Nichola Sellers, Paul Xavier, Justine Greenhorn, and Bella Said have also been invaluable for the data collection. They not only conducted the MRI sessions very professionally and with great dedication, but were also willing to spend numerous Sundays at the hospital to scan participants who I would otherwise not have been able to include in the study.
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And finally, I could not have done this without the ceaseless support from my family and friends. Words fall short to explain how crucial they have been along the way.
Thesis overview

Aim: To examine if and how nutrition can affect the brain structure alterations and the resulting cognitive problems that are caused by preterm birth.

This thesis is fitted with a colour-coded scheme. The colours of the dots above headings of the results chapters 7-10 show which participant groups are analysed. The data points in the figures also adhere to this system.

- Preterm
  - Preterm-Suboptimally Performing
  - Preterm-Normally Performing
- Control

Background

The first chapter starts with describing the effects of preterm birth on brain development and provides an overview of neurobehavioural and brain structural outcomes from infancy to adulthood. It also discusses the congenital and neonatal risk factors for poor outcomes in preterm infants. The effects of neonatal nutrition on
brain development and outcomes are discussed separately. Three types of studies are currently underrepresented in the literature: randomised controlled nutrition intervention studies, long-term studies, and studies that use advanced MRI methods.

The second chapter describes how MRI works and considers the underlying biophysical properties of the contrasts used in this thesis. Readers who are not very familiar with MRI research can use this chapter for reference. It also describes the models and techniques used for processing the raw MRI data, which are specific to this study. This includes a novel diffusion MRI model, which is not widely used yet.

Methods

The following two chapters contain the methods of the study. Chapter 3 outlines the details of the original nutritional intervention trial and briefly describes the cognitive follow-up studies at infancy, childhood, adolescence, and young adulthood. It then gives an overview of the previously published results of these studies, providing the context in which this adult follow-up study was conducted.

Chapter 4 describes the methods of the adult follow-up. This includes the procedures of the study visit, MRI protocol, and cognitive test battery as well as further processing, outcome measure selection, and statistical methods.
Results

All results chapters follow a similar format, as described below. They describe differences in outcomes on a set of cognitive test measures and brain MRI scans: Voxel-based morphometry of grey matter (T1), absolute brain volumes (T1), voxel-based changes in diffusion properties of white matter (DWI), white matter tract-averaged changes in diffusion properties (DWI), voxel-wise changes in magnetisation transfer (MT), and differences in network properties (T1+DWI).

Prematurity hypothesis: The effects of premature birth on the brain and cognition persist into adulthood.

● Chapter 5 investigates the first main hypothesis: people born preterm have on average lower cognitive function and altered brain structure compared to term born control subjects.

●● Chapter 6 identifies preterm subjects who perform suboptimally on the cognitive test battery. This group is then compared with normally performing preterm subjects and controls.
Human milk hypothesis: Neonatal human milk has a beneficial impact on cognitive and brain structure outcomes in people born preterm.

- Chapter 7 investigates the second main hypothesis by examining the relationship between the proportion of human milk in the neonatal diet and outcomes.

- Chapter 8: examines if human milk is associated with reduced infection/NEC and what the impact of neonatal infection/NEC is on cognitive function and brain structure in adulthood.
Nutrient content hypothesis: A neonatal diet high in nutrients has a beneficial impact on cognitive and brain structure outcomes in people born preterm.

Chapter 9 investigates the third main hypothesis by comparing participants who were allocated to a standard neonatal diet, consisting of term formula or banked donor breast milk, with participants who had been allocated a nutrient enriched diet in the form of a preterm formula.

Chapter 10: The effect of neonatal weight gain on adult outcomes is investigated after verifying that a high nutrient diet was indeed associated with increased neonatal weight gain.

Discussion

In chapter 11, results are first discussed with regards to prematurity, nutrition, and the short-term risk factors that were hypothesised to be affected by nutrition and to impact long-term brain outcomes; infection/NEC and neonatal weight gain. Next, MRI findings are integrated, and the relative sensitivities of the different MRI modalities are considered. Finally, the strengths and limitations of the original trial and follow-up study are discussed, along with avenues for future research.

Chapter 12 contains the conclusions of this thesis.
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<td>Axial Diffusivity</td>
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<tr>
<td>C</td>
<td>Control subjects group</td>
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<tr>
<td>CP</td>
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<td>Diffusion Weighted Imaging</td>
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<td>Diffusion Tensor Imaging</td>
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<td>FA</td>
<td>Fractional Anisotropy</td>
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<td>fODF</td>
<td>fibre Orientation Distribution Function</td>
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<td>GA</td>
<td>Gestational age</td>
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<td>ICV</td>
<td>Intracranial volume</td>
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<td>IQ</td>
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<td>Intra</td>
<td>Intra-cellular volume fraction</td>
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<td>MD</td>
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<td>MPM</td>
<td>Multi-Parametric Mapping</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>NEC</td>
<td>Necrotising enterocolitis</td>
<td>3</td>
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<td>NP</td>
<td>Normally performing preterm subjects group</td>
<td>6</td>
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<td>ODE</td>
<td>Orientation Dispersion Entropy</td>
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<td>PNT</td>
<td>Probabilistic Neighbourhood Tractography</td>
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<tr>
<td>PIQ</td>
<td>Performance Intelligence Quotient (WISC)</td>
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<td>PRI</td>
<td>Perceptual Reasoning Index (WASI-IV)</td>
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<tr>
<td>RD</td>
<td>Radial Diffusivity</td>
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<td>SGA</td>
<td>Small for Gestational Age</td>
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<td>Abbreviation</td>
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<td>SMT</td>
<td>Spherical Mean Technique</td>
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<td>SP</td>
<td>Suboptimally performing preterm subject group</td>
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<tr>
<td>VCI</td>
<td>Verbal Comprehension Index (WASI-IV)</td>
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<tr>
<td>VIQ</td>
<td>Verbal Intelligence Quotient (WISC)</td>
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1 Background

Each year approximately 15 million infants are born prematurely; that is, born before 37 weeks of completed gestation (Blencowe et al., 2012). It is a major cause of morbidity, with arguably the most important adverse long-term outcomes of prematurity relating to the brain. Improvements in neonatal care have led to increased survival rates of these infants, particularly for those at low gestational age (GA; Howson et al., 2013). Although much progress has been made over the last few decades in terms of treatment strategies, high rates of neurological injury and altered development remain in infants born preterm (Baron and Rey-Casserly, 2010). This presents an emotional and financial burden for the individual, the family, and society as a whole (Hack et al., 2007). In addition to the medical care services required during and immediately after delivery, there are costs associated with early intervention services, medical care after early childhood, special education services, and lost household and labour market productivity (Behrman and Butler, 2007), which in the UK alone equate to £3 billion a year (Mangham et al., 2009).

In this chapter, I will outline what developmental processes are shown by previous research to be disrupted after preterm birth and how this leads to changes in brain structure and cognitive function. The WHO defines moderately preterm birth as 32-37 weeks GA, very preterm birth as 28-32 weeks GA, and extremely preterm birth as
<28 weeks GA (WHO, 2015), but definitions and inclusion criteria vary per study. Low birthweight is defined here as <2500g, very low birthweight as <1500g, and extremely low birthweight as <1250 g unless otherwise specified.

I will then provide an overview of what is already known about the role of neonatal nutrition on these developmental processes and outcomes in preterm infants as well as what we do not know yet. In section 1.7 I will describe the three main gaps in the literature: there are very few randomised controlled nutrition intervention studies and none of these have followed up on the long-term. Furthermore, there are not enough studies that use the advanced MRI methods that are currently available. The current study provides a first attempt to fill these gaps.

1.1 Brain development during the preterm period

1.1.1 Developmental processes in utero during third trimester

Preterm birth occurs during the third trimester, at a time when multiple active developmental processes take place. The brain consists of many anatomical regions and tissue types which each have different developmental trajectories and critical periods.

White matter develops rapidly during the preterm period. This is a particularly critical phase for thalamocortical connections, as the fibres reach the subventricular plate.
and start forming synapses (Kostović and Jovanov-Milošević, 2006; Qiu, Mori and Miller, 2015). All major white matter tracts can be identified in the foetus on MRI scans after 32 weeks of gestation, forming a whole-brain structural network (Staudt, Krageloh-Mann and Grodd, 2000; Dubois et al., 2008, 2011; Qiu, Mori and Miller, 2015). Already in very early postnatal life the brain is organised in a way that enables efficient information transfer by favouring locally dense communication over long-distance connections (Ratnarajah et al., 2013). At 32 weeks most fibres have yet to be myelinated (Qiu, Mori and Miller, 2015).

The cortical grey matter also undergoes large changes during normal foetal development. The doubling in intracranial volume during the last trimester is mainly driven by a four-fold increase in cortical grey matter (Huppi et al., 1998; Nosarti et al., 2014), which is accompanied by significant changes in cortical surface structure. Whilst at 25 weeks of gestation the brain surface is still smooth, by 40 weeks it has gyri and sulci and resembles an adult brain (Ramel and Georgieff, 2014).

1.1.2 Disrupted developmental processes after preterm birth

Preterm delivery involves a complex interplay of factors. Injury to fragile, immature organ systems is common and can have life-long consequences (Behrman and Butler, 2007). Diffuse white matter injury is currently the most frequently observed type of brain injury amongst preterm survivors (Back and Miller, 2014). This type of white matter injury is primarily caused by degeneration of pre-oligodendrocytes,
which are ubiquitous in the brain throughout the preterm period. Pre-oligodendrocytes are vulnerable to hypoxia-ischemia and inflammation (Volpe, 2009a; Volpe et al., 2011). Although they can regenerate, affected pre-oligodendrocytes display arrested differentiation, preventing further normal myelination (Back and Miller, 2014).

There is evidence for some primary degeneration of neurons in preterm neonates, but most grey matter abnormality can be attributed to secondary neuronal degeneration related to white matter injury (Back and Miller, 2014). The latter includes degeneration of subplate neurons, which have a critical time window to form thalamocortical connections that falls within the preterm period (McQuillen and Ferriero, 2006).

1.2 Neurobehavioural outcome after preterm birth

A large number of studies have investigated the effects of disrupted brain development as a result of birth before 37 weeks GA on cognition and behaviour. The most prevalent outcome for preterm born children is an occurrence of cognitive deficits without major motor and sensory deficits (Bowen, Gibson and Hand, 2002; Marlow et al., 2005). Preterm infants generally show lower developmental quotient scores. Even in the absence of major sensory impairment, studies show abnormal sensory processing (Bart et al., 2011; Wickremasinghe et al., 2013), and lower social and emotional scores (Spittle et al., 2009) in preterm infants. Longitudinal follow-up
studies with preterm and very low birthweight subjects have shown that these individuals have significantly lower IQ scores from infancy until adulthood compared to term-born controls (Bhutta et al., 2002; Hack et al., 2002; Aarnoudse-Moens et al., 2009; Romeo et al., 2010; Kerr-Wilson et al., 2012; Breeman et al., 2015b; Eryigit-Madzwamuse et al., 2015). Meta-analyses including studies with preterm born infants <37 weeks of gestation and term born controls, show that the difference between preterm and term born school-aged children (mean age 5-14 years) is 11-12 IQ points (Bhutta et al., 2002; Kerr-Wilson et al., 2012). A large meta-analysis showed that extremely preterm children from pre-school to beyond school age had a significantly lower FSIQ and VIQ, and particularly PIQ scores. The IQ scores were consistently lower across age groups from pre-school through school age (Allotey et al., 2018).

Even in preterm birth survivors with IQ scores in the average range, performance on more specialised cognitive tasks was poor (Baron and Rey-Casserly, 2010). Numerous studies have also examined specific cognitive deficits in prematurely born individuals.

1.2.1 Cognitive domains affected in people born preterm

Processing speed
A recent meta-analysis that included data from 6163 children and adolescents born very preterm and 5471 term-born controls reported lower processing speed scores in
the preterm subjects (Brydges et al., 2018). Evaluating and responding to incoming information is at the basis of many cognitive processes. Preterm born individuals struggle especially in more complex tasks and situations, suggesting reduced processing efficiency (Anderson, 2014). This is reflected in increased inspection time and slower reaction times on difficult tasks as well as steeper reaction time slopes with respect to task difficulty in children (Rose and Feldman, 1996) and adults (Nosarti et al., 2007) born preterm. Mulder and colleagues argue that a processing speed impairment mediates executive functions and attention problems in very preterm individuals (Mulder, Pitchford and Marlow, 2011).

Attention

Very preterm and very low birthweight children aged 0-8 demonstrate problems with attention (Anderson and Doyle, 2003; van de Weijer-Bergsma, Wijnroks and Jongmans, 2008; Jaekel, Wolke and Bartmann, 2013). A meta-analysis reported a significant difference between preterm and term born children at ages ranging from 3.5 to 22.3 years in selective attention, sustained attention, and one, but not all, of the tasks assessing shifting attention. The extent of difficulties was related to GA (Mulder et al., 2009). Very preterm (<34 weeks GA) and low birthweight children are 2 to 3 times more likely to be diagnosed with ADHD than term-born peers (Johnson and Marlow, 2011), which is mostly due to their problems with attention rather than hyperactivity (Anderson and Doyle, 2003; Hack et al., 2009; Jaekel, Wolke and Bartmann, 2013). This can have a considerable impact on quality of life through
secondary problems in academic and social settings (Anderson, Howard and Doyle, 2010).

Executive functions

Attention problems in combination with other cognitive deficits are reflected in lower executive functioning capacities often seen in preterm born children and adolescents (Taylor et al., 2004; Vicari et al., 2004; Aarnoudse-Moens et al., 2009; Johnson et al., 2009). Mulder and colleagues noted that executive functioning has been defined in many different ways in the literature, generally as an umbrella term for cognitive processes that are required for goal-directed or future-oriented behaviour (Mulder et al., 2009). Factor analyses have shown that these include inhibition, working memory, planning, shifting, and fluency (Miyake et al., 2000; Lehto et al., 2003; Huizinga, Dolan and Van Der Molen, 2006; Mulder et al., 2009; Brocki and Bohlin, 2010).

Meta-analyses of studies until 2008 revealed lower performance in response inhibition, verbal fluency, planning ability, cognitive flexibility, and working memory in preterm born children and adolescents between the age 3.5-20 years compared to term born controls (Aarnoudse-Moens et al., 2009; Mulder et al., 2009). Another meta-analysis in very preterm individuals aged 4-17 showed lower executive functioning scores compared to controls (Brydges et al., 2018). These impairments can manifest themselves as a wide variety of problems in daily life such as an
inability to focus, problems with planning in advance, disorganisation, problems with shifting between tasks, disinhibition, and problems with holding information in working memory (Horwood, Mogridge and Darlow, 1998; Isaacs et al., 2000; Bowen, Gibson and Hand, 2002; Taylor et al., 2002; Anderson and Doyle, 2003; Johnson et al., 2009; Wolke et al., 2015). Executive functioning scores correlate and predict mathematics scores in school-aged children (Mazzocco and Kover, 2007) and there is evidence for contribution of executive function abilities to mathematical performance beyond general intelligence as measured by IQ (Blair, 2002; Costa et al., 2017). Maths computation tests include complex arithmetic problems that require organisation, focus and holding information in working memory, deficits in executive function (Bull and Scerif, 2001). Therefore, deficits in executive functioning might affect mathematical ability and may explain the poorer performance on maths tests by preterm children.

**Memory**

A wide range of studies report that preterm children have a generalised memory deficit, with studies reporting reduced abilities across domains, i.e. implicit, explicit, and working memory as well as across modalities, i.e. visual and verbal (Anderson, 2014). This was later further corroborated by Omizzolo and colleagues, who reported that very preterm (<30 weeks GA)/extremely low birthweight born children scored significantly lower than term born controls on visual and verbal measures of immediate memory, working memory, and learning ability (Omizzolo et al., 2014).
Language

The results of two meta-analyses suggest a generalized language impairment in populations of preterm children with mean ages 2-12. Lower scores compared to controls in receptive and expressive complex language tasks and in receptive and expressive semantics tasks were reported (Barre et al., 2011; van Noort-van der Spek, Franken and Weisglas-Kuperus, 2012).

Motor

Cerebral palsy (CP) refers to a group of non-progressive chronic conditions caused by injury to the immature brain that affects the control of movement and posture. The prevalence of CP increases dramatically with decreasing birthweight; in 2003 this ranged from 0.89 in normal birthweight infants (>2500g, this includes term born infants), 6.2 in infants with a birthweight between 1500 and 2499g, 35.9 in infants with a birthweight between 1000 and 1499, up to 38.2 at a birthweight of <1000g. The prevalence of CP decreased significantly between 1980 and 2003, except in the 1000-1499g birthweight category (Sellier et al., 2016).

Even children born preterm who do not develop CP sometimes show motor impairment. Meta-analyses including children between infancy and age 16 showed a significant motor impairment in very preterm and very low birthweight children (De Kieviet et al., 2009; Williams, Lee and Anderson, 2010). Prevalence of motor
impairment in the preterm population was 19% for moderate and 40.5% for mild-moderate impairment (Williams, Lee and Anderson, 2010).

Gross motor problems may hinder the child’s ability to play and explore the world, and thereby also the ability to participate in social activities. Fine motor skill deficits may also adversely affect the attainment of handwriting skills and progression in the educational system. This could contribute to the development of a delay in learning as well as behavioural problems (De Kieviet et al., 2009). A recent review by Oudgenoeg-Paz and colleagues (2017) on the relationship between early motor development and later cognitive skills concluded that there is likely to be a link, but there is only a small number of studies on this topic and the majority of these do not control for markers of early cognitive development. This means that the possibility remains that cross-sectional relationships between motor and cognitive development early in life account for the associations between motor and cognitive development later in life (Oudgenoeg-Paz et al., 2017).

Furthermore, brain injury associated with low gestational age and neonatal complications affect both motor and cognitive outcomes (Volpe, 2009a; Kwon et al., 2014). Nevertheless, motor impairment in combination with visuospatial and sensorimotor function contributed to poor academic performance after controlling for overall cognitive scores in a sample of preterm children aged 6 years. This suggests that impairment of motor function has a relatively small yet important additional contribution to school achievement (Marlow et al., 2007).
Generalised or specific impairments

From the previous sections it becomes clear that people born preterm have problems in multiple cognitive domains. This could be because there is a general deficit across domains, either due to a generalized cognitive impairment or due to a few selective, primary impairments that are important for various higher-order cognitive processes. Mulder and colleagues reported evidence in favour of selective, primary impairments, showing that verbal processing speed and working memory are significant and independent predictors of academic attainment in preterm children aged 9-10 years. Furthermore, processing speed mediated the significant effect of VP birth on selective tests of executive function (Mulder, Pitchford and Marlow, 2011). Although processing speed is consistently found to be related to executive functioning in preterm children, the same study and others also showed that preterm children had problems with executive functioning and response inhibition that were not accounted for by processing speed (Aarnoudse-Moens et al., 2009; Mulder, Pitchford and Marlow, 2011). Thus, performance in these primary domains cannot account for all the cognitive impairments reported in people born preterm.

Large, destructive haemorragic lesions are likely to have a significant impact on general cognitive functioning across domains, but only a small subset of people born preterm suffer from these (Larroque et al., 2003). White matter development, on the other hand, is almost inevitably affected by preterm birth since it spans the entire
third trimester and beyond (see section 1.1.2). It is therefore possible that the consequences of alterations in white matter integrity and structural connectivity may be shared amongst the majority of people born preterm. Since white matter is the substance that connects proximal and distal brain areas, any alterations may affect a wide, but consistent, range of cognitive processes. This would result in a general deficit across domains for preterm individuals. Section 1.4.2 will further discuss the changes in white matter integrity and structural connectivity in people born preterm and how these may relate to cognition. It is important to know the brain structural alterations and cognitive problems that are shared amongst the majority of people born preterm, even if they are relatively mild, so these can serve as outcome measures to test therapies or neonatal care strategies. Therefore, in this study results on specific cognitive tests will be reported. However, if an impairment on a specific test only affects a subset of people born preterm and therefore the number of subjects with impairment is low, this might not result in significant group differences when comparing preterm and control subjects. Consequently, the outcome measures that show group differences might not be the most representative reflection of cognitive ability profile for all people born preterm.

Each preterm individual has a unique profile of cognitive strengths and weaknesses. In line with the neuroconstructivist theoretical framework, which will be explained in more detail in section 1.7, gestational age and timing of neonatal insults could affect the type and severity of disruption of developmental processes, whilst environmental factors can mitigate the consequences. A study with 128 children born preterm at 11
years of age suggested that different factors affected different aspects of the neonatal profile. Major brain pathologies in MRI at term age (defined as consequences from intraventricular hemorrhages (grades 3 and 4), other major infarctions, increased extracerebral or ventricular space, or ventriculitis) were associated with lower FSIQ, with consistent contribution from all cognitive domains examined (verbal comprehension, perceptual reasoning, working memory, and processing speed), whilst birthweight z score was associated with processing speed and paternal education was associated with verbal comprehension (Nyman et al., 2017). Since these factors often vary widely between individuals of preterm populations, much larger sample sizes are required to disentangle the effects of multiple factors. Nevertheless, a deficit on one or more cognitive domains could serve as a valuable marker to identify structural brain alterations that are related to outcomes in later life. This was attempted on executive functioning outcomes in the Stockholm Neonatal Project cohort, where they found a significant effect of gestational age, intrauterine growth, lack of perinatal medical complications, and female sex on core executive functions up to 18 years (Stålnacke et al., 2019).

Unique cognitive profiles in people born preterm may appear as a generalised cognitive deficit on a group level, if a sufficient number of preterm individuals underperforms on all tasks, or it may not reach statistical significance in group comparisons with control subjects, if enough preterm individuals perform within the normal range on any given task. Therefore, in addition to looking at statistical comparisons per cognitive test, subjects with abnormal scores on any test will be
identified. The downside of this approach is that participants who have relatively mild, yet potentially important, deficits will be overlooked. Since the majority of people born preterm still perform in the normal range (Aarnoudse-Moens et al., 2009), focusing only on severe deficits will help a small subset of this population.

1.2.2 Quality of life in people born preterm

Considering the increased rates of learning disabilities and psychiatric disorders amongst preterm individuals, it is unsurprising that the rate of higher education completion is below average, which results in lower income later in life. Young adults born preterm are less likely to be in school or employed (Hille et al., 2007) and more likely to receive social support. However, the majority lead self-supported lives, and the differences in employment rate are small (Lindstrom et al., 2007; Moster, Lie and Markestad, 2008; Saigal, 2014). Interestingly, self-reported quality of life of adults born preterm is similar and in some domains higher in adults born preterm than adults born full-term (Vieira and Linhares, 2016).

In summary, people born preterm are at risk of significant cognitive and behavioural difficulties later in life. Although there are few studies with adults, results suggest that morbidity persists throughout development. Inclusion criteria, particularly in terms of gestational age and birthweight, is highly variable, which could explain some of the variance in study results. The following section will further discuss the effect of these and other risk factors on neurobehavioural outcome.
1.3 Determinants of neurobehavioural outcome after preterm birth

1.3.1 Risk factors and early life predictors

GA and birthweight

Increased GA and birthweight reduce chances of death within 24 hours, but only GA predicts survival mortality between the second day and the rest of the first year (Källén et al., 2015). Both GA and birthweight have commonly been related to neurodevelopmental outcome in the normal range (Bhutta et al., 2002; Taylor et al., 2002; Hack et al., 2004; Nosarti et al., 2008; Källén et al., 2015). It is difficult to disentangle the effects of birthweight and GA on neurodevelopmental outcome as the factors are strongly related.

A meta-analysis of 27 studies reported a strong association between GA and IQ in childhood and early adolescence (age 3-16 years; Kerr-Wilson et al., 2012), but it is unclear whether the relationship is linear (Johnson and Marlow, 2017). A larger and more recent meta-analysis showed that although extremely preterm children were at the highest risk of cognitive impairment, their cognitive scores were not much lower than those of very and moderately preterm (28-34 weeks) children. A further meta-analysis found no evidence for an effect of GA on IQ differences between low and appropriate birth weight individuals in adolescence and early adulthood (mean age 14-22 years), suggesting that birthweight might be a better predictor of
neurodevelopmental outcome than GA (Kormos et al., 2014). Again, the relationship between birthweight and outcome is not necessarily linear. There is a steep increase in the risk of mental disability and borderline intelligence associated with lower birthweight (Behrman and Butler, 2007). Brydges et al. showed that in very preterm children age 4-10 years, birthweight was associated with IQ scores, whereas GA was associated with executive function measures (2018). Neither of these relationships were significant in older children. These seemingly contradictory results from different studies and even meta-analyses might thus depend on the GA inclusion criteria, age at testing and the cognitive processes studied.

**Sex**

Male preterm infants are at a higher risk of mortality and morbidity including hospitalisation, neurodevelopmental problems, and structural brain abnormalities later in life (Lehtonen et al., 2011; Pogribna et al., 2013; Smithers-Sheedy et al., 2016). Even when taking perinatal, neonatal, and early childhood factors into account, male sex remains an independent risk factor for adverse cognitive outcomes (Hintz et al., 2006; Peacock et al., 2012; Skiöld et al., 2014). In male, but not female preterm subjects, VIQ scores decrease between adolescence and adulthood, resulting in lower VIQ scores in preterm born males only (Allin et al., 2008). Males seem to be more vulnerable to developmental aberration in general. The majority of developmental disorders are more common in males than in females (autism, ADHD, dyslexia, Tourette’s; American Psychiatric Association, 2013).
Socioeconomic status

Economic poverty is associated with poor birth outcomes, including preterm and low birthweight births. This is likely related to increased exposure to adverse environmental substances and stressors via pathways of epigenetic changes and inflammation (Brumberg and Shah, 2015). Therefore, infants born preterm are more likely to grow up in an impoverished environment.

Sociodemographic factors may particularly influence language skills and behavioural adjustment (Taylor et al., 2002). However, the effect of socioeconomic status on outcomes appears to be independent of prematurity (Wolke and Meyer, 1999; Brumberg and Shah, 2015). Intensive intervention programs at home have not shown any long-term benefits in very low birthweight groups, further suggesting limited interaction between preterm birth sequelae and social factors (Brooks-Gunn et al., 1994; Baumeister and Bacharach, 1996). An alternative explanation is that preterm children less able to take advantage of environmental stimuli due to their problems with processing of complex information (Wolke and Meyer, 1999). Whilst biological and social risk factors affect outcomes through different pathways and account for independent variance in outcomes, there is evidence that they sometimes may interact (Brumberg and Shah, 2015). Data from the “Early Childhood Longitudinal Birth Study Cohort” and the “Collaborative Project on Preterm and Small for Gestational Age” suggest that a higher socioeconomic environment can mitigate the effects of preterm birth and neonatal sequelae on cognition (Hille et al., 1994;
Hillemeier et al., 2011; Brumberg and Shah, 2015). Reversely, since poverty has a negative effect on brain development in children in general, a deficient environment may exacerbate the impact of prematurity on outcomes (Brumberg and Shah, 2015).

**Year of birth**

Advances in neonatal care have significantly improved since the 1980s. The introduction of surfactants, cryotherapy for retinopathy of prematurity, antenatal steroid use to enhance foetal lung maturity, and improvements of mechanical ventilation, nutrition, and training of clinical staff have resulted in decreased rates of severe developmental impairment (Raju et al., 2017). Nevertheless, a review reported that despite a reduction in medical complications and increased survival rates, high rates of neurobehavioural impairment continued to be reported well into the 1990s. There were no clear differences in reported functional outcomes between individuals born extremely preterm in the 1980s and those born extremely preterm after 1990 (Baron and Rey-Casserly, 2010), although the outcome on neurosensory outcomes has improved since the late 1990s (Doyle et al., 2011).

**Neonatal complications**

Intraventricular haemorrhage, periventricular leukomalacia, chronic lung disease, hypoxia, neonatal infection, and necrotizing enterocolitis are associated with poorer outcomes in childhood (Taylor et al., 2002; Rand et al., 2015). The combination of
intrauterine growth restriction and early infection is particularly associated with adverse neurodevelopment (Leviton et al., 2013). Neonatal pain-related stress contributes to the association between these neonatal medical conditions and developmental outcomes (Valeri, Holsti and Linhares, 2014). It is possible that social factors interact with neonatal complications if neonatal care provision is of lower quality in deprived areas, but the extent is very dependent on the health care system. However, there was no evidence for difference between areas of varying levels of deprivation in survival rates or care provision in the UK, at least between 1997 and 2005 (Smith et al., 2009). A population study using data from 39 New York hospitals concluded that although the largest proportion of racial differences in morbidity and mortality rates can be explained by variation in infant health risk, black and Hispanic preterm infants are more likely to be born at hospitals with higher morbidity and mortality rates. This suggests that in the USA, socioeconomic background could affect the consequences of neonatal complications (Howell et al., 2018).

1.3.2 Developmental trajectory

The Bavarian Longitudinal study reported consistently lower scores on on developmental and IQ tests between 5 months and 26 years of age in very preterm and very low birth weight subjects compared to term-born individuals (Breeman et al., 2015a). Effects of gestation and birthweight on intelligence were fully mediated by head circumference and head growth in the first four years of life, suggesting
limited capacity for adaptation beyond this period (Jaekel et al., 2018). Intelligence scores and attention problems were in fact more stable over time for the very preterm/very low birthweight group than the term born group, although this difference reduced when excluding individuals with severe impairment (Breeman et al., 2015b, 2015a). The Victorian Infant Brain Studies showed consistently lower language performance yet similar developmental trajectories in children born preterm at ages 2, 5, 7, and 13 years compared to a term born control group (Nguyen et al., 2018).

There is some evidence that as preterm children grow up, they perform increasingly worse compared to peers, potentially due to more complex environmental demands (O’Brien et al., 2004; Taylor et al., 2004; van Noort-van der Spek, Franken and Weisglas-Kuperus, 2012). However, there is also evidence of catch up (Rushe et al., 2001; Taylor et al., 2004; Ritter et al., 2013), depending on the skill assessed, the degree of prematurity, and environmental context (Taylor et al., 2004). Cognitive test scores between the ages of 8 and 17 years of participants in The Victorian Infant Study suggested that extremely preterm/low birthweight individuals improved more on the Rey Complex Figure copy accuracy compared to control subjects, but the effects reversed after adjusting for organisation score and parental occupation at age 8 years (Burnett et al., 2015).

There is mixed evidence about whether the association between GA and cognition decreases with age or remains stable during development (Aarnoudse-Moens et al., 2009; Kormos et al., 2014). However, the discrepancies between studies might be
due to reliance on cross-sectional analyses. Very few studies have followed-up beyond adolescence. In one longitudinal study design from infancy to early adulthood, the impact of male sex, maternal education and GA on the rate of development did not differ between preterm and term born children (Linsell et al., 2017).

It must be noted that interpretation of IQ scores at different ages is complicated, since different tests are used at different ages and there is an upward drift of IQ scores with increasing time from standardisation, known as the “Flynn effect” (Flynn, 1999; Behrman and Butler, 2007). The predictive validity of infant developmental assessments for later educational outcomes is low (Johnson and Marlow, 2016), but measures of cognitive ability during childhood are predictive of adult outcomes (Breeman et al., 2015b; Linsell et al., 2016; Costa et al., 2017). When investigating individual developmental trajectories from infancy through early adulthood, Linsell and colleagues (2017) found lower scores in individuals born extremely preterm compared to those born full term at every assessment, with no evidence of deterioration or recovery with age.

In summary, GA, birthweight, sex, socioeconomic status, neonatal course and the historical circumstances of neonatal care have a considerable impact on developmental outcome in individuals born preterm. These should therefore be taken into account when investigating cognitive outcomes in preterm populations.
1.4 Neuroimaging studies on brain structure in people born preterm

Magnetic Resonance Imaging (MRI) is a non-invasive tool that offers a variety of tissue contrasts. For more detailed explanations on each MRI method, see chapter 2. MRI has increasingly been used for visualisation and quantification of the effects of preterm birth on the brain.

1.4.1 Global and regional volumes (T1 + T2)

Infancy

Early MRI studies described lesions of the parenchyma, thinning of the corpus callosum (CC), and enlarged ventricles in preterm infants (Hack, 2000) as a result of intraventricular haemorrhage, cerebellar haemorrhage, and white matter injury (Ho et al., 2016). Volume reductions in cortical and deep grey matter (Inder, 2005; Thompson et al., 2007) as well as white matter (Mewes et al., 2006) were found in preterm infants scanned at term equivalent age compared to term born controls.

Childhood

Children born preterm show lower grey and white matter volumes compared to term born peers, specifically in the thalamus, cerebellum, hippocampus, and corpus callosum (de Kieviet et al., 2012). Particularly the posterior part of the corpus
callosum appears to be affected by preterm birth (Peterson et al., 2000; Caldu et al., 2006).

Longitudinal studies suggest that preterm birth results in long-term dynamic changes in brain structure. Compared to term born control subjects, preterm children show fewer grey matter decrease and fewer increases in white matter between the ages of 8 and 12 (Ment et al., 2009). Diffuse white matter damage in very preterm individuals is reflected in their globally smaller white matter volumes, including in bilateral, frontal, parietal, temporal, cingulate and periventricular areas as well as in the left insular and right cuneus regions of the occipital lobe (Giménez et al., 2006).

**Adolescence**

Bilateral areas of decreased grey matter volume have been found in adolescents born preterm, including in the cerebellum (Allin et al., 2001), temporal lobe (Peterson et al., 2000; Reiss et al., 2004), thalamus (Giménez et al., 2006; Nagy et al., 2009), hippocampus (Abernethy, Palaniappan and Cooke, 2002; Giménez et al., 2004, 2005; Nagy et al., 2009), and caudate (Abernethy, Palaniappan and Cooke, 2002; Abernethy, Cooke and Foulger-Hughes, 2004; Nagy et al., 2009). There are also reports of larger grey matter volumes in the right cingulate gyrus, the right middle temporal gyrus, and left parahippocampal gyrus as well as in the parietal and frontal lobe (Peterson et al., 2000; Kesler et al., 2004; Nosarti et al., 2008)
Adulthood

There is mixed evidence regarding catch-up of cerebral growth and maturation during adolescence through adulthood in individuals born preterm. Some studies find no differences between preterm and term-born individuals in overall grey and white-matter volume trajectories (Parker et al., 2008; Bjuland et al., 2014). However, other studies report accelerated grey matter brain maturation (Karolis et al., 2017), cortical maturation, corpus callosum growth (Allin et al., 2007; Nam et al., 2015) and cerebellar volume decreases (Parker et al., 2008) between adolescence and adulthood in preterm individuals relative to term born controls.

Differences between preterm and control subjects in adult studies are similar to those reported in adolescent studies, and include reduced overall grey and white matter, as well as grey matter reductions in temporal cortices (Allin et al., 2004; Bäuml et al., 2014; Nosarti et al., 2014; Meng et al., 2015), the thalamus (Bäuml et al., 2014; Nosarti et al., 2014; Meng et al., 2015), striatum (Meng et al., 2015), putamen, frontal cortex, insular cortex (Allin et al., 2004; Nosarti et al., 2014), caudate, frontal and occipital areas (Nosarti et al., 2014), hippocampus (Aanes et al., 2015), and internal capsule (Allin et al., 2004). Increases in grey matter volume have been reported in the medial/anterior frontal gyrus (Nosarti et al., 2014), and cingulate cortices (Meng et al., 2015). The total corpus callosum volume differences between term and preterm born individuals may become attenuated between adolescence and adulthood (Allin et al., 2007), but there is evidence that a significant reduction in
a cluster in the posterior section of the tract persists into adulthood (Nosarti et al., 2014).

**Relationship with cognitive performance**

A number of studies in preterm infants have identified one or more morphometric prognostic biomarkers that are predictive of neurodevelopmental outcome (Anderson, Cheong and Thompson, 2015; Cheong et al., 2016; Parikh, 2016). It has been suggested that regional decreased grey matter and white matter volumes in the brainstem, frontal, temporal and limbic regions mediates the relationship between preterm birth and reduced cognitive ability in adolescence (Nosarti et al., 2008). More specifically, there is evidence that perseveration errors, reflecting attention deficits, are associated with the volume of the fornix and dorsal cingulum (connecting medial frontal and parietal lobes), whereas visuospatial memory is associated with ventral cingulum volumes (connecting medial parietal and temporal lobes) in very preterm young adults (Caldinelli et al., 2017). Few other long-term studies used appropriate advanced image processing techniques, there is only limited evidence that volumetric differences on neonatal MRI are predictive of later cognitive functioning (Anderson, Cheong and Thompson, 2015).
1.4.2 White matter microstructure (DWI)

Microstructural changes in white matter that are not visible on T1-weighted and T2-weighted images can be quantified in vivo with Diffusion Weighted Imaging (DWI). A systematic review revealed that studies that tested DWI parameters at term-equivalent age and cognitive outcome measures between 18 months to 9 years were more likely to report prognostic biomarkers than studies examining volumetric markers (Parikh, 2016). The biophysical underpinnings of DWI contrasts and parameters are explained in more detail in chapter 2.

Low fractional anisotropy (FA) values reflect decreased myelination, axonal diameter, and fibre packing density (Beaulieu, 2002). A meta-analysis exploring differences in diffusion tensor metrics between preterm and term born individuals showed decreases in FA in the splenium, genu, and body of the corpus callosum, bilateral external capsule, left superior fronto-occipital fasciculus, left posterior thalamic radiation, right superior longitudinal fasciculus, left cingulum, left posterior corona radiata, and left posterior limb of the internal capsule. A decrease in FA was accompanied by an increase in radial diffusivity (RD; Li et al., 2015), indicating less myelination and/or lower axonal packing density (Beaulieu, 2013). Increased FA was found in regions of the corona radiata. Additionally, higher axial diffusivity in preterm subjects compared to control subjects has been reported at different ages (Li et al., 2015). Axial diffusivity is associated with greater fibre organisation, but it can also indicate a reduction in crossing fibres or decreased axon diameter (Beaulieu, 2002).
The meta-analysis included participants from infancy until young adulthood. Myelination starts at the second trimester of pregnancy and continues until adolescence, with different brain regions maturing at different times and different rates. Interpretation of the diffusion metrics in infants, children, and adolescents therefore depends on the location in the brain and the age of the participants (Li et al., 2015). The next sections discuss DWI findings for different developmental phases.

**Infancy**

Higher MD and lower FA values in the larger white matter tracts have been reported in preterm infants at term-equivalent age compared to term-born infants. These differences are widespread; in the sensorimotor tracts (Berman et al., 2005), the centrum semiovale (Anjari et al., 2007; Alexandrou et al., 2014), corona radiata (Alexandrou et al., 2014), internal capsule (Huppi et al., 1998; Pogribna et al., 2013), inferior longitudinal fasiculus (Alexandrou et al., 2014), external capsule (Constable et al., 2008; Nagy et al., 2009; Mullen et al., 2011; Alexandrou et al., 2014), and the corpus callosum (Anjari et al., 2007; Rose et al., 2008; Skiöld et al., 2010; Hasegawa et al., 2011; Thompson et al., 2011; Alexandrou et al., 2014; Akazawa et al., 2016).
**Childhood and adolescence**

Studies in preterm children and adolescents have reported lower FA in several white matter tracts, including the corpus callosum (Nagy et al., 2003; Vangberg et al., 2006; Skranes et al., 2007; Constable et al., 2008; Andrews et al., 2010; Mullen et al., 2011), internal capsule (Vangberg et al., 2006; Skranes et al., 2007), external capsule (Mullen et al., 2011), superior longitudinal fasciculus (Vangberg et al., 2006), and uncinate fasciculus (Constable et al., 2008; Mullen et al., 2011), inferior longitudinal fasciculus (Skranes et al., 2007). Of note, some studies, in particular those in more recent cohorts, report no differences in FA (Feldman et al., 2012; Loe, Lee and Feldman, 2013).

**Adulthood**

Studies that have been performed in young adults report reduced FA in the corpus callosum, superior longitudinal fasciculus, left superior corona radiata, (Allin et al., 2011; Eikenes et al., 2011), and bilateral superior corona radiata, uncinate fasciculus, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, external capsule, cingulum, and fornix, cerebellar peduncle, corticospinal tract, corticopontine tract, thalamus, posterior thalamic radiation/optical radiation, and stria terminalis (Eikenes et al., 2011). Using a tractography-based region of interest analysis rather than a whole-brain analysis, Salvan and colleagues found reductions in FA in adults born preterm compared to controls in the left hippocampal fornix, splenium of the
corpus callosum, inferior longitudinal fasciculus and inferior fronto-occipital fasciculus (Salvan et al., 2014), while Kontis et al. reported no significant differences in FA or MD between groups in the corpus callosum. The latter study did, however, find higher MD in the genu of the corpus callosum preterm females compared to term females (Kontis et al., 2009).

Higher FA in preterm adults compared to term born adults has been reported in the bilateral inferior fronto-occipital fasciculus, anterior corona radiata, uncinate fasciculus, left superior longitudinal fasciculus (Allin et al., 2011) and right superior longitudinal fasciculus (Allin et al., 2011; Eikenes et al., 2011).

Relationship with cognitive performance

FA in the corpus callosum has been associated with cognitive performance in individuals born preterm of varying ages (Kontis et al., 2009; Andrews et al., 2010; Feldman et al., 2012; Van Kooij et al., 2012; Murray et al., 2016). Altered microstructural organisation in the uncinate fasciculus (Eikenes et al., 2011; Kelly et al., 2016) and inferior fronto-occipital fasciculus (Eikenes et al., 2011; Kelly et al., 2016) have been linked to premature birth and to attention scores in children with the inattentive ADHD subtype (Constable et al., 2008; Mullen et al., 2011; Rossi et al., 2015; Shaw et al., 2015). The uncinate fasciculus is thought to be important for episodic memory, language and social emotional processing (Von Der Heide et al., 2013). Verbal IQ and vocabulary have been associated with FA values in the
uncinate fasciculus in 12 year old preterm children (Constable et al., 2008; Feldman et al., 2012), although this relationship was not unique to this tract (Feldman et al., 2012). The inferior fronto-occipital fasciculus connects visual and auditory association regions with the prefrontal cortex. It is involved in attention set-shifting, semantic processing and reading (Shaw et al., 2015). There is evidence that FA of the right inferior fronto-occipital fasciculus accounts for most of the variance in verbal IQ in preterm children (Feldman et al., 2012).

In summary, preterm birth is associated with decreased FA and increased RD and MD in most parts of the white matter, suggesting injury or aberrant development of the white matter. Changes in these diffusion parameters are associated with poorer performance on a range of cognitive tests. However, several studies find no changes in diffusion metrics, particularly those in adult cohorts. Some have reported local increases in FA. More studies, particularly long-term follow-up studies using advanced diffusion imaging techniques, are needed to characterise the pattern of white matter alterations in people born preterm.

1.4.3 Structural connectivity networks (T1 + DWI)

Early damage to white matter microstructure may impact the development of brain networks in preterm infants. Development of short-distance connections between spatially neighbouring cells, also called clusters, and the core connections between them appear to be prioritised and are relatively preserved in infants born preterm,
with effects on the whole-brain network persisting into adulthood (Kim et al., 2014; Karolis et al., 2016; Batalle et al., 2017). By contrast, connections between these important clusters and the rest of the brain, such as the connections between deep grey matter structures and the cortex, and cortico-cortical connections, appear to be affected by a paucity of white matter resources, and are significantly related to the degree of prematurity at birth (Batalle et al., 2017). Longer gestation is thus associated with greater connectivity strength and density, as well as increased network efficiency. Chapter 2 will describe how these network characteristics can be described and quantified by mathematics.

1.4.4 Functional connectivity networks (fMRI)

Although intrinsic networks are still developing during the third trimester of gestation, there is limited evidence of altered functional connectivity in preterm individuals. Resting state networks are complete in preterm and term born groups of infants (Damaraju et al., 2010; Doria et al., 2010; Smith et al., 2011; Smyser et al., 2016). The strength of long-range functional connections and complexity of networks, on the other hand, are reduced in very preterm infants at term-equivalent age (Lubsen et al., 2011; Smith et al., 2011; Smyser et al., 2016), indicating that intrinsic networks are present, but impaired in preterm infants.

A study in preterm and term born adults did not find significant differences in the strength or location of functional networks, but a time course analysis revealed that
the salience network was less connected to other networks in the brain (White et al., 2014). Alterations in the basal ganglia, thalamus, and salience networks were found in combination with grey matter differences in the caudate, thalamus and STG in preterm born adults. Decreased grey matter volume was related to increased functional connectivity of the thalamus and STG and decreased connectivity of the caudate in preterm born individuals compared to term born controls (Bäuml et al., 2014). Thus, there is less evidence for altered functional connectivity than there is for altered structural connectivity, along with some indication that functional changes may be driven by changes in grey matter volumes.

1.5 Risk factors and early life predictors of brain structure and function

GA and birthweight

GA has been linearly associated with brain volumes from infancy (Inder, 2005) to adolescence (Nosarti et al., 2008; Nagy et al., 2009), as well as local connectivity from infancy to adulthood (Ball et al., 2014; Karolis et al., 2016). Reports on the relationship between GA and white matter microstructure, on the other hand, show conflicting evidence (Pannek et al., 2014). Whilst GA on its own seems to have a small effect on tensor metrics in the preterm infant brain at term-equivalent age (Bonifacio et al., 2010; Alexandrou et al., 2014), and the effect of lesions on neurodevelopmental outcome has been found to be similar for infants born before 28 weeks and those born after 28 weeks of gestation (Vollmer et al., 2003). It is
possible that white matter microstructure is determined by brain injury and postnatal events rather than the degree of prematurity (Bonifacio et al., 2010).

Sex

Compared to full-term controls and females born preterm, preterm males exhibit lower FA values, decreased WM volumes, decreased GM volumes, and poorer performance across a range of cognitive tasks (Horwood, Mogridge and Darlow, 1998; Reiss et al., 2004; Kesler et al., 2008; Johnson et al., 2009; Benavides et al., 2019). VIQ, FSIQ, and vocabulary scores are positively correlated with FA in the uncinate fasciculus in preterm males, whereas in females VIQ and vocabulary scores are negatively correlated with FA in the right anterior uncinate fasciculus. (Constable et al., 2008). It is currently still not clear what exactly causes these sex differences.

1.6 Neonatal nutrition

Nutritional programming refers to the concept that a nutritional insult sustained during a critical phase of development can irreversibly alter a developmental trajectory (Lucas, 1991). Preterm infants, who already often suffer from postnatal brain injury, may be particularly vulnerable to nutritional deficits during the preterm period.
1.6.1 Nutritional requirements of preterm infants

Nutrients are essential for multiple developmental processes in the brain. Glucose, iron, branched chain amino acids and zinc support neuronal metabolism, allowing for neuronal differentiation (Jewell and Guan, 2013). Protein, energy, iron, zinc and long-chain polyunsaturated fatty acids are important for oligodendrocytes and astrocytes, both of which support optimal neuronal functioning via myelination, nutrient delivery, and neuronal trafficking. Glucose, protein, iron, zinc, long chain polyunsaturated fatty acids, and choline can influence synaptic functioning by affecting the number of receptors and neurotransmitter concentrations. Amino acids are also important building blocks for growth factors, which are required for nutrient-use, growth, and cell differentiation. Therefore, a deficit in any of these nutrients between 24 and 44 weeks post-conceptional age can have long-term neurobehavioural consequences (Ramel and Georgieff, 2014).

Preterm infants are at a different stage of development compared to term infants and therefore have different nutritional requirements (Koletzko, Poindexter and Uauy, 2014). Their energy expenditure is surprisingly high, with protein deficits reaching 25% in one week (Sunehag et al., 1993; Dusick et al., 2003). Presence of disease and infection can result in an even higher energy expenditure. Extremely premature infants that require ventilation expend 25% more energy than those who are not ventilated (Denne, 2001). Meeting these energy requirements in preterm infants is complicated by a reduced feeding tolerance as a result of an immature digestive
system (Tudehope, 2013). Parenteral nutrition is used in infants with low feeding
tolerance, but total parenteral nutrition only became routine practice around 1975
(Raju et al., 2017). Nutritional interventions could be a valuable tool to improve
immediate and long term outcomes in preterm infants (Edmond and Bahl, 2006).

1.6.2 Diet options for preterm infants

Human milk contains a number of bioactive components, including anti-infective and
anti-inflammatory agents, growth factors, and pre-biotics (Ballard and Morrow, 2013).
The macronutrient composition (protein, fat, and sugars) in human milk is unique for
our species and is remarkably similar across populations (Prentice, 1995). It is
characterised by a wide variety of specific proteins and peptides, non-protein
nitrogen-containing compounds, high proportions of palmitic and oleic acids, long
chain polyunsaturated fatty acids (LCPUFAs), and lactose (Ballard and Morrow,
2013).

Mother’s own milk (MBM) is highly recommended, but not always possible for
preterm infants. If available, donor breast milk or banked breast milk (BBM) can be
used as an alternative. Particularly in the US the use of BBM has increased in recent
years, following recommendations of the American Academy of Pediatrics (American
Academy of Pediatrics, 2012). BBM resembles MBM as it is human milk, but
pasteurisation and freezing processes reduce levels of macronutrients as well as
glycoprotein filaments, growth factors, and antimicrobial factors (Edmond and Bahl,
Processing of BBM has changed considerably. In the 1980s it was common practice to use drip milk, that is breastmilk that drips from the opposite breast while breastfeeding, from mothers of term born infants. This has a considerably lower nutrient density than the recommended expressed BBM that is currently used (Edmond and Bahl, 2006). However, for preterm infants the nutrient density of expressed BBM currently still falls below that of MBM. This is not only due to the effects of pasteurisation, but also to a reduction in macronutrient content over the period of lactation. BBM is typically provided by mothers of term born infants, who have been lactating for extended periods of time (Su, 2014).

Increased postnatal age and decreased gestational stage of the infant are associated with decreased macronutrient and mineral content of maternal breast milk (Gidrewicz and Fenton, 2014). Since the late 1980s, milk fortifiers are used to increase calorie, protein and mineral content of both MBM and BBM (Su, 2014).

Cow-based milk formula is another widely used alternative when MBM is not available or insufficient to meet full requirements. These do bear an increased risk of contamination with microorganisms Enterobacter sakazakii and salmonella (Edmond and Bahl, 2006). Special formulas for very-low-birth-weight/preterm infants were introduced in the early 1980s. These had a higher nutrient content compared with standard formulas (Greer, 2001).
1.6.3 The effect of neonatal diet on the preterm brain

*Human milk*

The active components in breast milk affect a myriad of processes in the developing infant. The well-established short-term benefits of breast milk on morbidity and mortality in infants are primarily due to the anti-infective properties of human milk (Horta and Victoria, 2013). Neonatal morbidities may have a negative impact on development. For example, inflammation and perinatal infection play an important role in white matter pathology in preterm infants (see section 1.1.2; Tudehope, 2013; Keunen et al., 2014). Reducing neonatal morbidities may therefore be one mechanism by which human milk improves neurodevelopmental outcome in preterm infants.

There is also evidence that human milk can promote brain growth and development. It is an important source of long-chain polyunsaturated fatty acids (LC-PUFAs), which play a role in neurogenesis (Lechner and Vohr, 2017) and myelination (Oshida et al., 2003). The cholesterol in breastmilk is also essential for myelin synthesis (Saher et al., 2005). Growth factors, and prebiotics in human milk mediate the formation and organisation of the central nervous system (Ramel and Georgieff, 2014), and pre-biotics, pre-biotic oligosaccharides, and certain amino acids may affect brain development via the immunological, endocrine, and neural pathways of the gut-brain axis (Keunen et al., 2014).
Breast-milk is positively associated with cognitive development even after controlling for social and demographic variables are statistically controlled for (Victora et al., 2016) and this effect persists into childhood (Lechner and Vohr, 2017). Few studies have examined the link between neonatal human milk intake and neurobehavioural outcome beyond childhood, but there is some evidence for an association with better neurocognitive abilities in adolescence (Isaacs et al., 2010) and adulthood (Sammallahti et al., 2017).

The few MRI studies on the effects of human milk on brain structure in preterm infants suggest a positive relationship with white matter volume and structure. A higher percentage of breast milk in the early diet is associated with higher relative white matter volumes and to VIQ scores in males (Isaacs et al., 2008; Isaacs, 2013). Duration of human milk feeding is associated with higher FA in the corpus callosum of preterm infants at term equivalent age (Pogribna et al., 2013).

Several confounding factors complicate interpretation of studies on the effects of breast milk. For example, mothers who choose to breastfeed tend to have higher education and socioeconomic status (Victora et al., 2016). Nevertheless, in a study by Lucas and colleagues, IQ scores did not differ significantly between children whose mothers wanted to provide breast milk but could not do so and those whose mothers chose not to (Lucas et al., 1992). A systematic meta-analysis including studies with both preterm and term infants found that although adjusting for confounding socioeconomic factors reduced the effect sizes, human milk was still
significantly associated with increased IQ scores (Horta, Loret de Mola and Victora, 2015). Breast feeding could also confer benefit through maternal bonding, skin-to-skin contact, and oral motor stimulation. IQ scores have been shown to be similar between children who were breast fed and those who received mother’s milk by nasogastric tube, supporting the hypothesis that there is a biological effect of the milk itself on development (Lucas et al., 1992).

**Nutrient content**

As was mentioned in section 1.6.2, (unfortified) human milk is deemed nutritionally inadequate to meet the requirements of very low birthweight infants (Morales and Schanler, 2007). Postnatal growth of preterm infants does not approach the in utero growth rates (Cole et al., 2014), which is likely due at least in part to nutrient deficiencies (Embleton, Pang and Cooke, 2001; Dusick et al., 2003; Denne and Poindexter, 2007). Two systematic reviews reported that preterm or very low birthweight infants who were fed formula (both preterm and term formula were considered) exhibited faster head and body growth rates than infants who received donor breast milk (Boyd, Quigley and Brocklehurst, 2007; Quigley et al., 2007). The association between BBM feeding and growth impairment persists even when milk was fortified to current standards (Madore et al., 2017). Growth during the period until term equivalent age is particularly crucial for neurocognitive abilities later in life (Sammallahti et al., 2014; Ranke, Krägeloh-Mann and Vollmer, 2015).
There are numerous observational studies in preterm infants that report a positive correlation between the amount of nutrient and energy intake during the first few weeks of life and growth (Dusick et al., 2003; Ehrenkranz et al., 2006; Stephens et al., 2009; Ehrenkranz, 2010; Eleni dit Trolli et al., 2012). A study in preterm infants with white matter damage demonstrated that a nutrient rich diet may not only confer benefit for head size and weight gain but may also increase axonal diameters in the corticospinal tract (Dabydeen et al., 2008). The results of this randomised controlled trial were so successful that it was terminated before the relationship with neurodevelopment could be assessed. Since then, several observational studies have provided evidence for links between growth and improved neurodevelopment (Ehrenkranz et al., 2006; Franz et al., 2009; Sammallahti et al., 2014; Ranke, Krägeloh-Mann and Vollmer, 2015; Jaekel et al., 2018).

Despite the clear associations between nutrition and growth and growth and cognitive outcome, linking nutritional intake to neurodevelopmental outcome has proven difficult. A meta-analysis of 15 preterm studies that specifically compared an intervention providing increased intake of multiple nutrients during the first four weeks after birth did not find significant effects on neurodevelopmental outcome at 12-18 months. The accompanying review did reveal that in studies with an enteral nutrition intervention, infants who received increased nutrition were approximately twice as likely to survive without neurodevelopmental impairment compared to the control groups (Chan et al., 2016). In addition, some studies included in the review demonstrated significant associations between protein and energy intake and
neurodevelopment (Tan, Abernethy and Cooke, 2008; Stephens et al., 2009; Cormack et al., 2011). The studies included in the analysis varied widely in terms of their intervention (protein or multiple nutrient supplementation to either maternal or donor breast milk, nutrient enriched formula, hyperalimentation, or implementation of a feeding programme) and control groups (unsupplemented maternal or donor breast milk, standard formula, no feeding programme). Moreover, non-randomised trials and observational studies were also included. A recent retrospective follow-up study of adults from a Finnish low birthweight cohort at age 25 did report an association between higher total neonatal energy intake from enteral and parenteral nutrition and PIQ, VIQ, and FSIQ scores at age 25, but did not find any effect on executive functioning outcomes (Sammallahti et al., 2017).

The randomised controlled trial by Lucas and colleagues showed that infants that were fed with a special nutrient enriched preterm formula (PTF) exhibited significantly faster rates of head circumference and length gain than infants fed with banked breast milk (Lucas et al., 1984). If weight is taken as a criterion for discharge from the hospital, then the average hospital stay of infants fed human milk (BBM or mother’s milk) would be three weeks longer than that of infants who were given PTF. Further advantages of PTF over banked donor breast milk were exhibited at 9 months of age (Lucas et al., 2001). Interestingly, despite the low nutrient and energy content of the donated drip breast milk, there were no differences in Bayley developmental indices at 18 months post-term between infants fed PTF and BBM. The advantage of PTF compared to standard formula on the developmental indices
remained between 9 and 18 months (Lucas et al., 1990). When comparing infants who received only BBM with infants who received only term formula (TF), the BBM group scored significantly higher on psychomotor development (Lucas et al., 1994). At 7 years, a high nutrient preterm formula diet was associated with higher VIQ, PIQ, and FSIQ scores compared to low nutrient banked donor breast milk or term formula (Isaacs, Morley and Lucas, 2009). However, when directly comparing term formula and preterm formula, the beneficial effect of the high nutrient diet was only observed for VIQ outcomes in males (Lucas, Morley and Cole, 1998). In an adolescent follow-up on a subgroup with low GA (<33 weeks), larger caudate volumes of preterm children in the high nutrient group (fed nutrient enriched formula) compared to children in the low-nutrient group (banked donor breast milk or standard term formula) were reported. Furthermore, the caudate volumes correlated with VIQ scores (Isaacs et al., 2008).

There are also studies investigating the effect of supplementation of specific nutrients, such as glutamine, LCPUFAs, minerals, pre- and probiotics (Agostoni et al., 2010). These are beyond the scope of this thesis, but results do highlight the potential long lasting effects of early nutrition on brain development after preterm birth (de Kievet, et al., 2012).

This section has outlined the multitude of ways in which nutrition can have an effect on brain development. Growth and development of both the cortex and the white matter are directly dependent on adequate supply of nutrients. It is conceivable that
specific brain structures may be affected by different environmental factors at different developmental stages during the preterm course and that specific nutrients have specific effects on brain structures. However, this is exactly the reason why these effects are unlikely to be picked up on by research studies. Since preterm subjects in these studies vary in perinatal characteristics, nutrition is unlikely to have the same specific, localised effect in all individuals. Effects that are less dependent on developmental stage are more likely to survive the statistical significance threshold in group analyses. For example, if nutrition can affect the inflammation-induced diffuse white matter injury, this will, as the name ‘diffuse’ indicates, have an effect across the brain.

*Interaction effect of sex and neonatal diet in preterm infants*

There is evidence to suggest that preterm males are more sensitive to malnutrition. Male children who received suboptimal standard term formula were at a cognitive disadvantage compared to girls, but this effect was abolished when given a nutrient enriched preterm formula. This difference in the effect of nutrition remained detectable in adolescence, when the relationship between percentage of maternal milk and IQ was stronger in males than females (Isaacs *et al.*, 2010). As was mentioned in section 1.3, preterm males are more vulnerable than preterm females. The sex-diet interaction effect could in part be due to the increased sensitivity of ill infants to inadequate nutrition (Lucas, Morley and Cole, 1998). Given that the sex differences decreased significantly between 12 and 16 years of age in another
cohort (Mullen et al., 2011), it is currently unclear whether the sex effects persist into adulthood.

1.7 Theoretical framework for development of people born preterm

Section 1.1 described the biological processes that are important for brain development that occur around the time of preterm birth. The brain still has an incredible amount of change during the preterm period (up until 40 weeks GA). There are numerous factors that affect brain development and long-term cognitive outcome after preterm birth. This section aims to provide a theoretical framework for brain, behaviour, environment influences and their interactions in development in chronological order. It will also highlight how variations and interactions between these factors result in heterogeneity in preterm populations.

Although it generally agreed upon that development is influenced by both genes and environment, different theories attribute different roles to each. This affects the way in which atypical development is researched. Traditionally, behavioural impairments in adulthood have been sought in domain-specific cognitive modules, where some modules are impaired, and others are intact (strict nativist approach). The ‘neuroconstructivist’ approach, on the other hand, emphasises the dynamic and non-localised nature of atypical brain development and emphasises that cognitive disorders lie on a continuum (Karmiloff-Smith, 1998). It states that cognitive development trajectories are constrained by the underlying neural structures
(Mareschal et al., 2007). Johnson and colleagues postulated that neuroimaging data support this view. Even within groups of people with developmental disorders, there is little support for the notion that discrete lesions to functional cortical areas can be observed on a group level. Furthermore, altered trajectories of development rather than a delay of a normal developmental trajectory could be responsible for the differences in behavioural outcomes in adulthood (Johnson et al., 2002). Both typical and atypical development are an adaptation to multiple interacting constraints (Mareschal et al., 2007). Within this framework, preterm birth changes the constraints and processes of representation construction, which is further determined by other biological and environmental factors throughout development, leading to a system in adulthood that is adapted to the specific set of constraints. The concepts of dynamic and non-localised development are useful in explaining the complex interactions between biological and environmental factors during brain development after preterm birth. The next few paragraphs will describe the variations in genes and environment within the preterm population that affect individual outcomes in adulthood.

Firstly, the causes of preterm birth vary. Preterm birth can be indicated when the mother suffers from (pre-)eclampsia or when the foetus is growth restricted. Spontaneous preterm birth can result from (a combination of) inflammation, uterine overdistension, vascular disease (Goldenberg et al., 2008). Preterm birth occurs more often in economically deprived populations (Beck et al., 2010). There are complex relationships between risk factors for preterm birth (i.e. ethnicity, family
history, maternal age, socioeconomic status, smoking, and air pollution) and biological mechanisms (genes, inflammation, vascular, neuroendocrine, and mechanical (Huang, Owen and Mukherjee, 2017).

The gestational age at the time of preterm birth also varies. An infant born at 25 weeks GA is at a different developmental stage at the time of birth than an infant born at 36 weeks GA. Some of the biological processes named in section 1.1.1 cover the entire preterm period, for example the formation and integration of a whole-brain structural network of white matter connections and perturbations at any stage during this process may or may not manifest similarly. Nevertheless, the brain develops in a precisely regulated sequence of events, and the timing of premature birth determines which processes are affected and this alters the developmental trajectory (Volpe, 2009a).

After birth, the preterm infant responds to its environment, which is considerably different from the uterine environment that term born infants were in at that stage. There are many factors that can alter brain structure itself during that period and beyond. Stimuli from the NICU environment (light, sounds, care-giving experiences), neonatal insults, stress, pain, and nutrition all potentially not only affect the brain structure development at that time but may also modulate the trajectory of further brain development. For example, different connections might be formed in response to external stimuli and pain, neonatal insults can damage brain tissue and reduce the ability to form new connections, stress could affect the immune system and impair
brain growth (Pickler et al., 2010). Section 1.3.1 demonstrated that neonatal complications are associated with adverse brain outcomes, but even in the absence of (visible) brain injury, the preterm brain may develop differently. Epigenetic modifications are susceptible to environmental stimuli and may be an important pathway linking early risk factors with neurodevelopmental effects. By changing the gene expression, acute exposures in early life can have long-lasting effects (Fitzgerald, Boardman and Drake, 2018).

Good post-discharge caregiving (uninterrupted access to food and warmth, parental attention and touch, absence of physical and mental stressors, and access to a stimulating environment) is an important moderator (Aylward, 1992). During early development in infancy, the brain structural changes that are already evident at term equivalent age might make learning more challenging (Salvan et al., 2014). Motor difficulties, including cerebral palsy, may impair learning by limiting the ability to explore the environment (see section 1.2.1).

At school age, the implications of the brain structure alterations between preterm and term born children may become more evident as they affect the ability to learn and develop and the cognitive demands become increasingly complex. Gross motor problems may hinder the child’s ability to play and explore the world, and thereby also the ability to participate in social activities. Fine motor skill deficits adversely affect the attainment of handwriting skills and the progression in the educational system (De Kieviet et al., 2009). Inattention makes it harder for preterm born children
to concentrate on the lessons and may be perceived as a behavioural problem by teachers, who may struggle to provide the attention and tools required to effectively communicate the academic material (Anderson, Howard and Doyle, 2010). People born preterm also tend to score higher on scales of neuroticism, cautiousness, and lower on extraversion, which may translate into a shy and timid personality and lower sociability (Ross et al., 1990; Schmidt et al., 2008). Since learning often requires interaction and communication, problems with sociability could have further implications for cognitive development. Furthermore, this personality phenotype might put people born preterm at risk for later (subclinical) behavioural and psychiatric problems (Montagna and Nosarti, 2016). Socioeconomic status may particularly affect language skills and behavioural management at this stage (see section 1.3.1; Taylor et al., 2002).

With adolescence comes a new phase of important brain development. Section 1.4 summarised that many of the brain structural alterations persisted from childhood to adulthood. Social factors become increasingly important in relationship to academic attainment and cognitive outcome (Cheung and Pomerantz, 2011), but risk factor studies suggest that early postnatal adversity remains important determinant of long-term outcomes (Linsell et al., 2015).

Successful transition to adulthood goes beyond biological maturation and is defined by markers based on societal norms, such as educational attainment, employment status, financial independence, marriage and parenthood (Furstenberg et al., 2003).
In a study in a relatively advantaged, homogenous population in Canada, the majority of low birthweight participants demonstrated a successful transition to adulthood. There were no significant differences in high school graduation rates, employment status, and independent living compared to normal birth weight controls (Saigal et al., 2006). Another study, set in inner-city Cleveland, painted a different picture for a cohort of very low birthweight survivors of which a significant proportion (41%) came from socioeconomically disadvantaged single-parent families. Here, a significantly lower proportion of very low birthweight young adults had graduated from high school compared to normal birthweight individuals (Hack et al., 2002). Fewer very low birthweight survivors who attended mainstream schools in England were or had been in higher education in young adulthood compared to their normal birthweight peers (Cooke, 2004).

The neuroconstructivist approach highlights how even small variations in the initial state could lead to different phenotypes and the development itself is considered to play an essential role in determining outcomes (Karmiloff-Smith, 1998). Furthermore, the understanding of the constraints on neural development plays a central role (Mareschal et al., 2007). Preterm birth itself changes the constraints on neural development, and thereby results in early brain structure alterations and associated cognitive deficits that persist into adulthood, but neonatal nutrition, adverse neonatal events, and socioeconomic influences may change the developmental trajectory and impact adult outcomes. The combination of these factors, their timing, and their unique interactions results in considerable heterogeneity in preterm populations.
regarding the amount and the nature of the brain structural changes and cognitive deficits. When taking this framework and given that the preterm birth occurs during rapid development of white matter in particular and by definition prior to extra-uterine exposure to stimuli, it is unlikely that preterm birth results in damage to specific cognitive modules or brain areas. Therefore, the whole brain and a wider range of cognitive domains should be examined in this population, and the potential heterogeneity between preterm born individuals should be taken into account.

1.8 Gaps in the literature

From this review on nutrition studies in preterm infants, a number of gaps in the literature can be identified.

Firstly, there is a paucity of randomised controlled nutrition intervention trials. The majority of studies on neonatal nutrition have been observational and retrospective. These allow for rapid testing of associations between nutritional intake and clinical or cognitive endpoints, but they are highly confounded by environmental circumstances such as the socioeconomic status and clinical condition of the infant. A randomised study design minimises the impact of confounding variables, and thus provides the most reliable evidence for a direct relationship between nutrition and cognitive outcome.
Secondly, long-term studies are needed to examine the effects of nutrition in adulthood, when the brain has finished developing and before aging effects become prominent. Improvements in neonatal care in the early 1980s significantly increased survival rates of preterm infants, in particularly those born extremely preterm (Raju et al., 2017). Therefore, the first large cohorts of preterm born individuals have just reached this developmentally stable stage of adulthood.

The importance of preterm nutrition was not widely recognised until a few years later. Therefore, there have been no nutrition intervention studies in preterm infants investigating the effects on brain structure or cognitive functioning in adulthood. Longitudinal studies that examine post-natal nutritional strategies are needed, because short-term changes do not necessarily result in long-term benefits. For example, increased neonatal weight gain has been a key outcome on which current nutritional recommendations in preterm infants are based, since neonatal growth rates have been positively associated with improved neurodevelopmental outcome in childhood (Ranke, Krägeloh-Mann and Vollmer, 2015). However, there are also reports of associations with increases in body fat percentage, insulin resistance, and cholesterol (Kerkhof et al., 2012), resulting in increased risk of developing type 2 diabetes and cardiovascular disease in later life (Singhal et al., 2003, 2004). It is important to determine the effect of different diets on cognitive outcome in adulthood to be able to evaluate the adverse and beneficial effects.
Modern MRI techniques can be applied to detect and characterise subtle differences in brain structure that were previously undetectable, such as subtle alterations in white matter microstructure and organisation. This could be particularly game changing in studies on people born preterm, since diffuse white matter injury is the most commonly observed brain structure alteration in this population (Back and Miller, 2014). Although some recent studies, particularly those in preterm infants, have used advanced sequences and processing pipelines (Ball et al., 2014, 2017; Batalle et al., 2016), diffusion imaging sequences and processing pipelines in the literature frequently do not meet the highest standards (Pieterman et al., 2015). Furthermore, many studies only reported IQ scores as a measure of cognitive ability (Isaacs et al., 2008; Breeman et al., 2015b). These are well validated and widely used measures in research and other settings, but they lack specificity. As the time between birth and follow-up increases, it becomes more difficult to recruit all original participants and environmental confounders have more opportunity to affect outcome measures. Therefore, it is even more important to use the most sensitive and specific tools available to detect the effects of preterm birth on the brain.

1.9 Addressing the gaps in the literature

The global incidence of prematurity is rising (Beck et al., 2010) and more preterm infants survive the neonatal period, including those born extremely premature. The population burden will continue to increase unless significant progress is made in reducing the sequelae and developmental impairments after preterm birth.
Nutrition is viewed as the single environmental variable with the widest range of possible effects on brain development (Walker, 2005). Furthermore, nutrition is easily modifiable, and holds promise as a relatively low-cost and low-risk intervention for improving outcomes in preterm populations. It is essential that the previously mentioned gaps in the literature are addressed, because neonatal practice is currently primarily based on short term outcomes that are deemed beneficial for development but have yet to be shown to result in long-term benefits.

Nutrition affects a myriad of biological processes. Breast milk contains numerous factors that are important for the developing brain, but unless fortified does not meet the nutrient requirements of preterm infants and might result in stunted growth (Martin, Ling and Blackburn, 2016). A diet high in energy and nutrients could promote neonatal growth, but although this is associated with improved cognitive outcomes at least until childhood, there might be a trade-off in terms cardiovascular and metabolic risk. In order to strike a balance between these effects and outcomes, high quality research is required to examine both the short-term and long-term effects of nutrition in preterm infants. Cognitive tests provide a good indication of academic attainment and everyday functioning, and neuroimaging can add value by examining the impact on the brain more directly.

This thesis addresses these three gaps in the literature signalled above: (i) It uses data from a randomised controlled nutrition intervention trial, (ii) studies people born preterm in adulthood, and (iii) uses state of the art MRI imaging and novel data
processing techniques in conjunction with cognitive tests to investigate brain and
cognitive outcomes. Chapter 3 provides more details on the study design after
which, in section 3.3, the relevance of this long-term follow-up study is discussed.
2 Introduction to Magnetic Resonance Imaging

2.1 Physical principles of MRI

Magnetic Resonance Imaging (MRI) is a non-invasive technique that uses the magnetic properties of protons in water molecules to generate images representing the structure and function of the body. Our bodies are made up of many water molecules which contain hydrogen atoms. Protons in hydrogen nuclei spin around their axes, generating a magnetic moment. When placed in a strong magnetic field ($B_0$), proton spins will align with the direction of the magnetic field. They precess either in a stable low-energy state (spin up) or in the opposite direction (spin down). Most protons are in the spin up state, resulting in a net magnetisation in that direction, aligned with the applied magnetic field. They precess around the $B_0$ at the Larmor frequency:

$$\omega = \gamma B_0$$

where $\omega$, the angular frequency of the spins, is calculated by multiplying the magnetic field strength by the constant $\gamma$, which is set at 42.56 MHz T$^{-1}$ for hydrogen (H$^1$). This means that the frequency with which the spins precess is directly proportionate to the field strength.
Protons can change states by releasing or absorbing energy in the form of electromagnetic radiation. A magnetic signal can be obtained from protons emitting electromagnetic radiation when they change from a high-energy state into a low-energy state. Radiofrequency (RF) coils emit RF pulses, producing an oscillating magnetic field that is perpendicular and orthogonal to $B_0$. Protons that precess at the frequency of the RF pulses start resonating with the pulses, and thereby resonate in phase with each other, and absorb this energy. This causes the magnetisation of the spins to tip into the transverse plane. How much the magnetisation is tipped depends on the strength of the RF pulse and on the amount of time the pulses are applied for. The extent to which the magnetisation is flipped into the transverse plane is described by the flip angle $\alpha$.

$$\alpha = \gamma B_1 t_p$$

where $B_1$ is the strength of the RF field and $t_p$ is the duration of the pulse. When the RF pulse is turned off, these protons emit radiofrequency energy and return to their low-energy state. This is called relaxation. The MRI RF receiver coil measures electromagnetic radiation that is emitted by protons returning to their low-energy state. This is the basis of nuclear magnetic resonance which is the physical phenomenon that underpins MRI.
2.1.1 T1 and T2 weighted imaging

In order to create useful images of body and brain it is important for the MRI signal to differ between tissue types. MRI contrast depends on four factors:

1) Proton density, the concentration of protons in the form of water and macromolecules in the tissue. The higher the proton density, the more protons emit a RF signal, and the higher the signal intensity.

2) The longitudinal relaxation time ($T_1$), which depends on how quickly protons return to their low-energy state after the RF pulse has been turned off (Figure 1). This, in turn, depends on how tightly the hydrogen atoms are bound. Tightly bound protons, for example the ones in fat tissue, release their energy much quicker than loosely bound protons in the CSF.

3) The transverse relaxation time ($T_2$), which depends on how quickly the spins start precessing out of phase again after the RF pulse has been switched off. This depends on energy transfer between spins (Figure 1). Transverse relaxation can also be caused by local inhomogeneities in the magnetic field ($T_2^*$).

4) Flow; rapidly flowing fluids such as arterial blood can result in a loss of signal.
TR=Repetition Time, TE=Echo Time

Figure 1: T1 and T2 relaxation curves

T1 and T2 relaxation are two simultaneous, but independent processes. There are multiple ways to exploit differences in magnetic properties in tissue types. The two most important parameters of an MR sequence are the repetition time (TR) and the echo time (TE). The TR is the time between the initial exciter RF pulses. An MRI echo is created by applying a second RF pulse following the initial exciter pulse that flips the spins 180°. The difference in precessing speed of the spins that caused the dephasing, now results in rephasing as the fastest spins catch up with the slower spins. This results in the formation of an echo and an increase of signal strength.
which then dies away. The TE is the time between the exciter RF pulse and the echo signal.

In a T1-weighted image, the differences in longitudinal relaxation are used for contrast, while the effect of transverse relaxation is kept to a minimum. The TR must be long enough to allow the protons in certain types of tissue to return to their relaxed state but short enough so that the protons in other types of tissue remain in the excited state. Tissues with a short T1 relaxation time appear bright, because they regain most of their longitudinal magnetization during the TR interval. The TE is also kept short, so the T2 relaxation does not have a large contribution to the signal strength. A long TE allows for the spins in tissues with short T2 to lose the signal, whilst tissues with a long T2 remain in phase for longer and produce a signal. TR is kept long to minimise the T1 weighting. Therefore, grey matter appears dark on a T1-weighted scan and light on a T2-weighted scan, while white matter appears brighter on a T1-weighted scan than on a T2-weighted scan (Figure 2).
In addition to signal contrast between tissue types, the location from which the signal was emitted needs to be pin-pointed. The signal needs to be encoded so the receiver knows where it comes from.

The slice encoding gradient is a magnetic field superimposed on the main magnetic field. This causes the protons to spin at slightly different frequencies, so when the RF pulse is applied, only the protons with the same resonant frequency as the applied RF pulse in a thin slice are excited. This means that the signal only comes from this slice.

The phase encoding gradient is switched on very briefly in a direction perpendicular to the slice encoding gradient. Again, this causes the spins in this direction to spin at...
different frequencies and dephase. When this gradient is switched off, the protons within the slice spin at the same frequency, but each has a different phase that depends on its location. The unique phases of the spins along this direction are thus used to encode position along this axis.

The final direction is encoded by a magnetic field gradient in a direction perpendicular to both the phase encoding and slice encoding directions during the acquisition of the echo signal. This encodes position according to the unique frequency of precession imparted by the gradient. Phase encoding can only be done one row at the time and has to be repeated many times to scan the whole slice.

A Fourier Transform calculation decomposes the MR signal into waves of different frequencies, phases, and amplitudes and can therefore detect the intensity of the signal coming from each voxel.

2.1.2 Diffusion-weighted imaging

Diffusion-weighted imaging (DWI) contrast is based on water displacement in brain tissues. Brownian water diffusion is hindered by structures within the brain tissue, such as cell membranes, macromolecules, and myelinated white matter fibres. As water diffuses more easily along the direction of axons than perpendicular to axons, diffusion properties reflect the underlying axonal organisation of the brain (Dudink et al., 2008; Qiu, Mori and Miller, 2015).
DWI sequences first apply a gradient that causes molecules to acquire phase shifts depending on their motion in the direction of the applied gradient. Subsequently, the same gradient amplitude applied in the opposite direction rephases static spins but spins that are diffusing and therefore moving do not rephase completely since they do not experience an exactly opposite gradient pulse the second time. The resultant phase shift thus results in imperfect formation of the echo signal and hence signal attenuation. The b-value is a sequence parameter that is determined by a combination of the strength and the timing of the gradients. Increasing the gradient amplitude and duration or widening the interval between gradient pulses increases the b-value. Sequences with higher b-values produce images with stronger diffusion effects. The signal after application of the diffusion gradients can be described as

\[ S = S_0 e^{-bD} \]

where \( S_0 \) is the MR signal without gradients, \( b \) is the b-value, and \( D \) is the diffusion coefficient. A b-value of around 1000s/mm\(^2\) is commonly used in diffusion MRI.

As the gradient can only be applied in one direction, multiple images with gradients in different directions have to be acquired in order to get an approximation of water diffusion in three-dimensional space. If there is a large amount of diffusion in the direction of the gradient, the signal will be low for that acquisition. Conversely, if the diffusion is restricted in the direction of the applied gradient, the signal will be stronger. Assuming that the directions are balanced, averaging all diffusion-weighted
images cancels out the directionality of diffusion and gives a map of the overall magnitude of diffusion. This is called the directionally averaged diffusion coefficient ($D_{av}$) and describes the spatially averaged diffusivity of water in a voxel. It can serve as an indicator of brain maturation and/or injury (Huppi et al., 1998; McKinstry et al., 2002) and reflects the overall density of the restricting tissue microstructure.

**Diffusion Tensor Imaging**

The information about diffusion across many different gradient directions can be represented in one three-dimensional shape, the diffusion tensor (Figure 3). This is a mathematical construct in the form of a geometric object made up of three principal diffusivities (eigenvalues, $\lambda_1$, $\lambda_2$, $\lambda_3$) of the corresponding three principal directions (eigenvectors $v_1$, $v_2$, $v_3$). The assumption is made that the diffusion follows a 3D Gaussian distribution. Axial diffusivity (AD) and radial diffusivity (RD) quantify diffusivity parallel and perpendicular, respectively, to the direction of axonal fibres in brain tissue (Pierpaoli et al., 1996). Axial diffusivity is associated with axonal integrity, while radial diffusivity is associated with myelination (Sun et al., 2008).

Fractional anisotropy (FA) values reflect how much diffusion is restricted in one direction relative to other directions, i.e. the proportion between the AD and RD (Pierpaoli et al., 1996). FA values range from zero to one with lower values representing anisotropic diffusion as seen in free water and higher values representing anisotropic diffusion. In the brain, anisotropic diffusion is primarily due
to cylinder shaped structures such as neural fibres with intact membranes that hinder perpendicular water diffusion. Myelination can modulate the degree of anisotropy. Therefore, higher FA is often attributed to higher white matter integrity with more densely packed fibres, and myelination.

**Figure 3**: Diffusion Tensor with principal diffusivities (eigenvalues, $\lambda_1$, $\lambda_2$, $\lambda_3$).

White matter is a complex biological tissue which complicates interpretation of water diffusion (Beaulieu, 2002). The classic diffusion tensor only gives one principal direction per voxel, while in many white matter voxels there are multiple fibre orientations (e.g. crossing fibres). In voxels with crossing fibres, the diffusion tensor does not provide accurate directionality and FA values are low even when the fibre bundles themselves are coherent and well-myelinated (Wheeler-Kingshott et al., 2003). Additionally, the direction of the eigenvalues and eigenvectors are also affected by the noise and shape of the tensor. Therefore, drawing conclusions about myelination and axonal integrity from AD and RD without examining the geometrical
shape of the diffusion properties could be misleading (Wheeler-Kingshott et al., 2003).

The mode of anisotropy (MO) can give some further information in this case. MO is orthogonal to FA and represents the second-order geometric properties of the tensor as a continuous measure. Voxels with a single fibre population result in a linear, cigar-shaped tensor (positive MO values), and voxels with crossing fibres of two approximately equal fibre populations give a planar tensor (negative MO values; Figure 4).

![Figure 4: MO tensor shapes](image)

Constrained spherical deconvolution

Newer methods such as constrained spherical deconvolution provide a better solution to the crossing fibre problem (Tournier et al., 2004). CSD requires acquisitions of diffusion images in all directions at multiple (at least two) b-values as
well as an image with no diffusion weighting; a multi-shell sequence. Higher b-values have a higher angular contrast, and therefore allow for more accurate reconstruction of the white matter fibre tracts, but this comes at the cost of a lower signal to noise ratio. Multi-shell sequences combine the advantages of low and high b-values.

The underlying assumption with CSD is that the diffusion signal originates from the various fibre populations in a particular voxel. The diffusion characteristics are identical for all white matter fibre bundles in the brain and they add up linearly. Any differences in anisotropy can be attributed to partial volume effects. Thus, since the diffusion-weighted attenuation profile for a typical fibre bundle is a convolution of the white matter diffusion profile measured over the surface of a sphere and the distribution of fibre orientations within a voxel, the fibre orientation distribution function (fODF) can be obtained using spherical deconvolution (Tournier et al., 2004). The fODF is estimated from the data itself, without prior assumptions about the tissue characteristics. The robustness of this calculation is greatly enhanced by a non-negativity constraint, since negative fODF values are physically impossible (Tournier, Calamante and Connelly, 2007). However, CSD cannot discriminate crossing and fanning fibres due to the symmetric nature of the diffusion profile (Jbabdi and Johansen-Berg, 2011). The multi-shell CSD approach uses the multi-shell response function curves for grey matter, white matter, and CSF tissue types to estimate the fODFs of these tissue types separately. This significantly improves the apparent fibre density and fibre orientation estimates, especially at the tissue interfaces. Since white matter tissue in the brain is surrounded by CSF and grey
matter, many voxels that are classified as white matter also contain CSF or grey matter (Jeurissen et al., 2014). Use of the multi-shell multi-tissue-type CSD rather than the single shell CSD has been shown to result in more accurate fibre tracking due to greater sensitivity to fibre crossings (Sotiropoulos et al., 2013).

Spherical Mean Technique

A second problem with the traditional tensor is that even within a fibre bundle the axons rarely have the same orientation. The spherical mean technique (SMT) aims to factor out the effects of fibre dispersion within a fibre bundle and of crossing of bundles without making assumptions about prior knowledge about the fibre orientation distribution (Kaden, Kruggel and Alexander, 2016). It is based on the insight that the fibre orientation distribution does not have an effect on the diffusion signal when you take the average of the signal over all gradient directions. The magnitude of the averaged signal only depends on voxel-averaged microscopic diffusion processes. This relatively new technique produces per-axon diffusion coefficients that are more sensitive to the microanatomy of the fibres than FA since they are not confounded by the microdomain orientation distribution. Figure 5 shows three schematic examples of microstructural organisation that occurs in the brain and corresponding tensor shape and diffusion metric values of both the tensor model and SMT. The top example represents free diffusion, which is approached in the ventricular space, the middle shows two fibre populations cross in orthogonal directions, and the bottom a single, coherent fibre population. We assume that the
microstructural properties of the fibres are all equal. The tensor and SMT models can accurately describe the top and bottom examples, but tensor FA fails to distinguish the top and the middle very well. SMT does show similar values in the middle and
bottom example, representing similar white matter microstructural properties. FOD and MO can be used to differentiate between the middle and bottom examples.

<table>
<thead>
<tr>
<th>Fibres</th>
<th>Tensor</th>
<th>Tensor values</th>
<th>SMT values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1.png" alt="Tensor" /></td>
<td>FA: low</td>
<td>FA: low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD: high</td>
<td>AD: high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD: high</td>
<td>RD: high</td>
</tr>
<tr>
<td></td>
<td><img src="image2.png" alt="Tensor" /></td>
<td>FA: high</td>
<td>FA: high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD: high</td>
<td>AD: high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD: low</td>
<td>ODE: low</td>
</tr>
<tr>
<td></td>
<td><img src="image3.png" alt="Tensor" /></td>
<td>FA: high</td>
<td>FA: high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD: high</td>
<td>AD: high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD: low</td>
<td>ODE: high</td>
</tr>
</tbody>
</table>

*Figure 5: Schematic examples of different fibre organisations and associated tensor shapes and diffusion metric values.*
Neural fibres themselves consist of multiple compartments with different water diffusion. A multi-compartment microscopic diffusion model aims to identify diffusion properties in each of the compartments. The SMT multi-compartment model distinguishes between an intra-neurite domain and extra-neurite compartment. There are three main assumptions; i) there is no myelin compartment, because the water between the myelin layers has a very fast T2-relaxation, ii) the intra- and extra-neurite water pool have similar signal decay, iii) the microscopic diffusion process around the neurites can be described by a first-order tortuosity approximation $\lambda_{\text{I}}^{\text{ext}} = (1 - \nu_{\text{int}})\lambda$.

First, the MR signal is averaged over all directions for each shell with a particular b-value. Then, the measured mean signals are fit to the model using a least-square approach. Hereby, the parameters of the microscopic diffusion model are estimated. The axon orientation distribution can then be calculated using spherical deconvolution (Kaden, Kruggel and Alexander, 2016). This provides a measure of orientation dispersion entropy (ODE) defined as the entropy (or divergence) of the fibre orientation relative to the uniform spherical distribution. Values are high in areas with a single fibre bundle with a coherent structure and orientation (Kaden et al., 2016).

Both tensor and SMT parameters are used to describe white matter microstructure (Beaulieu, 2002; Ennis and Kindlmann, 2006; Kaden, Kruggel and Alexander, 2016). Interpretation of the different metrics can be found in Table 1.
Table 1: Interpretation of MRI diffusion metrics with regards to white matter microstructure

<table>
<thead>
<tr>
<th>Diffusion metric</th>
<th>Model</th>
<th>Interpretation of increased values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional anisotropy</td>
<td>FA</td>
<td>Tensor + SMT&lt;sup&gt;a&lt;/sup&gt; Increased fibre density, increased fibre coherence, increased myelination</td>
</tr>
<tr>
<td>Axial diffusivity</td>
<td>AD</td>
<td>Tensor + SMT&lt;sup&gt;a&lt;/sup&gt; Increased fibre orientation coherence, axonal integrity</td>
</tr>
<tr>
<td>Radial diffusivity</td>
<td>RD</td>
<td>Tensor + SMT&lt;sup&gt;a&lt;/sup&gt; Reduced myelination</td>
</tr>
<tr>
<td>Mean diffusivity</td>
<td>MD</td>
<td>Tensor + SMT&lt;sup&gt;a&lt;/sup&gt; Reduced myelination, loss of axonal integrity, increase in extracellular volume</td>
</tr>
<tr>
<td>Mode of anisotropy</td>
<td>MO</td>
<td>Tensor&lt;sup&gt;b&lt;/sup&gt; Planar/linear anisotropy (crossing fibres)</td>
</tr>
<tr>
<td>Intra-neurite volume</td>
<td>intra</td>
<td>SMT&lt;sup&gt;c&lt;/sup&gt; Neurite density, excluding myelin component</td>
</tr>
<tr>
<td>Orientation Dispersion Entropy</td>
<td>ODE</td>
<td>SMT&lt;sup&gt;c&lt;/sup&gt; Directional tissue heterogeneity</td>
</tr>
</tbody>
</table>

SMT=Spherical Mean Technique
<sup>a</sup> (Kaden et al., 2016 or Pandit et al., 2013)
<sup>b</sup> (Ennis and Kindlmann, 2006)
<sup>c</sup> (Kaden et al., 2016)

Tractography

Mapping the diffusion tensors for every voxel does not only provide information about the degree of anisotropy of diffusion in that voxel, but also about the principal direction of diffusion. This information can then be used to reconstruct white matter tracts and visualise structural connectivity in the brain (Huisman, 2010). Fibre tracking algorithms are based on the assumption that neighbouring voxels with similar anisotropic diffusion directions are part of the same white matter tract.
An inherent and fundamental limitation of all diffusion MR tractography algorithms is that they rely on water diffusion as an indirect probe of axon orientation. Even using more advanced techniques of diffusion mapping, the models remain an approximation of the intra-voxel axon geometry. Especially long-range connections are prone to tractography errors, because they surpass regions where multiple fibre bundles come together, which can cause the algorithms to jump between white matter tracts. Determining termination points is another challenge for the algorithms, since the shape of sulci result in a multitude of possible axon configurations with the same diffusion profile (Jbabdi and Johansen-Berg, 2011). Furthermore, the resolution of MRI scans does not allow tracking of individual fibres.

There are multiple algorithms, each with their own advantages and disadvantages. The two main approaches are deterministic and probabilistic tractography. Deterministic tractography uses the diffusion tensors to trace fibre pathways from seed regions by following the principal diffusion direction from voxel to voxel (Mori and Van Zijl, 2002). Probabilistic tractography on the other hand models the uncertainty in the data by creating a number of potential pathways from seed points and assigning the probability of connectivity to individual voxels based on the number of those pathways that go through those voxels (Behrens et al., 2003).

Despite recent advances in tracking algorithms, an international tractography competition in 2015 showed that, on average, the algorithms only recovered one third of the volume of fibre bundles. Additionally, they created four false positive
bundles for every true positive fibre bundle, meaning that they visualised fibre bundles that were not part of the ground truth. Streamline filtering techniques such as Spherical-deconvolution Informed Filtering of Tractograms (SIFT) improve the specificity of tractography by optimizing the signal prediction error (Maier-Hein et al., 2016). SIFT uses the fibre densities estimated by CSD to create a tractogram in which the number of streamlines between two regions is proportional to the cross-sectional area of the connecting fibres (Smith et al., 2013).

**Probabilistic Neighbourhood Tractography**

White matter is organised in fibre bundles, each connecting different brain regions and thereby facilitating a different set of cognitive functions. It is therefore also informative to look at structural changes across the entire tract. To identify individual tracts, probabilistic neighbourhood tractography (PNT) uses a reference tract as a topological guide (Clayden, Storkey and Bastin, 2007). Information from the voxels that are associated with that tract can then be extracted.

**Network analysis and graph theory**

Graph theory can be used to characterise and compare structural and functional connectivity in the brain. This approach describes the brain mathematically as a large network consisting of nodes (brain regions) and edges (connections between regions). Nodes can be determined based on parcellation of the cortex from a
structural T1-weighted scan. The parcellated image is then registered to the images that are used to derive connections, such as diffusion images with tractography, in order to see which brain regions are connected. The connections are represented in a connectivity matrix, where the presence or absence of a connection is indicated for each combination of nodes. In an unweighted matrix, the presence of a connection is simply indicated by a 1 as opposed to 0. In a weighted matrix, the strength of the connection is represented by a continuous variable.

The topology of a network determines the speed and manner in which information transfer takes place. Brain organisation is shaped by a trade-off between maximising the ability to effectively transfer information and minimising the material and metabolic costs associated with connections (Bullmore and Sporns, 2012). Therefore, graph theory variables might indicate how the brain is organised and how well it functions. A multitude of variables can be derived from the connectivity matrix, describing different characteristics of the network.

Path length between two nodes is the minimum number of edges between the nodes. This reflects how quickly brain regions can exchange information. Efficiency is the inverse of the path length between nodes. Triangular connections between groups of three neighbouring nodes are called ‘clusters’. Many complex networks consist of multiple modules, each with densely interconnected nodes and clusters. Global efficiency reflects how efficiently the network can exchange information at the
global level and is a measure of the capacity for parallel information transfer and integrated processing (Bullmore and Sporns, 2009).

Tractography algorithms are not without error and produce both false positive and false negatives. Whilst a stricter streamline threshold will improve specificity by reducing false negatives, it will also reduce sensitivity. It has been shown that improved specificity is at least twice as important as sensitivity when using connectomics to extract network properties (Zalesky et al., 2016). This also suggests that it is good practice to apply streamline filtering techniques when using whole-brain tractography to create a connectome. Furthermore, a priori settings that require streamlines to end in grey matter are too restrictive, because the anisotropy is low in grey matter, which makes it difficult to generate trajectories (Conturo et al., 1999). Setting the termination of streamlines in the interface between grey and white matter may be more appropriate (Maier-Hein et al., 2016).

2.1.3 Multi-Parametric Mapping

Multi-Parametric Mapping (MPM) was specifically designed to provide quantitative images with absolute values that are comparable across different MRI scanners and different time points. The acquisition consists of T1-weighted, proton density (PD)-weighted, and magnetisation transfer (MT) -weighted images, which are then used to calculate the parameter maps of the MT saturation, signal intensity, the longitudinal relaxation rate $R_1$ and transverse relaxation rate $R_2^*$. 
Magnetisation transfer enables detection of protons bound to macromolecules, many of which are located in myelin. It is based on the phenomenon that nuclei exchange magnetic energy, which increases the observed R1 recovery rate. The sequence applies an off-resonance pulse that excites protons that are bound in macromolecules. The relaxation time of bound protons is too short to image, but the protons exchange their magnetic energy with free water protons. This reduces the signal coming from the water which can be measured. Therefore, voxels with a significant concentration of macromolecules, such as myelin, appear darker than voxels with low macromolecular content (Stikov et al., 2015). The magnetisation transfer ratio (MTR) can be calculated by comparing the signal coming from an image with the MT pulse to an image without the MT pulse (Tofts, Steens and van Buchem, 2003). The MTR is usually calculated using the following formula, where $M_s$ is the signal magnitude with MT saturation and $M_0$ the signal magnitude without MT saturation (Dousset et al., 1992).

$$MTR = \left(1 - \frac{M_s}{M_0}\right) \times 100$$

The MTR reflects the quantity of bound protons in a tissue. It is the fraction of magnetisation left after application of the saturation pulse. CSF has very low MTR values, white matter has high MTR values, and grey matter has intermediate values (Tofts, Steens and van Buchem, 2003). A reduction in MTR is thought to reflect a reduction in myelination and to a lesser extent a reduction in the number of axons (Brochet, Petry and Dousset, 2003).
2.2 Justification of MRI sequence choice

The effects of preterm birth itself and neonatal factors on the brain often co-exist in preterm populations. Using multiple MRI modalities with different sensitivities and limitations allows for better examination of differences in brain structure and the underlying biological processes (Table 2).

As was outlined in chapter 1.4, previous studies, mainly in children and adolescents, have shown that people born preterm have smaller brain volumes and therefore have fewer neurons and connections to work with. T1-weighted images will be used to segment and quantify brain volumes, and DWI can be used to characterise how densely packed the axons are within the white matter tissue.

Chapter 1.1.2 describes how the white matter is particularly vulnerable to preterm birth, which affects not only the quantity but also the quality of the brain connections. Damage to pre-oligodendrocytes as a result of preterm birth or neonatal insults could result in problems with myelination. As was discussed in this chapter, the SMT model on multi-shell diffusion data is better equipped to measure the axon characteristics and fibre organisation than the traditional tensor model. DWI RD and FA are most often used to derive information about myelination, but MT images are particularly sensitive to quantities of macro-molecules such as myelin.
Axonal development can be hampered by paucity of nutritional resources, resulting in lower white matter quantity and alterations in fibre coherence, which might lead to altered development of structural network organisation. Structural organisation of the network as a whole is further examined using tractography and quantified with graph theory measures.

Voxel-based analysis provides information about local differences in white matter microstructure irrespective of the tract(s) the affected axons are part of, whilst tract-averaged measures, using probabilistic neighbourhood tractography- PNT (Clayden et al., 2011), highlight any alterations that are specific to a certain tract rather than a certain location in the brain.
<table>
<thead>
<tr>
<th>To be examined</th>
<th>Sequence</th>
<th>Subheading Results</th>
<th>Most relevant outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity (quantity of brain tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>Volume per structure</td>
<td>Volume of subcortical structures + total grey and white matter volumes</td>
</tr>
<tr>
<td></td>
<td>DWI</td>
<td>White matter-Diffusion voxel based</td>
<td>Grey matter volume (voxel-based) SMT intra (voxel-based)</td>
</tr>
<tr>
<td></td>
<td>DWI</td>
<td>Network</td>
<td>Edge density</td>
</tr>
<tr>
<td>White matter myelination</td>
<td>DWI</td>
<td>White matter: diffusion voxel-based</td>
<td>SMT + tensor FA/RD (voxel-based) Tensor FA (tract averaged)</td>
</tr>
<tr>
<td></td>
<td>MPM-MT</td>
<td>White matter: MT-voxel-based</td>
<td>MT (voxel-based)</td>
</tr>
<tr>
<td>White matter fibre coherence</td>
<td>DWI</td>
<td>White matter: diffusion voxel-based</td>
<td>SMT + tensor FA, SMT ODE + tensor MO (voxel-based)</td>
</tr>
<tr>
<td></td>
<td>DWI</td>
<td>Network</td>
<td>Shortest path length, global efficiency, local efficiency, cluster coefficient</td>
</tr>
</tbody>
</table>

DWI=Diffusion Weighted Imaging, MPM=Multi-Parametric Mapping, MT=Magnetisation Transfer, SMT=Spherical Mean Technique, ODE=Orientation Dispersion Entropy
3 Methods - Original nutrition intervention trial

3.1.1 Participants

The original trial included all infants with a birthweight below 1850 g who were admitted to the special care baby units in Ipswich, Cambridge, King’s Lynn, Norwich, and Sheffield between 1982 and 1985. Only babies with severe congenital abnormality known to affect growth or neurodevelopment, or those who died within the first two days were excluded from the study. Guardian(s) were asked for consent to participate in a nutritional intervention trial. There was no selection bias in this cohort, since all guardian(s) granted consent.

3.1.2 Trial design

The 926 participating preterm newborns were randomised to different diets within 48 hours after birth. Within each study, randomisation was stratified by birthweight (<1200g and 1200-1849g). Since the mother was given a choice as to whether or not she wished to provide breast milk, there were two parallel trials. Infants whose mothers wished to provide breast milk were entered in the supplement trial and received the randomised diet as a supplement to their mother’s milk. Infants whose mothers elected not to express breast milk were entered in the primary trial and
received the randomised diet as their sole source of nutrition. All nutrition, including MBM, was given via a naso-gastric tube as much as possible, but very sick infants were fed intravenously for a period (Morley and Lucas, 1993).

Infants remained part of the trial until they reached 2000g or were discharged from the neonatal unit, whichever occurred first. Some infants with relatively few problems left the trial at an early stage, because they were referred back to their local hospital. After finishing the trial, infants were fed according to their mother’s choice.

It was not possible to blind clinical staff to the diet, as the diets are clearly different in clinical practice, but all researchers involved in follow-up studies were blinded to group allocation.

3.1.3 **Intervention**

The intervention diet was a preterm formula especially designed for this study. This new formula contained protein and fats of similar quality, but higher in quantity. In addition, the formula was enriched with sodium, calcium, phosphorus, copper, zinc, vitamins D, E, and K, water-soluble vitamins, carnitine and taurine. Control diets were not fortified since milk fortifiers did not exist at that time. All infants in the trial received vitamin D supplements. In centres with a breast milk bank (Ipswich, Cambridge, and King’s Lynn), banked donor breast milk (BBM) was used as a control diet. In Sheffield and Norwich, the control diet consisted of term formula (TF).
Nutrient content of each diet is listed in Table 3. The BBM used at this time had a nutrient content much lower than MBM, as drip milk is effectively very low-fat foremilk that drips from the contralateral breast during breast feeding and was donated by mothers of term born infants. Term breast milk contains lower levels of protein, sodium, chloride, calcium, zinc, copper, and folate than preterm transitional breast milk. BBM was also pasteurized and frozen, reducing the levels of macro and micro nutrients as well as bioactive factors. By contrast, MBM was not pasteurized and only occasionally frozen.

The volume of milk intake was increased in accordance with tolerance to meet a target of 180 ml/kg/day for TF and PTF and 200 ml/kg/day for BBM (Lucas et al., 1994; Lucas, Morley and Cole, 1998).

Table 3: Nutrient content of trial diets per 100 mL

<table>
<thead>
<tr>
<th></th>
<th>PTF</th>
<th>TF</th>
<th>BBM</th>
<th>MBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>80</td>
<td>68</td>
<td>46</td>
<td>62</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.0</td>
<td>1.5</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>4.9</td>
<td>3.8</td>
<td>1.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>70</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>35</td>
<td>29</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

PTF=preterm formula, TF=term formula, BBM=banked breast milk, MBM=maternal breast milk
Nutrient content of diets per 100 mL BBM values are based on 340 pools (Lucas et al., 1984; Morley and Lucas, 1993)
3.1.4 Data collection

Enteral nutritional intake in mL was recorded daily (parenteral nutrition was not included) and samples of human milk (banked donor breast milk or maternal milk) were collected from each pooled 24-hour milk collection.

In addition to daily nutritional intake, detailed notes of perinatal events, obstetric events, and clinical course were taken. Extensive data were collected on foetal, neonatal, and environmental factors. A research nurse and paediatrician in each centre were responsible for collecting anthropometrical measures and samples for biochemical and immunological studies. The median number of days in the study was 39.5.

3.2 Cognitive follow-up studies

3.2.1 Follow-up studies descriptions

At nine months post expected delivery date, measures of growth and cognitive development were recorded for infants born in Ipswich, Cambridge, and King's Lynn hospitals. The Knobloch, Pasamanick and Sherard's developmental screening inventory was used to measure development across five domains: adaptive, gross motor, fine motor, language, and personal/social (Knobloch, Pasamanick and Sherard, 1966). At 18 months, the full cohort was followed up. This time the Bayley
scales of development were used, providing indices of mental (MDI) and psychomotor (PDI) development (Bayley, 1969).

Ninety-two percent of children from the original cohort who had survived were seen again at age 7.5-8 (see table 6 for population characteristics) during a childhood follow-up visit, in which an abbreviated IQ test (WISC; Wechsler, 1974) and neurological examination were conducted.

Children who had been born at 30 weeks GA or less and were classified as normal on the neurological examination at age 7.5-8 years were invited back for a follow-up during adolescence. The 112 subjects aged 13-19 years (mean age 15 years) who participated, completed the WISC-III or WAIS-III IQ test depending on exact age (Wechsler, 1991, 1997). Of these, 76 also underwent a T1 MRI brain scan.

A total of 167 participated again as young adults, aged 20. They completed an IQ test, as well as the CANTAB cognitive test battery (Sahakian et al., 1988) and T1 and DWI brain scans, but these data have yet to be published.
Figure 6: Overview of study design with sample sizes at each follow-up

Knobloch= Knobloch Passamanic & Sherrards test adaptive score, Bayley=corrected Bayley Mental Development score, FSIQ=Full Scale IQ.
3.2.2 Published results of follow-up studies

This section provides an overview of the published results from the trial, on which hypotheses 2 and 3 of this thesis are based, i.e. the beneficial effect of human milk and a high nutrient diet on outcomes in people born preterm. Publications are summarised in table 4 on page 134.

*Human Milk*

At the 18 month follow-up visit, there were no differences in cognitive development between the BBM and PTF groups, and both groups outperformed the TF group. This is surprising since BBM has lower energy, protein, fat, and mineral content than TF (Lucas *et al.*, 1994). BBM and MBM were also equally protective against necrotising enterocolitis (NEC; Lucas and Cole, 1990). This supports the hypothesis that human milk has components that benefit brain development via mechanisms other than the nutritional level.

To explore this further, infants previously fed BBM in study 1 were compared with babies previously fed TF in study 2. The analysis was confined to the groups in trial A, who received the study diet as their sole diet, to avoid confounding biological effects of human milk as well as to factor out the social and educational biases associated with a mother's choice to breastfeed. There was a significant advantage for the BBM fed group in motor development at 18 months compared to the TF fed group. At 18 months, motor development scores were higher in the BBM group than the TF group. Mental development scores were also higher, but the difference did
not reach statistical significance. These differences were most apparent in infants who were born small for gestational age (SGA).

The comparison between BBM and TF was non-randomised because the BBM group was part of study 1 and TF of study 2. Conveniently, PTF was used in both studies and could therefore be used for interstudy comparison. The developmental scores of the PTF group in study 1 (BBM study) were lower than those of the PTF group in study 2 (TF study), suggesting that the difference between BBM and TF may have been even larger than was revealed by the direct comparison (Lucas et al., 1994).

If benefits of human milk on neurodevelopment can be observed for the low nutrient banked donor milk, even larger benefits should be seen for mother’s milk. Indeed, even after adjusting for social and demographic factors, children whose mothers chose to provide breast milk had significantly higher motor development scores at 18 months post-term. It is of course possible that not all relevant confounding factors were identified and adjusted for. Since mothers sometimes lived at some distance from the hospital, expressing milk was a time-consuming activity and required a considerable amount of intrinsic motivation. This may have related to the greater commitment and investment in the child’s development (Morley and Lucas, 1993).

The childhood follow-up study carefully attempted to disentangle the effects of sociodemographic factors and breast milk. After adjusting for social class, mother’s
education, days of ventilation, and sex, children whose mothers chose to provide breast milk still had a significant 7.6 IQ point advantage over the children whose mothers chose not to express breast milk (Morley and Lucas, 1993). There were multiple indications that the beneficial effect of breast milk on cognition in infancy and childhood could not solely be attributed to socioeconomic and environmental factors. There was a dose-response relationship between the percentage of mother’s milk in the diet and IQ, particularly for VIQ, while success in providing breast milk was not related to the mother’s social class or education. There were no interaction effects between the percentage breast milk and social class or mother’s education and IQ, and the effect was not driven by the small group of infants who had been breastfed on discharge. Furthermore, the IQ scores of children whose mothers chose to provide breast milk but were unable to do so were comparable to those of children whose mothers chose not to provide breast milk, but significantly lower than those of children who did receive mother’s milk (Lucas et al., 1992).

At adolescence, VIQ was significantly correlated with the proportion of MBM in the infant diet. In males, the proportion of MBM in the diet also correlated with PIQ and FSIQ, while in females none of the relationships between MBM and IQ scores reached statistical significance. MRI scans showed a significant correlation between the proportion MBM, total brain volume and total white matter volume. This effect was again driven by the males. White matter volume correlated significantly with both VIQ and FSIQ in the total group. In males, there was an additional significant relationship between total brain volume and VIQ. Exclusion of the BBM group
(whose diet consisted of 100% human milk) did not change the relationship between MBM intake and IQ scores (Isaacs et al., 2010). This study demonstrated that the beneficial effect of (maternal) breast milk remained observable in adolescence, and that the effect is strongest in males.

**Nutritional Plane**

As can be seen in Table 3, PTF has a much higher nutritional content than TF and BBM. Compared to BBM, intake of preterm formula was associated with faster postnatal increases in length and head circumference as well as greater adaptive, language and personal-social development at nine months post term. The strongest effect of diet on developmental outcome at 9 months was seen in males, and in infants born small for gestational age (SGA). In the group who received the trial diet as a supplement to mother’s milk, the effect on developmental scores at nine months was stronger for infants for whom the allocated diet accounted for more than 50% of their intake (Lucas et al., 1989). At 18 months, however, no significant differences could be observed in developmental outcome between infants fed BBM and infants fed preterm formula. There were small advantages for SGA infants and boys, but these did not reach statistical significance (Lucas et al., 1994).

Study 2 compared infants previously fed PTF with infants fed TF. In this study, the effects of nutrition on growth were less marked, but babies fed PTF still had significantly faster weight gain and head growth than those fed TF (Morley and
Lucas, 1993). The effect on developmental outcome, however, was larger than in study 1 (Lucas et al., 1990, 1994). Infants in the preterm formula group scored higher on the mental and psychomotor development index as well as the social quotient. The mental index score group difference did not reach statistical significance. Consistent with the 9-month follow-up in study 1, the effect of diet was largest in males and SGA infants (Lucas et al., 1990; Morley and Lucas, 1993). The effect of diet was larger when children who received <50% of their enteral intake as trial diet or those who were in the trial for less than two weeks were excluded. (Lucas et al., 1990).

This pattern was also observed at age 7; children in the TF group had significantly lower VIQ than those in the PTF group. When restricting the group analysis to children who had received at least 50% of their enteral intake as trial diet for more than two weeks, the advantage of preterm formula was seen for both VIQ and FSIQ scores. Again, the effect was more marked in males (Lucas, Morley and Cole, 1998).

For the adolescent follow-up study, only those born at 30 weeks GA or less were included, and the two studies were grouped together. BBM and TF groups were collapsed into a standard nutrient group, which was compared to a high nutrient group consisting of those who had received PTF. In this subset of participants born very prematurely, childhood VIQ, PIQ, and FSIQ scores were increased in the high compared to the standard nutrient group. The effects of diet on VIQ persisted until adolescence with lower VIQ scores in both the original BBM and TF groups.
compared to the PTF groups. No effect of sex or SGA on IQ was found when these factors were included in the analyses (Isaacs, Morley and Lucas, 2009). MRI scans obtained during adolescence showed significantly larger caudate volumes in the high nutrient group compared to the standard nutrient group. Caudate volumes were significantly correlated with VIQ scores, but only in males and only in the standard nutrient group (Isaacs et al., 2008).

In summary, the past published trial reports indicate that both human milk and high nutrient content have beneficial effects on cognitive development in preterm infants. BBM appears to have a protective effect against NEC and developmental delay in early in life, but like TF, is low in nutrient content and associated with lower IQ scores in childhood and adolescence.
### Table 4: Results of previous follow-up studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants age</th>
<th>Outcome measures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Lucas et al., 1984)</td>
<td>0-2 months</td>
<td>Days to regain birthweight, Steady state weight gain, Head circumference gain, Length gain, Incidence of NEC</td>
<td>Study 1: BBM slower growth and relatively lower weight in relation to head circumference and length gain during hospital stay compared to PTF</td>
</tr>
<tr>
<td>(Lucas and Cole, 1990)</td>
<td>BBM and MBM intake associated with reduced NEC incidence.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lucas et al., 1989)</td>
<td>9 months*</td>
<td>Knobloch et al. Developmental screening inventory (Knobloch, Pasamanick and Sherard, 1966)</td>
<td>PTF higher overall development quotient compared to BBM. Largest effect in SGA infants and males.</td>
</tr>
<tr>
<td>(Lucas et al., 2001)</td>
<td>BBM higher overall development quotient compared to BBM. Largest effect in SGA infants and males.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lucas et al., 1990)</td>
<td>18 months*</td>
<td>History and physical examination, Bayley scales of infant development, MDI: mental development index, PDI: psychomotor development index</td>
<td>PTF higher PDI compared to TF, effect largest in trial 1, males, and SGA infants. No differences between BBM and PTF, except in SGA infants. BBM higher PDI compared to TF. MBM higher MDI compared to no MBM.</td>
</tr>
<tr>
<td>(Lucas et al., 1994)</td>
<td>No significant differences between BBM and PTF in developmental scores.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lucas et al., 2001)</td>
<td>PTF higher weight and length compared to TF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lucas et al., 1992)</td>
<td>7 years</td>
<td>Wechsler Intelligence Scale for Children (revised Anglicised version: WISC-R UK; Wechsler, 1974)</td>
<td>Higher VIQ, PIQ, and FSIQ scores of MBM compared to no MBM. Larger proportion of MBM in diet was associated with higher IQ scores.</td>
</tr>
<tr>
<td>(Lucas, Morley and Cole, 1998)</td>
<td>TF lower VIQ compared to PTF in males.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Isaacs, Morley and Lucas, 2009)</td>
<td>PTF higher VIQ, PIQ, and FSIQ compared to TF/BBM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Isaacs et al., 2004)</td>
<td>Higher VIQ scores between age 7 and 15. A decline in VIQ is associated with temporal and frontal volumes and a decline in PIQ is associated with volumes in the occipital and temporal lobe regions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Isaacs et al., 2008)</td>
<td>Decline in mean IQ scores between age 7 and 15. A decline in VIQ is associated with temporal and frontal volumes and a decline in PIQ is associated with volumes in the occipital and temporal lobe regions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Isaacs, Morley and Lucas, 2009)</td>
<td>PTF larger caudate volumes and higher VIQ compared to TF/BBM. Caudate volumes correlates with VIQ in low TF/BBM in males.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Isaacs et al., 2010)</td>
<td>PTF higher VIQ compared to TF/BBM. Effect is principally accounted for by verbal comprehension index score.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NEC=necrotising enterocolitis, BBM=banked donor breast milk, PTF=preterm formula, MBM=maternal breast milk, TF=term formula, SGA=small for gestational age, PDI=psychomotor development index, MDI=mental development index PIQ=performance IQ, VIQ=verbal IQ, FSIQ=full scale IQ

* corrected for gestational age
3.3 Relevance of adult follow-up

The study presented here addresses the gaps in the literature that were identified in section 1.7; a lack of randomised controlled nutrition trials with long-term follow-ups, and insufficient use of modern MRI techniques. The cohort studied here is one of the first large preterm study cohorts to have reached adulthood thanks to significant decreases in mortality since the 1980s (Raju et al., 2017). The importance of preterm nutrition was not universally recognised until later, in part due to the convincing early results of this trial. This therefore presents the first opportunity to study the effects of preterm nutrition in adulthood, with the added benefit of an intervention trial design.

Inevitably, nutritional standards have changed in the past 30 years. The term formula and even the preterm formula in this trial contain less energy and protein than current preterm formulas (Su, 2014). According to current recommendations, drip milk, as used in this trial, is not suitable as donor milk because of its low energy content (Baumer, 2004). Nowadays, even expressed donor milk is often fortified for preterm infants (Brown et al., 2016). When this trial was conducted, there were no special feeding recommendations for preterm infants. The first set of nutrition recommendations for preterm infants were only published in 1987, by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN, 1987). Guidelines have changed considerably between then and the latest update in 2010 (Agostoni et al., 2010, see table 5).
Nevertheless, the study remains relevant for a number of reasons. First, it is important to understand, and identify markers of, the long-term effects of neonatal nutrition such that an adequate risk-benefit analysis can be conducted. Second, despite improved nutritional standards, nutritional deficits and poor growth remain problematic. Third, this study is particularly well placed for exploration of mechanisms that may drive the effect of nutrition on outcome, since the range of nutritional intake in this group is much wider than would be possible in a trial with current nutritional standards. Nowadays it would be unethical to randomise infants to BBM or TF with very low nutritional density. Insights into the biological effects of nutrition on the preterm brain can be used to inform decisions on future nutritional recommendations. Fourth, there is no consensus yet as to whether cow’s milk-based formulae are a good alternative to those that are based on human milk. As human milk fortifiers (made from human milk rather than cows’ milk) have recently gained

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Table 5: Feeding recommendations by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in 1987 and 2010

<table>
<thead>
<tr>
<th></th>
<th>1987(^a)</th>
<th>2010(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid (ml/kg/day)</td>
<td>135-200</td>
<td>135-200</td>
</tr>
<tr>
<td>Energy (kcal/kg/day)</td>
<td>110</td>
<td>110-135</td>
</tr>
<tr>
<td>Protein (g/kg/day)</td>
<td>35</td>
<td>29</td>
</tr>
</tbody>
</table>

\(^a\) (ESPGHAN, 1987)  
\(^b\) (Agostoni et al., 2010)
traction in North America, the comparison between exclusive human milk and bovine milk has become highly relevant once again.

Finally, modern tools may be better able to characterise the effects of premature birth and neonatal nutrition than those that have been employed previously. Previous studies have identified the cognitive tests that are most likely to pick up specific deficits in preterm born adults. Additionally, this project takes advantage of the tremendous development in MRI methods and analysis techniques to identify and quantify subtle differences in brain structure.
4 Methods - adult follow-up

4.1 Participants

4.1.1 Recruitment

The current study is an adult follow-up of the cohort described in chapter 3 (aged 33.5 ± 1.2 years). Individuals from the original cohort who participated at the 7-year follow-up were contacted, with priority given to subjects who had taken part as young adults and those who participated in a cardiac study around the age of 25 years. In total, there were contact details available for 377 people, of whom 94 indicated that they would be interested in taking part and 8 subjects declined. No response was received from the other 275 people. It is plausible that many of them had moved house or changed their e-mail address.

Recruitment process preterm subjects

Figure 7 demonstrates the recruitment flow for the preterm subjects. In 2011, participants who had taken part in the 20-year follow-up were asked by letter if they would be happy to be contacted to take part in a future follow-up study. The 94 subjects had responded positively were contacted during the first wave of
recruitment. Subsequently, a recruitment letter was sent out to the 78 subjects who had not responded in 2011. Then, 38 more subjects were contacted from a sample who had participated in a cardiac study at Oxford University at age 25. Finally, old notebooks with addresses of the participants at age 10 were transcribed and another 167 letters were sent out.

*Figure 7: Recruitment flow preterm subjects*
Eight subjects indicated that they did not wish to participate in any further studies, nine subjects indicated that they could not participate at the moment, but that they would potentially be willing to do so in the future. Thirteen subjects initially indicated that they would like to take part, but it was not possible to arrange a study visit. Table 6 compares the population characteristics of the preterm participants who participated in the previous follow ups, along with those who participated in the current study and those who did not.
Table 6: Population characteristics of subject groups included in studies at birth, childhood, and adulthood and differences between subjects who were followed-up here, in adulthood, compared to the sample followed-up in childhood but not in adulthood

<table>
<thead>
<tr>
<th></th>
<th>Neonate</th>
<th>Childhood</th>
<th>Adulthood</th>
<th>Number of subjects</th>
<th>697</th>
<th>t-test ( t ), ( \chi^2 ) test, ( F ) test, ( W ) test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at time of study mean (SD), years</td>
<td>0</td>
<td>7.6 (0.16)</td>
<td>33.5 (1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex male, %</td>
<td>50</td>
<td>50</td>
<td>46</td>
<td></td>
<td>50</td>
<td>0.23(^b)</td>
<td>0.63</td>
</tr>
<tr>
<td>Maternal education &lt; A-levels, %</td>
<td>51</td>
<td>51</td>
<td>36</td>
<td></td>
<td>52</td>
<td>6.3(^b)</td>
<td>0.01</td>
</tr>
<tr>
<td>Gestational age mean (SD), weeks</td>
<td>30.8 (2.8)</td>
<td>31.1 (2.7)</td>
<td>30.1 (2.4)</td>
<td></td>
<td>31.2 (2.7)</td>
<td>-2.77(^a)</td>
<td>0.007</td>
</tr>
<tr>
<td>Birthweight mean (SD), g</td>
<td>1369 (316)</td>
<td>1401 (294)</td>
<td>1307 (302)</td>
<td></td>
<td>1411 (292)</td>
<td>1.12(^a)</td>
<td>0.08</td>
</tr>
<tr>
<td>Birthweight z-score mean (SD)</td>
<td>-0.8 (1.3)</td>
<td>-0.8 (1.3)</td>
<td>-0.7 (1.1)</td>
<td></td>
<td>-0.9 (1.3)</td>
<td>32553(^d)</td>
<td>0.34</td>
</tr>
<tr>
<td>Small for gestational age, %</td>
<td>35</td>
<td>35</td>
<td>28</td>
<td></td>
<td>36</td>
<td>1.30(^b)</td>
<td>0.25</td>
</tr>
<tr>
<td>Mechanical ventilation (no/1-5 days/&gt;6 days), %</td>
<td>48/29/23</td>
<td>54/27/18</td>
<td>45/31/24</td>
<td></td>
<td>55/27/18</td>
<td>0.38(^c)</td>
<td>0.54</td>
</tr>
<tr>
<td>Standard nutrient group, %</td>
<td>50</td>
<td>50</td>
<td>46</td>
<td></td>
<td>50</td>
<td>0.27(^b)</td>
<td>0.60</td>
</tr>
<tr>
<td>Human milk mean (SD), %</td>
<td>51 (44)</td>
<td>50 (43)</td>
<td>51 (40)</td>
<td></td>
<td>49 (44)</td>
<td>0.36(^a)</td>
<td>0.72</td>
</tr>
<tr>
<td>Infection/NEC, %</td>
<td>14</td>
<td>13</td>
<td>15</td>
<td></td>
<td>11</td>
<td>0.01(^b)</td>
<td>0.90</td>
</tr>
<tr>
<td>FSIQ age 7 mean (SD)</td>
<td>-</td>
<td>100 (16)</td>
<td>108 (14)</td>
<td></td>
<td>100 (16)</td>
<td>4.43(^a) &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>VIQ age 7 mean (SD)</td>
<td>-</td>
<td>100 (19)</td>
<td>107 (17)</td>
<td></td>
<td>99 (19)</td>
<td>3.63(^a) &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>PIQ age 7 mean (SD)</td>
<td>-</td>
<td>101 (17)</td>
<td>108 (16)</td>
<td></td>
<td>100 (17)</td>
<td>4.00(^a) &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

\(^a:\) t-test, \(^b:\) chi-squared test, \(^c:\) One way ANOVA
Recruitment process control subjects

This is the first follow-up visit for which an equal sample of term born control subjects was recruited. Inclusion criteria were individuals aged 25-40 years, born after 37 weeks of gestation, fluent in English, with no history of neurological disease, no exclusions to MRI, and no prior experience of cognitive testing. Participants from the preterm cohort were asked if they had any family members or friends who met the selection criteria and would be willing to take part. As not all preterm participants were able to bring a control participant, additional full-term participants were recruited through personal connections, our study facebook page (www.facebook.com/ucl.preterm), and online webpages such as gumtree, craigslist, Do-it.org, and the website of volunteer centre Camden. Leaflets were distributed at dentist and GP practices and pharmacies in the Bloomsbury area, London. Figure 8 shows the proportion of subjects recruited via the different channels.
4.2 Data collection

4.2.1 Study visit

Participants completed a Quality of Life questionnaire (described below) prior to the study visit. Participants were scheduled undergo an MRI scan and cognitive testing as close in time as possible, most often on the same day. Participants were asked to sign an informed consent form before the start of the test and/or MRI scan. Whether the MRI scan or the cognitive testing occurred first depended on the availability of the participant and the MRI scanner appointment slots. Before the MRI scan, the radiographer on duty or someone associated with the study went through an MRI safety checklist and made sure the participant did not carry any metal items into the...
scanner to ensure his or her safety. Including preparatory procedures, the MRI session took approximately 1.5 hours. The cognitive testing session took on average approximately 2.5 hours. Participants were reimbursed for their travel and lunch expenses and received a CD with their T1-weighted brain scan.

Cognitive test examiners were blinded to both preterm/control group membership as well as the nutrition group of the participants. Since I recruited participants and booked study visits, I was not blinded to group membership. I could therefore run the MRI scans, but the cognitive tests were administered by seven students and research assistants. All examiners adhered to a written testing protocol that included detail down to the level of individual sentences. Professor Paul Burgess tested and trained the first examiners, and subsequent examiners were trained and assessed by the previous examiners before undertaking any cognitive testing.

All examiners tested both preterm and full-term participants. After the cognitive testing, the batteries were scored by the examiner who had administered the tests and by a second-scorer. The double-scoring system was introduced in January 2017 and there were four second-scorers. All discrepancies between the two scorers were discussed and resolved in meetings held once every three months. Inter-rater reliability was at least 0.87 (see table 7). Inter-rater reliability is the proportion of the number of ratings that are in agreement relative to the total number of ratings.
Table 7: Inter-rater reliability of selected cognitive test scores

<table>
<thead>
<tr>
<th>Cognitive test scores</th>
<th>Inter-rater reliability</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLT trial A6 (correct items)</td>
<td>0.96</td>
<td>0.94-0.97</td>
</tr>
<tr>
<td>CVLT trial B (correct items)</td>
<td>0.96</td>
<td>0.95-0.97</td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td>0.87</td>
<td>0.82-0.90</td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td>0.96</td>
<td>0.95-0.97</td>
</tr>
<tr>
<td>HSCT 1 timea (s)</td>
<td>0.94</td>
<td>0.91-0.95</td>
</tr>
<tr>
<td>HSCT 2 timea (s)</td>
<td>0.98</td>
<td>0.97-0.98</td>
</tr>
<tr>
<td>HSCT 2 errora</td>
<td>0.90</td>
<td>0.86-0.93</td>
</tr>
<tr>
<td>Arithmetic (correct items)</td>
<td>0.99</td>
<td>0.99-0.99</td>
</tr>
<tr>
<td>Pegboard both hands (score)</td>
<td>1.00</td>
<td>1.00-1.00</td>
</tr>
<tr>
<td>Pegboard assembly (score)</td>
<td>0.90</td>
<td>0.86-0.93</td>
</tr>
<tr>
<td>Zoo map errora</td>
<td>0.92</td>
<td>0.89-0.94</td>
</tr>
<tr>
<td>Verbal fluency phonetic</td>
<td>0.99</td>
<td>0.97-0.99</td>
</tr>
<tr>
<td>Verbal fluency semantic</td>
<td>0.96</td>
<td>0.95-0.97</td>
</tr>
<tr>
<td>VCI</td>
<td>0.92</td>
<td>0.90-0.95</td>
</tr>
<tr>
<td>PRI</td>
<td>0.95</td>
<td>0.93-0.96</td>
</tr>
<tr>
<td>FSIQ</td>
<td>0.70</td>
<td>0.60-0.77</td>
</tr>
</tbody>
</table>

CI=Confidence Interval

<0.50=poor, 0.50-0.75=moderate, 0.75-90=good, >0.90=excellent

4.2.2 Cognitive test administration protocol

Standardised tests of general intelligence as well as tests to examine more specific domains of cognition were administered to all participants. The tests
and outcome measures are described in Table 8. Particular emphasis was placed on selecting tests with a high ecological validity and sensitivity. The tests chosen here aimed to assess those abilities that were likely to be affected in this population born preterm. This was based on this literature as well as on the problems in daily life that participants of this study had reported during previous follow-up visits. When selecting tests for the cognitive battery, four main factors were taken into account; whether the tests were reliable, whether they covered all cognitive abilities described in section 1.2.1, whether they covered both generalised and some specific cognitive abilities and whether the total length of the test battery did not exceed three hours. Tests that had been normed on a population similar to the current cohort were preferred. To avoid repetition effects, tests with a memory component (i.e. the Rey Complex Figure and verbal list learning) should not have been administered to the preterm participants during previous studies.

The executive function domain can be split into three orthogonal variables: response inhibition, updating working memory representations (updating), and shifting between tasks or mental sets (shifting; Miyake et al., 2000). Updating is strongly correlated with IQ, but shifting and inhibition are not and should therefore be assessed separately (Friedman et al., 2006). Within the memory domain, both verbal and non-verbal components were assessed. The final set of outcome measures covered assessment of the cognitive abilities that were likely to be affected in this population as comprehensively as possible.
Rationale for tests chosen

Computer tests (Volle et al., 2011): The computer tests were developed specifically for an adult population in London with neurological difficulties. Particularly given the potential effects of selective attrition in this long-term follow up study, the performance differences in this population are likely to be small (Eisner et al., 2019). It is a great advantage to have the automatic and precise measurement of reaction times offered by a computerised programme. The prepare task measured simple reaction times and the ability to maintain attending behaviour over a varying inter-stimulus interval. Reaction time tasks are widely used and sufficiently sensitive to pick up on variability in healthy adult populations (Hultsch, MacDonald and Dixon, 2002). They pick up on non-specific differences, because performance will change with any cognitive difficulty or transient factors such as fatigue or motivation. A reaction time task can also detect specific variation, for example in the medial area 10 (Gilbert et al., 2006). Outlier trials were removed, because some trials with extremely long reaction times compared to other trials in the same subject had a very large effect on the mean reaction time. These outlier trials are unlikely to reflect the ability of an individual to respond quickly to a stimulus. The aim was to remove the trials for each subject that did not represent the performance of that subject, whilst keeping the inter-subject variability intact. This was done using Median Absolute Deviation (MAD) per subject; the trials where the RT was at least 3 times the median deviation
from the median of that particular subject were removed.

The inhibition task is one step up towards measuring executive function. It is sensitive to problems related to prefrontal cortex function (Hu et al., 2018). Requiring multi-tasking, the switch task is again a step up in the executive function hierarchy and covers a different cluster of symptoms than inhibition (Volle et al., 2011). Multi-tasking deficits are associated with poor social and professional outcomes even if IQ is preserved (Bell-McGinty et al., 2002).

The Hayling Sentence Completion Test (Burgess and Shallice, 1997) is a test of executive functioning of ecological validity and is relatively independent of IQ (Cervera-Crespo and González-Alvarez, 2016). The Zoo map (Emslie et al., 2003) is a planning test with high ecological validity. Participants of this cohort had reported difficulty navigating new environments. The Zoo map error scores were calculated by subtracting the errors in Map 2 (with route instructions) from those in Map 1 (without route instructions). The COWAT verbal fluency test measures verbal executive function (Benton and Hamsher, 1976).

Verbal memory was assessed with the AMIPB verbal list learning (Coughlan and Hollows, 1985), and the Rey Complex Figure (Meyers and Meyers, 1995) was used to test non-verbal memory.

The graded difficulty arithmetic test (Jackson and Warrington, 1986) is a pure arithmetic test. Mathematical deficits were previously found in this population at age 15 (Isaacs et al., 2001), which is why we decided to include these tests in the battery.
Fine motor skills and dexterity were measured using the pegboard test (Tiffin and Asher, 1948). This test can indicate executive dysfunction when looking at rule violations (Tolle et al., 2019), but a practice trial was used to reduce potential rule violation issues. During the practice trials, rule violations were pointed out and the testing trial was not started until the participant appeared to have understood the rules.
**Table 8: Cognitive test battery description**

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
<th>Outcome measures selected based on theoretical grounds</th>
<th>Cognitive domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>WASI-II (Wechsler, 2011)</td>
<td>A brief measure of intelligence using four subtests.</td>
<td>FSIQ, PRI (PIQ), VCI (VIQ)</td>
<td>Visual spatial orientation, Constructional ability, General intelligence</td>
</tr>
<tr>
<td></td>
<td>Block design: The subject has to replicate two-dimensional geometric patterns using two-color cubes</td>
<td>Block design, Matrix reasoning, Vocabulary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Similarities</td>
<td>Vocabulary, Similarities</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vocabulary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Computerised reaction time tests (Volle et al., 2011)</td>
<td>The stimuli were presented on a screen (36 cm x 27 cm) with a 1280 x 800 resolution of a HP notebook PC (Compaq 6730s), running Microsoft Windows XP. Instructions were given on the screen and the examiner gave additional explanations whenever the subject asked for it. After the instructions, participants were given 10 practice trials before the test started. Stimuli were presented on a white background on the PC.</td>
<td>Prepare</td>
<td>Mean RT, mean RT 2.5s delay, mean RT 5s delay, mean RT 10s delay, mean RT 10s delay pictures, mean RT 10s delay words, mean RT 10s delay correct words, mean RT all correct numbers, mean RT all correct pictures, mean RT all correct accuracy words, mean RT all correct accuracy pictures, mean RT all correct accuracy numbers, accuracy all, accuracy numbers, accuracy all</td>
</tr>
<tr>
<td></td>
<td>Participants were shown pictures and words and they had to press a key as quickly as possible when an item appeared on the screen. The intra stimulus interval duration varied.</td>
<td>Prepare</td>
<td>mean RT, mean RT 2.5s delay, mean RT 5s delay, mean RT 10s delay, mean RT 10s delay pictures, mean RT 10s delay words, mean RT 10s delay correct words, mean RT all correct numbers, mean RT all correct pictures, mean RT all correct accuracy words, mean RT all correct accuracy pictures, mean RT all correct accuracy numbers, accuracy all</td>
</tr>
<tr>
<td></td>
<td>Switch: Participants were shown pairs of words, numbers and pictures. For word pairs they had to press the mouse key that is in the direction of the word that contained the letter p. For pictures they had to press the key in the direction of the item that costs the least and for numbers they had to press in the direction of the even number.</td>
<td>Switch</td>
<td>mean RT, mean RT words, mean RT correct words, mean RT correct numbers, mean RT pictures, mean RT correct pictures, mean RT all correct accuracy words, mean RT all correct accuracy pictures, mean RT all correct accuracy numbers, accuracy all</td>
</tr>
<tr>
<td></td>
<td>Inhibit: Similarly to the prepare task, participants were shown a series of pictures, one at a time. They had to press the spacebar as soon as a picture appeared on the screen. The only difference with the prepare task is that they should not press the key when the picture depicted an animal.</td>
<td>Inhibit</td>
<td>False positives</td>
</tr>
</tbody>
</table>

*Table 8 continues on the next page*
### Table 8: Cognitive test battery description

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
<th>Outcome measures</th>
<th>Outcome measures selected based on theoretical grounds</th>
<th>Cognitive domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>California Verbal Learning Test (Dell et al., 1987)</td>
<td>Participants were asked to repeat words from list A, consisting of 16 words drawn from four semantic categories. The words were read to them. They did not have to recall the words in order. After five trials, the list is changed to list B, and after one trial of list B, the subjects were asked to recall words from the first list again without hearing it again.</td>
<td>List A&lt;br&gt; Total recall trials 1–5&lt;br&gt; Percentile score trial 1–5&lt;br&gt; Recall trial 6&lt;br&gt; Percentile score trial 6</td>
<td>List A&lt;br&gt; Total recall trials 1–5&lt;br&gt; Recall trial 6&lt;br&gt; List B&lt;br&gt; Recall trial B</td>
<td>Verbal memory&lt;br&gt; Episodic memory&lt;br&gt; Language</td>
</tr>
<tr>
<td>Verbal fluency Control Oral Word Association Test (Benton and Hamre)</td>
<td>This test consists of a semantic and a phonemic part. Participants were asked to name as many words as possible in one minute with a certain characteristic. Proper names of people or places are not allowed, and derivatives of the same word are not counted. In the phonemic part the characteristic was starting with a specific letter (three trials: F, A, and S), while in the semantic part, the characteristic was the category animals.</td>
<td>Phonetic&lt;br&gt; Score F&lt;br&gt; Score A&lt;br&gt; Score S&lt;br&gt; Score F+A+S</td>
<td>Total Score F+A+S+category Language animals</td>
<td>Executive functioning</td>
</tr>
<tr>
<td>Hayling sentence completion (Burgess and Shallice, 1997)</td>
<td>Participants were instructed to finish incomplete sentences read by the examiner. In trial A, the response should be a word that fits well within the context of the sentence. In trial B, the response should be a word that does not fit at the end of the sentence and is unrelated to the content. This tests the ability to inhibit responses.</td>
<td>Trial A&lt;br&gt; Total response time&lt;br&gt; Error score&lt;br&gt; Scaled total response time&lt;br&gt; Error score</td>
<td>Trial A&lt;br&gt; Error score&lt;br&gt; Trial B&lt;br&gt; Error score&lt;br&gt; Total response time</td>
<td>Executive functioning</td>
</tr>
<tr>
<td>Purdue Pegboard (Tiffin and Asher, 1948)</td>
<td></td>
<td>Score left hand&lt;br&gt; Score right hand&lt;br&gt; Score both hands&lt;br&gt; Score left + right + both hands</td>
<td>Score left hand&lt;br&gt; Score right hand&lt;br&gt; Score both hands&lt;br&gt; Assembly</td>
<td>Fine motor skills</td>
</tr>
</tbody>
</table>

*Table 8 continues on the next page*
### Table 8: Cognitive test battery description

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
<th>Outcome measures</th>
<th>Outcome measures selected based on theoretical grounds</th>
<th>Cognitive domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoo Map (part of the Behavioural Assessment of the Dysexecutive Syndrome battery (Emslie et al., 2003))</td>
<td>Participants are asked to plan a route on a map of a zoo to visit six out of twelve possible locations. The locations that should be visited are specified, but in the first trial, the participant has to come up with a route that does not use the same path twice. The second trial involves the same elements, but the order in which the places should be visited is given in instructions.</td>
<td>Map 1: Raw score, Error score, Time error&lt;br&gt;Map 2: Raw score, Error score&lt;br&gt;Total: Raw score, Profile score, Total profile score (including planning time map 2)</td>
<td>Map 1: time&lt;br&gt;Map 2: Map 1 error</td>
<td>Prospection&lt;br&gt;Executive functioning&lt;br&gt;Planning</td>
</tr>
<tr>
<td>Rey Complex Figure (Meyers and Meyers, 1995)</td>
<td>Participants are asked to copy a visual line figure freehand. After three minutes (immediate recall) and then again after thirty minutes (delayed recall), they are asked to draw the same figure from memory. They do not know in advance that they will have to draw the figure again from memory.</td>
<td>Copy score: Raw score, Time (s), T score, Percentile score&lt;br&gt;Immediate recall: Raw score, Percentile score, T score, Time (s)&lt;br&gt;Delayed recall: Raw score, T score, Percentile, Time (s)</td>
<td>Copy: Raw score, Immediate recall, Raw score, Delayed recall&lt;br&gt;Copy 2: Raw score</td>
<td>Attention&lt;br&gt;Planning&lt;br&gt;Visual memory</td>
</tr>
<tr>
<td>The Graded Difficulty Arithmetic Test (Jackson and Warrington, 1986)</td>
<td>Participants were asked to solve addition and subtraction problems that are read to them by the examiner. The examiner stops after three consecutive incorrect answers.</td>
<td>Addition: Total correct, Total correct in &lt;10s&lt;br&gt;Subtractions: Total correct, Total correct in &lt;10s&lt;br&gt;Total: Total correct, Total correct in &lt;10s, Scaled score</td>
<td>Total: Total correct, Total correct in &lt;10s</td>
<td>Mathematical ability&lt;br&gt;Verbal working memory</td>
</tr>
</tbody>
</table>

RT=reaction time
4.2.3 Questionnaires

The questionnaires completed by the participant prior to the study visit can be found in the supplementary material. The questionnaires consisted of:

- Basic questions: i.e. date of birth, contact details, occupation, maternal education, highest level of education, and income
- Quality of Life Questionnaire: A self-report measure about life satisfaction in 16 domains, rated using a 5-point Likert scale.

4.2.4 MRI acquisition protocol

Participants were scanned on a Siemens Prisma 3 T MRI system, using a self-shielding gradient set with maximum gradient strength of 80 mT m$^{-1}$ and a 64 channel quadrature head coil for which there was dedicated research scanning time.

T1-weighted volume were acquired with 1mm isotropic voxels. Repetition time for the T1 images was 2300 ms, and echo time was 2.74 ms. Multi-shell diffusion MRI was acquired with 2mm isotropic voxels using multi-band echo-planar diffusion-weighted images with an optimized two-shell protocol: two 60-direction shells of $b = 1000$ s mm$^{-2}$ and $b = 2200$ s mm$^{-2}$, interleaved with 14 T2-weighted ($b = 0$) volumes. The echo time was 60ms and repetition time was 3050 ms. MPM scans are reconstructed on the scanner from a T1, PD, and MT-weighted sequences with a repetition time of 24.50 ms and echo time was 2.34 ms and a voxel size of 1mm isotropic.
4.2.5 Missing data and exclusion criteria

One preterm participant was excluded from analyses as she had suffered from a severe concussion and reported cognitive problems and fatigue as a result.

The computer tests were completed on an old machine and e-prime would sometimes fail to start, which resulted in missing data from 15 preterm and 12 control subjects. One subject could not complete the Purdue pegboard test due to a broken hand. One participant did not complete the IQ test due to a mistake by one of the examiners. The examiners made a judgment call to exclude the scores of specific tests if they thought the scores were not representative of the participants’ ability (e.g. due to external factors such as distractive noises, severe lack of motivation, misunderstanding of the task instructions).

Three preterm participants and one control did not undergo an MRI scan due to metal implants that were potentially not MRI compatible, one preterm subject suffered from claustrophobia. One control subject was late for the MRI scan visit and only completed the T1-scan. Diffusion data from five subjects (3 preterm and 2 control) was excluded as the FA skeleton contained voxels with partial volume effects of CSF, causing outliers in the data.
4.3 Data processing

4.3.1 Cognitive test measure processing

In order to facilitate the interpretation of the different variables, the scores where a higher value could be considered a worse performance (i.e. error scores and response times) were multiplied by -1. Hereby, generally a higher score could be considered better performance. However, it is important to note that this relationship is not always this simple. For example, in the Zoo map, a short time to complete the task is not good if the participant also had a lot of errors.

4.3.2 Cognitive test outcome measure selection

Determining which cognitive outcome variables should be used in further analyses depends critically upon theoretical understanding of the test measures as well as empirical grounds. Raw scores were favoured over scaled scores. As some tests in this test battery were developed for clinical populations, not all subtest measures are sensitive enough for a relatively high performing group of participants, such as the intrusions in the verbal list learning task or the error score of trial A in the Hayling sentence completion test.

Outcome measures of the same test are often dependent and therefore strongly correlated. Principal Component Analysis (PCA) was used to reduce the number of
variables per test by showing which subtest measures explain a similar variance in the data, so that only subtest measures that explained unique variance in the data were taken as outcome measures.

PCA is a data-driven, mathematical procedure that transforms a set of observations into uncorrelated variables called principal components. The first principal component explains as much of the variance in the data as possible, and each subsequent principal component accounts for the variance remaining. The variables that loaded most strongly on each of the principal components were chosen as ‘indicator variables’ for each cognitive test. For example, in the verbal list learning test, it becomes clear from the plot in figure 9 that there are two main principal components. The total trial A1-A5 and trial A6 load mainly on principal component 1 and recall trial B loads mainly on principal component 2. It is therefore not necessary to include both trial A1-A5 and trial A6 scores. As a rule of thumb, PCA factor(s) explaining more than 70% of variance were considered, and then the variable that most strongly loaded on that factor was selected for subsequent analyses. However, the final decision of which measures were selected as outcome measures was again based on theoretical grounds as described in section 4.2.2.
Figure 9: Principal component analysis of cognitive test measures

(Figure 9 continues on next page)
Figure 9: Principal component analysis of cognitive test measures

RCFT=Rey Complex Figure test,
HSCT=Hayling Sentence Completion Test,
PC=principal component
The first principal component of the verbal fluency score explained 77% of the variance and phonological and semantic fluency scores loaded equally on this component. Therefore, a combined verbal fluency score was calculated by averaging the phonological score (which in itself is an average of the F, A, and S trials) and the semantic score (the animal trial). The measures verbal fluency trial A6, Zoo map time error, arithmetic <10s, immediate recall, Pegboard left and right hand, were dropped from the list of cognitive variables.

4.3.3 MRI pre-processing

\textit{T1}

T1-weighted images were converted to NIfTI-1 format using the TractoR software package http://www.tractor-mri.org.uk (Clayden et al., 2011). T1-weighted volume data was parcellated into 32 cortical regions per hemisphere using FreeSurfer 5.3; http://surfer.nmr.mgh.harvard.edu. The T1-images were also processed using FSL (Jenkinson et al., 2012; Patenaude et al., 2012). The fsl anat pipeline was used for brain extraction and FIRST for parcellation of subcortical structures. Since FreeSurfer provides a better cortical parcellation, and FIRST a better subcortical parcellation, the two were combined using an in-house script. Volumes for the caudate, thalamus, hippocampus, and putamen from the FIRST parcellation were
used, as well as cortical white matter and ventricular volumes from the FreeSurfer algorithm.

**Diffusion Weighted Imaging**

The Tracto-R diffusion pipeline was applied to the multi-shell diffusion data (Clayden *et al.*, 2011). First, the DICOM files were converted into a 4D file in NIfTi format with the images in 64 directions and B0 field maps. The raw data was corrected for susceptibility-induced distortions using the FSL topup function (Andersson, Skare and Ashburner, 2003) and for eddy current induced distortions using FSL eddy function (Andersson and Sotiropoulos, 2016).

FSL was also used to fit a diffusion tensor and create tensor maps (Jbabdi *et al.*, 2012), and SMT was used to create microscopic diffusion maps (Kaden *et al.*, 2016). SMT maps were adjusted for rician noise after calculating the median of the voxel-wise estimate of the rician-distributed noise in the field map (Kaden, Kruggel and Alexander, 2016).

The structural and diffusion-weighted images were coregistered using NiftyReg with default settings (http://cmictig.cs.ucl.ac.uk/wiki/index.php/NiftyReg). MRTrixo 3 Multi-Shell Multi-Tissue Constrained Spherical Deconvolution was used for tractography of the whole brain, seeding dynamically in the white matter and stopping at the grey matter-white matter interface (Christiaens *et al.*, 2015).
Probabilistic Neighbourhood Tractography (PNT)

Probabilistic neighbourhood tractography is an automated method for extraction of major white matter tracts using a reference tracts as a topological guide (Clayden, Storkey and Bastin, 2007). As the study sample only included adults without major morphological brain abnormalities in the white matter (such as large lesions which were checked for visually), a pre-trained model was used for the reference tracts. At the moment, this is limited to the arcuate fasciculus, the anterior thalamic radiations, the cingulum, corticospinal tract, inferior longitudinal fasciculus, the uncinate fasciculus, the splenium and the genu of the corpus callosum (Table 9). Then, FA values were averaged across the voxels of these tracts in each individual.

<table>
<thead>
<tr>
<th>Tract name</th>
<th>Connecting regions/structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcuate fasciculus</td>
<td>Caudal temporal cortex - inferior parietal cortex - frontal lobe</td>
</tr>
<tr>
<td>Anterior thalamic radiations</td>
<td>Thalamus – prefrontal cortex</td>
</tr>
<tr>
<td>Cingulum</td>
<td>Cingulate gyrus – entorhinal cortex</td>
</tr>
<tr>
<td>Corticospinal tract</td>
<td>Spinal cord – somatosensory cortex</td>
</tr>
<tr>
<td>Corpus callosum genu*</td>
<td>Left-right medial/lateral frontal cortex</td>
</tr>
<tr>
<td>Corpus callosum splenium*</td>
<td>Left-right occipital lobes</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus</td>
<td>Temporal lobe – occipital lobe</td>
</tr>
<tr>
<td>Uncinate fasciculus</td>
<td>Hippocampus/amygdala - orbitofrontal</td>
</tr>
</tbody>
</table>

* Not analysed separately for left and right hemispheres
**Structural connectome**

The organisation of the brain can be described as a structural network of which lends itself to topological analyses. Nodes represent grey matter regions, determined by parcellation of the T1-weighted images (as described above) and edges represent the axonal pathways that transfer information between neurons. A connectivity matrix was created from the whole-brain tractography and graph metrics were extracted using the TractoR software (Clayden *et al.*, 2011). Summary measures can provide information about network characteristics (see table 10).

*Table 10: Description of graph theory metrics that were used examine the structural network connectome*

<table>
<thead>
<tr>
<th>Graph theory metric</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge density</td>
<td>The number of edges in a network relative to the maximum possible number of edges</td>
</tr>
<tr>
<td>Shortest path length</td>
<td>The minimum number of edges that must be traversed to get from one node to another</td>
</tr>
<tr>
<td>Global efficiency</td>
<td>The average inverse shortest path length in the network</td>
</tr>
<tr>
<td>Local efficiency</td>
<td>The average inverse shortest path length on the neighbourhood of the node</td>
</tr>
<tr>
<td>Cluster coefficient</td>
<td>The number of triangular connections between groups of three nodes</td>
</tr>
</tbody>
</table>
Multi Parametric Mapping

The Voxel-Based Quantification (VBQ) toolbox (Draganski et al., 2011) running on SPM 12 (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) and Matlab 2017a (Mathworks, Sherborn, MA, www.mathworks.com) was used to construct proton density (PD*), longitudinal relaxation rate (R1), magnetisation transfer saturation (MT), and effective transverse relaxation rate (R2*) maps in NIfTi format. The maps were visually screened for movement distortions and artefacts. For some images, the origin needed to be reset manually. The MT images were used for segmentation of the tissue types. A proton density mask was created by thresholding the map at >120, which was used to exclude the skull from the MT images and improve the segmentation.

4.4 Statistics

4.4.1 Covariates

Group differences between preterm and control subjects in chapter 5 and 6 were calculated using an ANCOVA model, including sex, age, and maternal education (higher or lower than A-levels) as covariates. Perinatal characteristics were added as covariates for the tests examining the effects of neonatal nutrition within the preterm group in chapters 7-10. GA, birthweight, and need for mechanical ventilation have shown to be related to brain structural and cognitive outcomes (Lucas et al., 1992;
Linsell et al., 2016). Since GA and birthweight were strongly correlated ($r=0.70$, $p<0.001$), a binary SGA variable was chosen as a covariate instead of birthweight. This accounted for the variance in birthweight beyond GA by identifying subjects with a birthweight <10$^{th}$ percentile for their GA. Data for days of ventilation was skewed, with many infants not receiving any ventilation, and few receiving more than a month of ventilation. Four categories were created based on clinical significance and distribution of the data; no ventilation, between 1 and 5 days, between 6 and 14 days, and more than 15 days. For volumetric analyses, total intracranial volume (ICV) was added as an extra covariate to examine changes in regional volumes over and above total brain volume.

4.4.2 Numeric outcome measures

Statistical analysis on numeric values from cognitive test scores, brain volumes, PNT tract averaged FA, and graph theoretical network measures was performed in R version 3.5.0 (‘R: A language and environment for statistical computing’, 2013). Details on the statistical tests applied are outlined in each chapter.

4.4.3 Voxel-based MRI analyses

Voxel-Based Morphometry

Voxel-based morphometry (VBM) is a technique that uses statistical parametric mapping to investigate regional differences in brain anatomy on a voxel-by-voxel
basis. This allows for unbiased examination of grey and white matter concentration and volumes.

VBM was performed using FSL's VBM tool (Douaud et al., 2007), which involves spatial normalisation of the images to MNI space, segmentation (grey matter, white matter and other tissue), smoothing, and statistical analysis. At the final step, ICV was included as an additional covariate and results were corrected with threshold-free cluster enhancement (TFCE) correction at the p=0.05 level to account for multiple comparisons across voxels. This provided a statistical parametric map that indicates voxels where grey matter concentration differed significantly between groups or correlated with a continuous variable (Ashburner and Friston, 2000; Winkler et al., 2014). Regions with significant results were named using the Harvard-Oxford cortical and subcortical atlases (Desikan et al., 2016).

_Tract-based spatial statistics (TBSS)_

Voxel-wise differences in DTI indices were assessed using tract-based spatial statistics (TBSS; Smith et al., 2006). This method creates a study-specific template by applying nonlinear registration of all individual FA maps to standard space and averaging them. A white matter ‘skeleton’ is created by applying a threshold on the mean FA image, consisting of voxels at the core of the white matter tracts common to the group. The warp fields from the non-linear registration are applied to all other diffusion maps. The subjects’ nearest maximum diffusion values are then projected
onto the skeleton, which aims to remove extra spatial variability across subjects. The skeleton maps are then entered in a GLM and analysed using voxel-wise permutation testing in combination with threshold-free cluster enhancement (TFCE) correction (Winkler et al., 2014). Tracts were identified using the JHU atlas white matter labels. FSL provides the probabilities of a certain cluster being a member of the different labelled tracts in the atlas (Oishi et al., 2010). An extent threshold of 10 voxels was chosen to reduce false positives displayed.

4.4.4 Diffusion metrics and cognitive test scores

Since diffusion metrics within a model are directly proportional to one another, it is not informative to relate all diffusion metrics to cognitive scores if the differences are primarily driven by one of the parameters. For example, changes in FA can be the result of a stable AD in combination with changes in RD. If this is the case, only FA will be analysed in relationship to cognitive measures. In the SMT model, intra-neurite volume fraction and orientation dispersion entropy provide additional information and will be analysed separately in case of significant group differences.

Voxel-Based Quantification (VBQ)

The DARTEL function of the VBQ toolbox in SPM uses the grey and white matter images of each subject to create a study-specific template. All grey and white matter images were registered to the template and transformed to MNI space. The final
transformation was then applied to all maps. The maps can be normalised to MNI whilst preserving the quantitative values within a particular tissue class. Finally, the standard SPM GLM was used for statistical analyses. VBQ calculates voxel-wise statistical differences or correlations in the quantitative MPM images. Results are family wise error (FWE) corrected to account for multiple comparisons across voxels. An extent threshold of 10 voxels was chosen to reduce false positives displayed.

4.5 Ethics

Ethics approval was granted by the UCL ethics committee in 2015. An amendment to the informed consent form to include an additional item asking for consent to share the data with other researchers in an anonymised manner was approved on 01/06/2016.
5 Comparison between adults born preterm and at term

5.1 Background

As was previously underlined in section 1.2.1, it is important to know the brain structural alterations and cognitive problems that are shared amongst the majority of people born preterm. Chapter 1 provided an overview of studies that examined differences in brain structure and cognitive function between preterm born and term born individuals. These report below average performance on cognitive tests, in particular in the domains of executive functioning, attention, spatial memory, language and some motor functions (Vohr, 2010). These are related to the white matter abnormalities commonly observed in people born preterm. In addition, lower grey matter volumes in people born preterm, whether caused by primary neuronal injury or secondary degeneration and/or dysmaturation after white matter injury, could also affect cognitive function (Nosarti et al., 2008).

The few long-term studies on preterm individuals report continuity from childhood through adulthood with regards to cognitive outcome (Johnson and Marlow, 2017) and brain structure (Nosarti, Murray and Hack, 2010). Recent studies in preterm adult populations have consistently reported reductions in grey matter volumes in the thalamus, caudate, hippocampus, putamen, and temporal cortex (Bäuml et al., 2014;

White matter abnormalities seem to persist into adulthood. Not only do white matter volume remain smaller in very preterm individuals in early adulthood (Nosarti *et al.*, 2014), studies also report widespread reductions in FA (Allin *et al.*, 2011; Eikenes *et al.*, 2011; Salvan *et al.*, 2014) as well as local increases in FA (Allin *et al.*, 2011; Eikenes *et al.*, 2011). Kontis and colleagues only reported higher MD in the genu of the corpus callosum preterm females compared to term females (Kontis *et al.*, 2009). Using the regular tensor FA models, it is impossible to say if these changes in white matter microstructure should be attributed to reduced myelination, reduced fibre density, reduced fibre coherence or a combination of these factors. More advanced diffusion sequences and models and other MR modalities such as MPM (see chapter 2) could further help separating these effects.

Reductions in white matter density and organisation also has an effect on the organisation and capacity of the entire structural network, the connectome. Since the field of connectomics is relatively young, very few studies have investigated this in preterm populations. The picture that is emerging shows that a reduction in white matter connections in people born preterm, which is also reflected by lower white matter volumes, leads to the development of a network characterised by preserved core connectivity at the cost of reduced local connectivity (Kim *et al.*, 2014; Karolis *et
This is reported from infancy until young adulthood, but more studies in adulthood with a control group of at least equal size are needed to investigate if this indeed remains a characterising feature of preterm brain structure in adulthood. Furthermore, network organisation could reflect beneficial adaptation to early damage or a paucity of resources and might therefore have a unique relationship to cognition in those who do show a network structure different from term born controls.

5.2 Aims

This chapter examines if and to what extent the effect of premature birth on cognitive outcome and brain structure persists into adulthood.

5.3 Hypotheses

Prematurity hypothesis: The effects of premature birth on the brain and cognition persist into adulthood.

Cognition

- Preterm born adults have lower scores on all cognitive test measures compared to term born controls.
Grey matter

- Preterm born adults have lower grey matter volumes compared to term born controls.
  - Thalamus, cerebellum, temporal lobe, caudate, and hippocampus.
- Preterm born adults have higher grey matter volumes compared to term born controls.
  - Cingulate cortex and frontal lobe.

White matter

- Preterm born adults have widespread reductions in white matter integrity.
  - Reduced fractional anisotropy (FA), increased radial diffusivity (RD) and increased mean diffusivity (MD).
  - Reduced MT values.
- Preterm born adults have reduced white matter capacity.
  - Reduced white matter volume and reduced number of connections (edge density).
- Preterm born adults have reduced white matter organisation.
  - Reduced fibre coherence/orientation density entropy (ODE).
  - Global network organization (global efficiency) is preserved, but local connectivity (local efficiency) is reduced.
- White matter alterations are associated with lower performance on cognitive tests.
5.4 Methods

5.4.1 Data collection

The MRI acquisition details as well as the study population descriptions, cognitive test measures, computation of brain volumes, voxel-based imaging analysis techniques (TBSS, VBM, VBQ), and connectivity are described in chapter 4.

5.4.2 Statistical analyses

Group differences in age were examined with a Mann-Whitney test and differences in sex and maternal education were examined with a chi-square test. Age, sex, and maternal education were included as covariates in all analyses. Intracranial volume (ICV) was added as an additional covariate for VBM and brain volume analyses. An ANCOVA was used to calculate group difference in cognitive test measures, brain volumes, tract-averaged FA, and graph theory measures. The relationship between diffusion metrics and cognitive test scores was examined with a linear regression. P-values adjusted for multiple comparisons were calculated with the Benjamini-Hochberg method across metrics of the same subtopic (Benjamini and Hochberg, 1995). The JHU atlas was used to label the tracts in the TBSS analysis (Eickhoff et al., 2007).
Partial correlation analyses between diffusion metrics and cognitive test scores were done, adjusting for age, sex, and maternal education.

5.5 Results

5.5.1 Demographics

Control subjects were significantly younger than preterm subjects (p<0.001). There were no significant differences in maternal education and sex (Table 11).

Table 11: Characteristics of preterm and term born control subjects

<table>
<thead>
<tr>
<th></th>
<th>Preterm (n=71)</th>
<th>Control (n=72)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (SD), years</td>
<td>33.5 (1.0)</td>
<td>30.9 (4.0)</td>
<td>1357.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>46</td>
<td>47</td>
<td>&lt;0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maternal education &lt; A-levels, %</td>
<td>36</td>
<td>44</td>
<td>0.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Wilcoxon test, <sup>b</sup> Chi-squared test

Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

5.5.2 Cognition

Preterm subjects scored significantly lower on the verbal list learning task, RCFT, verbal fluency, arithmetic, and the IQ test, adjusted for sex, age, and maternal education after multiple comparisons correction. There were no significant
differences in outcomes of the computer tasks, the HSCT, the zoo map test, and the pegboard test (Table 12).

Table 12: Comparisons of preterm and control groups in cognitive test scores

<table>
<thead>
<tr>
<th>Cognitive test scores</th>
<th>Preterm (n=71)</th>
<th>Control (n=72)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df^b F p</td>
</tr>
<tr>
<td>Computer prepare (s)</td>
<td>174 (556)</td>
<td>-365 (279)</td>
<td>1,106 195.3 0.04</td>
</tr>
<tr>
<td>Computer switch (s)</td>
<td>-107 (112)</td>
<td>-105 (95)</td>
<td>1,109 0.0 1.00</td>
</tr>
<tr>
<td>Computer inhibit (error)</td>
<td>-1 (2)</td>
<td>-1 (1)</td>
<td>1,107 0.3 0.32</td>
</tr>
<tr>
<td>Verbal list trial A6 (correct items)</td>
<td>11 (3)</td>
<td>12 (2)</td>
<td>1,132 1.9 &lt;0.001</td>
</tr>
<tr>
<td>Verbal list trial B (correct items)</td>
<td>6 (2)</td>
<td>7 (2)</td>
<td>1,131 1.3 0.002</td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td>33 (3)</td>
<td>34 (2)</td>
<td>1,131 1.5 0.004</td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td>19 (7)</td>
<td>24 (5)</td>
<td>1,131 5.3 &lt;0.001</td>
</tr>
<tr>
<td>HSCT 1 time (s)</td>
<td>-9 (11)</td>
<td>-8 (9)</td>
<td>1,132 0.3 0.88</td>
</tr>
<tr>
<td>HSCT 2 time (s)</td>
<td>-33 (36)</td>
<td>-20 (22)</td>
<td>1,132 8.7 0.13</td>
</tr>
<tr>
<td>HSCT 2 error</td>
<td>-2 (2)</td>
<td>-1 (1)</td>
<td>1,131 0.6 0.07</td>
</tr>
<tr>
<td>Arithmetic (correct items)</td>
<td>16 (6)</td>
<td>19 (4)</td>
<td>1,132 4.0 &lt;0.001</td>
</tr>
<tr>
<td>Pegboard both hands (score)</td>
<td>4 (3)</td>
<td>3 (2)</td>
<td>1,131 -0.5 0.36</td>
</tr>
<tr>
<td>Pegboard assembly (score)</td>
<td>16 (8)</td>
<td>17 (6)</td>
<td>1,131 1.3 0.36</td>
</tr>
<tr>
<td>Zoo map error</td>
<td>-1 (2)</td>
<td>-1 (2)</td>
<td>1,132 0.5 0.22</td>
</tr>
<tr>
<td>Zoo map 1 time (s)</td>
<td>-200 (100)</td>
<td>-178 (102)</td>
<td>1,130 9.0 0.65</td>
</tr>
<tr>
<td>Verbal fluency (correct items)</td>
<td>63 (16)</td>
<td>74 (17)</td>
<td>1,132 10.3 0.001</td>
</tr>
<tr>
<td>VCI</td>
<td>106 (16)</td>
<td>113 (14)</td>
<td>1,133 8.2 &lt;0.001</td>
</tr>
<tr>
<td>PRI</td>
<td>107 (14)</td>
<td>113 (13)</td>
<td>1,132 7.5 0.001</td>
</tr>
<tr>
<td>FSIQ</td>
<td>107 (15)</td>
<td>115 (12)</td>
<td>1,132 9.2 &lt;0.001</td>
</tr>
</tbody>
</table>

RCFT=Rey Complex Figure Test; HSCT=Hayling Sentence Completion Test, VCI=Verbal Comprehension Index, PRI=Perceptual Reasoning Index, FSIQ=Full Scale Intelligence Quotient.

ANCOVA test of group differences, adjusted for age, sex, and maternal education. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

* Values multiplied by -1, higher scores indicate better performance. ^ degrees of freedom may vary due to missing data
\^ Age not included as covariate, adjusted for age at scoring
5.5.3 Brain structure

*Grey matter volume – voxel-based*

Bilateral lower grey matter volumes were observed in the preterm group compared to the control group. Cortical areas with significant group differences include the middle temporal lobe extending into the central opercular cortex, the fusiform gyrus, and the parahippocampal gyrus as well as the orbitofrontal cortex, the cerebellum, and occipital cortex. In the subcortical structures, differences were observed in the thalamus, hippocampus, caudate, and the right amygdala (Figure 10).

*Figure 10: VBM results showing differences between preterm and control subjects*
Volume – per structure

Bilateral thalamus and hippocampus were significantly smaller, and both ventricles were significantly larger in preterm subjects compared to control subjects (Table 13).

Table 13: Comparisons of preterm and control groups in brain volumes between

<table>
<thead>
<tr>
<th>Brain volumes (mm$^3$)</th>
<th>Preterm (n=68)</th>
<th>Control (n=71)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df</td>
</tr>
<tr>
<td>Cortical white matter left</td>
<td>227052 (34429)</td>
<td>232694 (28101)</td>
<td>1,126</td>
</tr>
<tr>
<td>Cortical white matter right</td>
<td>229027 (33233)</td>
<td>234895 (28022)</td>
<td>1,126</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>9404 (6671)</td>
<td>6818 (4564)</td>
<td>1,126</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>8499 (4860)</td>
<td>6334 (4061)</td>
<td>1,126</td>
</tr>
<tr>
<td>Thalamus left</td>
<td>7747 (900)</td>
<td>8343 (922)</td>
<td>1,126</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>7576 (861)</td>
<td>8151 (881)</td>
<td>1,126</td>
</tr>
<tr>
<td>Caudate left</td>
<td>3498 (500)</td>
<td>3694 (429)</td>
<td>1,126</td>
</tr>
<tr>
<td>Caudate right</td>
<td>3633 (559)</td>
<td>3826 (471)</td>
<td>1,126</td>
</tr>
<tr>
<td>Hippocampus left</td>
<td>3528 (460)</td>
<td>3795 (374)</td>
<td>1,126</td>
</tr>
<tr>
<td>Hippocampus right</td>
<td>3514 (416)</td>
<td>3819 (412)</td>
<td>1,126</td>
</tr>
<tr>
<td>Putamen left</td>
<td>5018 (578)</td>
<td>5255 (554)</td>
<td>1,126</td>
</tr>
<tr>
<td>Putamen right</td>
<td>5071 (626)</td>
<td>5254 (561)</td>
<td>1,126</td>
</tr>
<tr>
<td>ICV</td>
<td>1381728 (182358)</td>
<td>1459623 (178710)</td>
<td>1,127</td>
</tr>
</tbody>
</table>

* not adjusted for ICV

* ANCOVA test of group differences, adjusted for age, sex, maternal education, and intracranial volume (ICV). Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.
*White matter: MT – voxel-based*

No clusters of significant group differences in white matter MT values exceeded the extent threshold.

*White matter: Diffusion – voxel-based*

Preterm subjects show higher FA in the anterior corona radiata compared to term born controls as well as higher AD on the right side where the superior corona radiata and superior longitudinal fasciculus cross. The shape of the tensor, MO is more cigar-like in the bilateral superior longitudinal fasciculus. Additionally, preterm subjects demonstrated widespread decreases in fibre orientation dispersion entropy (Figure 11).
Figure 11: TBSS results showing differences in diffusion metrics between preterm and control subjects

(Figure continues on next page)
Figure 11: TBSS results showing differences in diffusion metrics between preterm and control subjects

<table>
<thead>
<tr>
<th>Tensor</th>
<th>SMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of Anisotropy (MO)</td>
<td>N/A</td>
</tr>
<tr>
<td><img src="image1" alt="Tensor MO" /></td>
<td><img src="image2" alt="SMT MO" /></td>
</tr>
<tr>
<td>Orientation Dispersion Entropy (ODE)</td>
<td>N/A</td>
</tr>
<tr>
<td><img src="image3" alt="ODE Tensor" /></td>
<td><img src="image4" alt="ODE SMT" /></td>
</tr>
</tbody>
</table>

N.S.=non-significant, N/A=not applicable: no metric equivalent in model

Statistical brain maps showing significant differences between subjects in diffusion metrics within the white matter skeleton between preterm and control subjects (p<0.05 TFCE corrected). Red: preterm > control, blue: preterm < control. Boxplots of diffusion metric values in voxels with significant group differences, adjusted for sex, age, and maternal education. Left: diffusion tensor model metrics, right: spherical mean technique (SMT) model metrics.
Diffusion metrics and cognitive test scores

For those voxels and diffusion metrics that showed significant group differences, mean values were related to cognitive test scores. After multiple comparisons correction, increased FA in the frontal area with significant group differences was associated with lower scores on the verbal list A6 ($\beta=-16.3$, $p=0.004$) and arithmetic scores ($\beta=-36.5$, $p=0.004$; Figure 12).

![Figure 12: Associations between tensor FA and cognitive test scores in voxels with tensor FA differences between preterm and control subjects](image)

The mode of anisotropy was significantly associated with verbal list (A6: $\beta=-7.5$, $p<0.001$, B: $\beta=-6.6$, $p<0.001$), RCFT delayed recall ($\beta=-15.5$, $p=0.002$), arithmetic ($\beta=-14.8$, $p=0.002$), verbal fluency ($\beta=-33.6$, $p=0.02$), VCI ($\beta=-38.9$, $p=0.002$), and FSIQ ($\beta=-34.4$, $p=0.003$; Figure 13).
Figure 13: Associations between tensor MO and cognitive test scores in voxels with tensor MO differences between preterm and control subjects
Figure 14 shows a significant positive correlation between ODE and verbal list trial B scores ($\beta=18.7$, $p=0.002$), verbal fluency ($\beta=120.1$, $p=0.009$), PRI ($\beta=95.4$, $p=0.009$), and FSIQ ($\beta=95.0$, $p=0.008$).

![Figure 14: Associations between SMT ODE and cognitive test scores in voxels with SMT ODE differences between preterm and control subjects](image)

**White matter – Diffusion per tract**

FA in the left cingulum and left uncinate fasciculus was lower in the preterm group compared to the control group (Table 14). In addition, FA in the right cingulum was lower in the preterm group in females ($F(1,66)=11.06$, $p=0.001$).
Table 14: Comparisons of preterm and control groups in white matter tract FA

<table>
<thead>
<tr>
<th>Tract averaged FA</th>
<th>Preterm (n=67)</th>
<th>Control (n=70)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df</td>
</tr>
<tr>
<td>Arcuate fasciculus left</td>
<td>0.55 (0.04)</td>
<td>0.56 (0.03)</td>
<td>1,125</td>
</tr>
<tr>
<td>Arcuate fasciculus right</td>
<td>0.51 (0.04)</td>
<td>0.52 (0.03)</td>
<td>1,125</td>
</tr>
<tr>
<td>Anterior thalamic radiations left</td>
<td>0.42 (0.03)</td>
<td>0.43 (0.03)</td>
<td>1,125</td>
</tr>
<tr>
<td>Anterior thalamic radiations right</td>
<td>0.39 (0.03)</td>
<td>0.39 (0.03)</td>
<td>1,125</td>
</tr>
<tr>
<td>Cingulum left</td>
<td>0.54 (0.05)</td>
<td>0.56 (0.05)</td>
<td>1,125</td>
</tr>
<tr>
<td>Cingulum right</td>
<td>0.49 (0.05)</td>
<td>0.52 (0.05)</td>
<td>1,125</td>
</tr>
<tr>
<td>Corticospinal tract left</td>
<td>0.5 (0.05)</td>
<td>0.48 (0.05)</td>
<td>1,125</td>
</tr>
<tr>
<td>Corticospinal tract right</td>
<td>0.54 (0.04)</td>
<td>0.54 (0.04)</td>
<td>1,125</td>
</tr>
<tr>
<td>Corpus callosum genu</td>
<td>0.55 (0.07)</td>
<td>0.56 (0.04)</td>
<td>1,125</td>
</tr>
<tr>
<td>Corpus callosum splenium</td>
<td>0.61 (0.08)</td>
<td>0.65 (0.04)</td>
<td>1,125</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus left</td>
<td>0.55 (0.04)</td>
<td>0.56 (0.04)</td>
<td>1,125</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus right</td>
<td>0.55 (0.04)</td>
<td>0.55 (0.04)</td>
<td>1,125</td>
</tr>
<tr>
<td>Uncinate fasciculus left</td>
<td>0.47 (0.04)</td>
<td>0.49 (0.03)</td>
<td>1,125</td>
</tr>
<tr>
<td>Uncinate fasciculus right</td>
<td>0.45 (0.03)</td>
<td>0.46 (0.03)</td>
<td>1,125</td>
</tr>
</tbody>
</table>

ANCOVA test of group differences, adjusted for age, sex, maternal education, and intracranial volume (ICV). Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold. ± relationship differs between males and females.

Network

There were no differences between preterm and control subjects in graph theory network measures (Table 15).
Table 15: Comparisons of preterm and control groups in network measures

<table>
<thead>
<tr>
<th>Network measures</th>
<th>Preterm (n=67)</th>
<th>Control (n=69)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df</td>
</tr>
<tr>
<td>Edge density</td>
<td>37.11 (3.14)</td>
<td>38.11 (2.47)</td>
<td>1,124</td>
</tr>
<tr>
<td>Shortest path length</td>
<td>1.25 (0.06)</td>
<td>1.23 (0.05)</td>
<td>1,124</td>
</tr>
<tr>
<td>Global efficiency</td>
<td>0.43 (0.02)</td>
<td>0.44 (0.01)</td>
<td>1,124</td>
</tr>
<tr>
<td>Local efficiency</td>
<td>0.46 (0.01)</td>
<td>0.46 (0.01)</td>
<td>1,124</td>
</tr>
<tr>
<td>Cluster coefficient</td>
<td>0.42 (0.01)</td>
<td>0.43 (0.01)</td>
<td>1,124</td>
</tr>
</tbody>
</table>

*ANCOVA test of group differences, adjusted for age, sex, and maternal education.*
5.6 Results summary

Cognition

- Results supporting the hypotheses:
  - Preterm born adults scored lower on the verbal list learning, RCFT, arithmetic, verbal fluency, and the IQ test compared to term born controls.

- Results not supporting the hypotheses:
  - There were no significant group differences in computer, HSCT, pegboard, and zoo map scores.

Grey matter

- Results supporting the hypotheses:
  - Preterm born adults had larger ventricles and smaller bilateral thalamus and hippocampus volumes. Voxel-based analysis additionally showed reduced grey matter volume in the caudate, right amygdala, middle temporal lobe extending into the central opercular cortex, the fusiform gyrus, and the parahippocampal gyrus as well as the orbitofrontal cortex, the cerebellum, and occipital cortex.

- Results not supporting the hypotheses:
  - There were no increases in grey matter volume in the cingulate cortex and frontal lobe.
White matter

- Results supporting the hypotheses:
  o Preterm subjects had widespread decreases in fibre coherence (ODE).
  o White matter alterations were associated with lower cognitive test scores.
  o FA in the left cingulum and left uncinate fasciculus, and in females additionally in the right cingulum, was lower in the preterm group compared to the control group.

- Results not supporting the hypotheses:
  o The tensor model showed higher anisotropy (FA) in preterm born adults in the corona radiata as well as higher diffusivity in the principal direction (AD) and a more linear tensor (MO) at the crossing of the superior longitudinal fasciculus and posterior corona radiata compared to control subjects.
  o There were no group differences in FA or AD with the SMT model.
  o There were no group differences in white matter volume.
  o There were no group differences in MT values.
  o There were no group differences in network measures.
5.7 Discussion

5.7.1 Cognition

Preterm subjects had statistically significantly lower average scores in some, but not all cognitive tests. There was an 8-point difference in unadjusted FSIQ scores (d=0.58). This is a smaller difference than the 12-point IQ that was reported in a meta-analysis (Bhutta et al., 2002; Kerr-Wilson et al., 2012). VIQ and PIQ scores were 7 (d=0.48) and 6 (d=0.46) points lower in the preterm group compared to the control group, respectively.

Although not statistically significant, preterm subjects also scored lower on the computer prepare (d=0.35), computer switch (d=0.02), HSCT (1 time: d=0.09, 2 time: d=0.44, 2 error: d=0.44), pegboard assembly (d=0.17), and zoo map (time: d=0.22). Preterm subjects only outperformed the control subjects on the pegboard both hands measure (d=0.25), but this was not statistically significant. These results suggest that, at least on average, people born preterm continue to have lower cognitive abilities compared to term born individuals in adulthood.

Processing speed

On average, the preterm group took 59% longer to complete the “prepare” computer test than the control group, picking up on a difference in psychomotor speed, but the
variation within the preterm group was large and the difference did not survive multiple comparisons correction. A recent meta-analysis has shown lower processing speed scores in children and adolescents born preterm compared to age-matched term born controls. Age did not significantly account for variation in effect sizes between studies, but some caution is warranted for interpretation of these results as there were only few measures of processing speed in a subset of studies including participants 11-17 years (Brydges et al., 2018). The study in this meta-regression with the oldest mean age (17 years) did not find significant differences in psychomotor reaction time in preterm and term born adolescents (Burnett et al., 2015). This, in combination with the results presented here, suggests that differences in psychomotor processing speed may not persist beyond childhood in the majority of people born preterm.

*Executive functioning*

There was no evidence for impaired attention shifting as measured by the “switch” computer test, nor for inhibition as measured by the HSCT trial 2 measures and “inhibit” computer tests. A study on another British cohort of similar age reported no differences in selective attention allocation and response inhibition between preterm and control adolescents (Nosarti et al., 2006). Other studies on attention shifting and inhibition in preterm populations in childhood and adolescence have reported mixed outcomes (Mulder et al., 2009; Mulder, Pitchford and Marlow, 2011). The variation in results could be due to the differences in prematurity of the study populations. The
minimal GA in this cohort is 26 weeks, since survival of more extremely preterm infants was not possible at the time of the start of the study. A systematic review revealed that selective attention skills are significantly associated with GA and that these skills catch up with age only in preterm children with GA of more than 26 weeks (Mulder et al., 2009).

Although holding information in working memory is still required, the arithmetic test used here relied relatively little on executive functioning abilities. This indicates that at least some preterm individuals have a deficit in mathematical ability, as was suggested previously in this population during adolescence (Isaacs et al., 2001).

The finding of lower verbal fluency scores in the preterm group compared to the control group is in line with the literature. On average, preterm individuals could name 9 fewer items in 2.5 minutes across four trials of different phonological and semantical constraints. The gap in verbal fluency skills between preterm and term born individuals seems to increase during childhood (Mulder et al., 2009) and persist into adulthood (Nosarti et al., 2007, 2014; Dutt et al., 2011).

In contrast, there were no group differences in HSCT scores, while studies in adolescents from the Victorian Infant Collaborative Study cohort and young adults from the UCLH cohort did report significant differences in the HSCT scores between preterm and control subjects (Nosarti et al., 2007, 2014; Allin et al., 2011; Burnett et al., 2015). The largest difference between the groups would be expected to be in the
trial 2 time, where the participant is required to suppress their initial response and come up with an unrelated word. Indeed, the response time is longer for the preterm subjects in this trial and the group difference in this trial is larger than that of trial 1. However, the variability within the preterm group is large, and the difference does not reach statistical significance. There were also no group differences in Zoo map 1 scores, while this has been reported in children (Haebich et al., 2018).

It must be noted that in the studies with significant findings on HSCT and Zoo map scores, preterm subjects had lower FSIQ scores (100.2-100.5) than those of subjects in the current study (FSIQ: 107). It is possible that differences in HSCT and Zoo map scores could not be identified here, because the overall level of cognitive functioning was slightly higher in this study. However, the control sample in the aforementioned studies also had slightly lower IQ scores than the control current sample (110-112 and 115, respectively), and the difference in FSIQ scores between the preterm and control groups were similar to this study. Given the large standard deviation in cognitive scores in this study, it is also possible that there were no significant group differences because some of the preterm individuals do not have any deficits in these skills. It is possible that executive functioning is particularly important for the willingness and ability to come and participate in the study. People with severe problems in executive functioning might struggle to organise a visit to London and may therefore have been less likely to participate in the study.
Alternatively, it is possible that adults born preterm have managed to compensate for cognitive difficulties to some extent by cognitive strategies and/or altered neural structure. The HSCT and zoo map are tests with a higher ecological validity, reflecting real-life situations which allow for more compensation and strategy use than tests of lower level cognitive processes.

Memory

Preterm subjects showed reduced performance in all tests with a memory component; verbal memory (verbal list), visuospatial memory (RCFT delayed recall), and working memory (arithmetic). This is in accordance with an extensive body of research reporting memory deficits across different domains and modalities (Anderson, 2014; Omizzolo et al., 2014).

Language

The verbal list, verbal fluency, and VCI measures support previous evidence of impaired generalised language ability in preterm born individuals, summarised in two meta-analyses (Barre et al., 2011; van Noort-van der Spek, Franken and Weisglas-Kuperus, 2012).
Motor

There were no significant group differences in the Purdue Pegboard scores. Previous studies consistently reported a manual dexterity impairment in very preterm and very low birthweight children (Taylor et al., 2000; de Kieviet et al., 2009), slower gains in Purdue pegboard scores in extremely low birthweight children during childhood (Taylor et al., 2004), and lower scores in adolescence (Skranes et al., 2012). However, in line with results from the present study, a study in preterm children did not show a significant difference in fine motor performance using the pegboard subtest of the Zurich Neuromotor Assessment (Wehrle et al., 2016). This task is similar to the Purdue Pegboard and requires brass pegs to be placed, inverted, and replaced in twelve holes in a board using only one hand (Largo, Fischer and Caflisch, 2002).

Cognition discussion

Although the group differences are not significant for all measures, mean scores are consistently lower and SD larger in the preterm compared to the control group. It is possible that the differences are small, the tests are not sensitive enough, or there are some preterm individuals with significantly impaired performance, but the effect is masked by other preterm subjects who perform in the normal range. In other words, it is possible that some preterm subjects score significantly worse than any of the control subjects, but on average, the preterm subjects score in the normal range.
In addition, it is possible that the cognitive profiles of the individual preterm subjects do not fully overlap due to the many biological, environmental, and behaviour interactions described in section 1.7. On a group level, some of the tests might therefore not show significant differences. Standard deviations are even larger in some tests that do show significant effects between preterm and control subjects, such as the RCFT, HSCT, arithmetic, and IQ test measures. Chapter 6 will explore if there is indeed a subset of underperforming preterm individuals who do show brain structure alterations compared to term born controls. This subset will be identified with a method that circumvents the group averaging effect.

This variation in outcome within the preterm population is not surprising since their medical histories and neonatal trajectories also vary widely. A study of the Stockholm Neonatal Project showed that preterm adolescents with a lower risk neonatal profile, i.e. born after 28 weeks of gestation, with an appropriate birthweight and no perinatal complications, did not perform worse than term-born controls on any of the IQ, memory, executive functioning, visuomotor and verbal measures. Extremely preterm, SGA and those with neonatal complications did score below the control group on some of the measures (Lundequist et al., 2015). This phenomenon could also mask group differences in brain structure.
5.7.2 Brain structure

**Grey matter**

Both voxel-based and per structure analyses showed larger ventricles, smaller bilateral thalamus and hippocampal volumes in the preterm compared to the control group. The voxel-based analysis further showed lower grey matter volume in the right amygdala and bilateral middle temporal lobe, central opercular cortex, and orbitofrontal cortex. Reduced temporal grey matter volumes have been reported in child and adolescent preterm populations (Peterson et al., 2000; Kesler et al., 2004), in young adults (aged 19-20 years; Nosarti et al., 2014) and in adults in their mid-twenties (Meng et al., 2015). In line with the current results, studies in adults also reported lower grey matter volume in the caudate, putamen, the medial frontal gyrus and medial occipital gyrus (Nosarti et al., 2014; Meng et al., 2015), and in the (right) hippocampus and fusiform gyrus (Meng et al., 2015). Temporal and periventricular regions are particularly vulnerable to ischaemic stress due to their low perfusion (Sakamoto and Ishii, 2000). Early neonatal hypoxic-ischemic events can disrupt developmental processes that occur during the third trimester of gestation, such as cell migration from the subventricular zone and maturation of pre-oligodendrocytes (Volpe, 2009a). The observed grey matter volume reductions could be a result of increased atrophy, secondary maturational effect of white matter injury (Boardman et al., 2010), or exaggerated synaptic pruning during adolescence (Gogtay et al., 2004). Lower grey matter volume in the temporal and inferior frontal cortex in the
preterm group is in line with previous studies in preterm adolescents that reported a thinner cortex in these areas (Frye et al., 2010).

There were no areas of increased grey matter volume, which has been observed previously in preterm populations in childhood (Kesler et al., 2004), adolescence (cortical thickness; Frye et al., 2010) and young adulthood (Nosarti et al., 2014).

There is a fair amount of concordance between the reports on the two preterm adult populations previously studied by Meng and Nosarti and colleagues and the current study, despite the differences in age at the time of testing. This study therefore further demonstrates that extensive structural brain alterations remain present in adulthood and brain development after preterm birth is, at least in some respects, distinct.

**White matter**

There were no areas with lower FA in the preterm subjects compared to the controls in the voxel-wise TBSS analysis. This is in contrast with previous studies that used whole-brain, voxel-wise analyses to compare preterm adolescents and adults with term born controls (Table 16).
Table 16: Schematic overview of FA differences (threshold >10 voxels) between preterm and term born participants reported in the literature

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years</td>
<td>34</td>
<td>15</td>
<td>18</td>
<td>19</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Analysis method</td>
<td>TBSS, PNT</td>
<td>SPM, TBSS</td>
<td>SPM, TBSS</td>
<td>TBSS</td>
<td>TBSS</td>
<td>TBSS</td>
</tr>
<tr>
<td>Arcuate fasciculus</td>
<td>R L -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior thalamic radiations</td>
<td></td>
<td>R L -</td>
<td></td>
<td></td>
<td></td>
<td>R L -</td>
</tr>
<tr>
<td>Cingulum</td>
<td>R L -</td>
<td></td>
<td></td>
<td>R L -</td>
<td>R L -</td>
<td>R L -</td>
</tr>
<tr>
<td>Corticospinal tract</td>
<td>R L -</td>
<td></td>
<td></td>
<td>R L -</td>
<td>R L -</td>
<td>R L -</td>
</tr>
<tr>
<td>Corpus callosum genu (forceps minor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum splenium (forceps major)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum body</td>
<td>X</td>
<td></td>
<td></td>
<td>R L -</td>
<td>R L -</td>
<td>R L -</td>
</tr>
<tr>
<td>Superior longitudinal fasciculus</td>
<td>X</td>
<td>R L -</td>
<td>R L - R L +</td>
<td>R L - R+</td>
<td>R L -</td>
<td></td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus</td>
<td></td>
<td>R L -</td>
<td></td>
<td>R L -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncinate fasciculus</td>
<td>L -</td>
<td></td>
<td>R L - R L +</td>
<td>R L -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior fronto-occipital</td>
<td>X</td>
<td>R L -</td>
<td>R L +</td>
<td>R L -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corona radiata</td>
<td>X</td>
<td></td>
<td>L - R L +</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Internal capsule</td>
<td>X</td>
<td>R L -</td>
<td>R -</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fornix</td>
<td>X</td>
<td></td>
<td>L -</td>
<td>R L -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This thesis
R: right hemisphere, L: left hemisphere
+ preterm subjects higher FA values compared to term born subjects, - preterm subjects lower FA values compared to term born subjects, X: structure not analysed

The tract-averaged FA values in this study did show decreases in bilateral cingulum tracts and the left uncinate fasciculus in the preterm group, which is in line with previous findings (Vangberg et al., 2006; Meng et al., 2015; Vollmer et al., 2017).
Previous studies that used tractography to delineate fibre bundles reported lower FA values in the corticospinal tract at the level of internal capsule in preterm born adolescents (Groeschel et al., 2014), and in the hippocampal fornix, splenium, inferior longitudinal fasciculus, and the fronto-occipital fasciculus, but no differences in FA in tracts between the right anterior cingulate gyrus and caudate in preterm born young adults (Salvan et al., 2014). The absence of significant group differences in diffusion parameters in the corpus callosum is consistent with previous studies in samples of similar age and history of brain injury. Groeschel and colleagues did report increased MD and decreased FA in the corpus callosum between adults born preterm and at term, but no differences were found after exclusion of the subjects with significant preterm brain injury. Similarly, a paper by Kontis and colleagues with a sample of participants who were screened for significant brain injury reported no FA differences in the corpus callosum in adults born preterm compared to people born at term (Kontis et al., 2009; Groeschel et al., 2014). It was suggested that reported differences between preterms and controls in diffusion parameters in the corpus callosum may be influenced by an increase in partial volume effects with CSF as a result of callosal thinning (Groeschel et al., 2014). It has been mentioned before that the participants in the sample presented in this thesis are relatively high functioning and less likely to have suffered from significant brain injury than those who were not followed up into adulthood.

TBSS analysis of the diffusion images showed a local increase in FA in the preterm group at the crossing of the anterior corona radiata, the inferior fronto-occipital
fasciculus, and the anterior thalamic radiations. A meta-analysis including preterm individuals from infancy until adolescence and a study in adults have reported increased FA in small bilateral clusters of the corona radiata (Allin et al., 2011; Li et al., 2015). FA in that area combined with a larger cluster in the inferior fronto-occipital and uncinate fasciculi did not significantly relate to IQ or executive function (Allin et al., 2011). In the current study, higher FA in the corona radiata cluster was also not associated with IQ or most of the executive functioning scores, but it was related to lower scores on the verbal list and arithmetic tests. Increased FA in the frontal cortex could reflect structural adaptation to continuous increased recruitment of the frontal cortex in cognitive processes (Kolb et al., 2012). However, when extracting SMT’s ODE from the voxels with increased FA in preterm subjects compared to the control subjects, a higher FA is associated with lower fibre coherence (Figure 15). Since there was no significant increase in SMT FA values, this suggests that the increase FA can be attributed to a decrease in fibre coherence rather than compromised white matter integrity.
The preterm brain also shows increased AD and a more linear (cigar-like) shape of the diffusion tensor in the parietal lobe. Higher AD has been reported in preterm populations of different ages (Li et al., 2015). Higher AD could indicate better fibre organisation. However, the clusters with significant differences in AD are located in areas of crossing fibres, where an apparent change in AD could in reality be caused by a change in RD (Wheeler-Kingshott et al., 2003). Furthermore, since the diffusion tensor is confounded by orientation dispersion, increases in AD values in areas with
multiple fibre populations can be due to a decrease in effect on diffusion from another fibre bundle crossing instead of a difference in white matter microstructure (Groeschel et al., 2014). This explanation is supported by results from the MO and SMT measures. Figure 15 shows that higher values of AD are associated with higher fibre coherence (ODE). There was higher MO in the same region as the increase in AD, and this is also associated with higher fibre coherence. Higher MO and AD in the same area has been associated with reduced cognitive capacity in elderly people (Douaud et al., 2011). There were no significant differences in SMT FA or SMT AD, further supporting that there are no differences in anisotropy or axial diffusion in any of the individual fibre populations.

Outside of the areas with increases in tensor FA, tensor AD, and MO, there were widespread decreases in fibre coherence in the preterm compared to the control group. Since the regular tensor model cannot extract fibre orientation information, this is not a well-documented phenomenon yet. One study in preterm children used neurite orientation dispersion and density imaging (NODDI). This model of diffusion, similar to SMT, aims to quantify effect of neurite orientation dispersion and remove this from anisotropy measures (Zhang et al., 2012). The results also showed higher axon dispersion and lower FA but no difference in axon density in preterm subjects compared to controls (Kelly et al., 2016).
**Discussion brain structure**

There were no significant differences in caudate, hippocampus, and putamen volumes and no decreases in FA or group differences in network measures as was hypothesized based on previous studies. This might be due to the sample recruited for this follow-up study, which has on average slightly higher overall cognitive functioning compared to other studies. The longer the time to follow-up, the higher the chance of selective drop-out. The current preterm sample has a higher maternal education and higher IQ scores at age 7 than the original cohort.

Furthermore, subjects with ventriculomegaly were excluded from the TBSS analysis, because the white matter skeleton overlapped with their ventricles. Excluding those subjects with early damage to the periventricular zone might have biased the sample towards preterm subjects with intact white matter structure. Periventricular leukomalacia is a common white matter injury in preterm infants, which disrupts oligodendrocyte maturation and the subsequent reduction in myelination is hypothesised to underlie the altered diffusivities observed in people born preterm (Nagae et al., 2007). FSIQ scores of preterm subjects who were not included in the TBSS analysis were on average lower (107) than scores of those who were included in the TBSS analysis (111), although this difference was not statistically significant (t(18)= 0.19, p = 0.84).
5.8 Conclusion

Preterm born adults demonstrate lower cognitive scores and reduced grey matter volumes as well as widespread reductions in fibre coherence. There is no strong indication of reduced white matter integrity or changes in the structural network.

Some preterm individuals clearly perform significantly worse compared to term born controls, while others appear to function within the normal range. The large variability in outcomes within the preterm subject group is a testament to the complex interplay of perinatal influences and potentially adaptive responses thereafter. This variability could potentially mask some of the effects of preterm birth in a simple group comparison. It is important to discover what determines whether a preterm infant ends up with significant cognitive deficits that impact their daily lives in adulthood or develops like term born peers. The next chapter will therefore identify those preterm subjects with poor cognitive outcomes and then investigate how this relates to their brain structure and neonatal history.
6 Comparison between suboptimally performing preterm, normally performing preterm, and term born adults

6.1 Background

Chapter 5 showed that preterm subjects had larger ventricles, smaller total brain volume as well as differences in local grey matter volumes. However, in contrast to some previous studies, no decreases in FA or other metrics of microstructural integrity were found. This is surprising, since white matter is especially vulnerable to preterm birth and subsequent sequelae (Volpe, 2009a). Furthermore, in accordance with the literature, preterm subjects in this study did show problems with executive functioning, arithmetic and visuospatial memory, all of which rely heavily on white matter to integrate information processed in different brain regions. The standard deviation in test scores in the preterm group was large, indicating substantial heterogeneity in the preterm group.

Previous studies have identified multiple risk factors after preterm birth that are associated with poor cognitive outcome later in life, including male sex, low GA, low birthweight, SGA, low maternal education, mechanical ventilation, receiving a low nutrient plane diet and less human milk (Morse et al., 2000; Walther, Den Ouden and Verloove-Vanhoeick, 2000; Isaacs, Morley and Lucas, 2009; Isaacs et al., 2010;
Linsell et al., 2015). One study showed that adolescents with few early risk factors; 
> 28 weeks GA, with appropriate birthweight and no perinatal complications, 
functioned on par with term-born peers (Lundequist et al., 2015). The multiple hit 
hypothesis proposes that cumulative exposure to multiple perinatal risk factors 
exacerbates white matter injury (Barnett et al., 2018).

There is a large variation in these risk factors between participants in this cohort. All 
infants born in the five dedicated centres within the study period with a birthweight 
below 1850g were included, resulting in a diverse cohort with birthweight ranging 
from 529g to 1847g and GA between 24 and 39 weeks. Some infants had a 
relatively uncomplicated neonatal trajectory, whereas others suffered from a 
prolonged period of illness and even had to undergo surgery. Due to the nature of 
the study, they received different neonatal nutrition, which has been associated with 
their brain structure and cognitive outcomes (Isaacs et al., 2008, 2010). As a result, 
some preterm individuals might have ended up performing in the normal range, 
whereas others may show a significant impaired performance later in life.

It is likely that those individuals who experience cognitive difficulties show the 
structural alterations that have been reported in preterm populations in the literature. 
This could be seen in the grey matter reported in chapter 5, but considering that 
many of the cognitive tests require information integration between several different 
brain regions, performance is most likely to be affected by widespread white matter 
microstructure differences. Alternatively, those who perform normally may have
developed compensatory brain structure which could be reflected in their structural network properties.

Given the neuroconstructivist approach to developmental outcomes, it is unlikely that the preterm population as a whole suffers from domain specific deficits (Karmiloff-Smith, 1998). Considering this heterogeneous sample as one preterm group could mask the true potential impact of preterm birth and its sequelae on the brain and cognitive outcome by including highly performing preterm subjects with relatively intact brain structure. This effect is potentially exaggerated in long-term follow-up research such as the current study, since selective attrition is difficult to prevent. People with fewer difficulties in their daily lives find it easier to participate in further research. Furthermore, participants might not all have problems in the exact same brain areas and cognitive domains. Hypothetically, if half of the preterm participants performed more poorly compared to controls on one test, but there was normal performance on a second test, while the other half of preterm subjects were only impaired on the second test and not the first, there might not be a significant group difference on either test, even though all preterm subjects have impaired performance on at least one test (Shallice and Shallice, 2009).

As was discussed in section 1.2.1, people born preterm can experience difficulty in a range of cognitive domains and there is substantial variability in cognitive profiles within the population. The test battery used in this study is tailored to pick up on the general and specific deficits that people born preterm are more likely to suffer from.
IQ tests are well validated and widely used to estimate cognitive abilities on a continuous scale, but they do not capture problems with executive functioning, fine motor control or arithmetic as well as the Zoo Map, the Pegboard test, and the graded difficulty arithmetic tests, respectively.

Calculating a general sum of fails allowed for exploitation of the specificity of the cognitive tests. A minimum threshold can be set to identify those who struggle in multiple of these areas and who are therefore particularly likely to experience difficulties in daily life. This also helps to conserve robustness and maintain a low risk of false positives.

Identifying those preterm subjects that are performing suboptimally not only enables a better understanding of the potential impact of preterm birth and its consequences for brain development, it can also elucidate which neonatal factors are important for preventing cognitive difficulties later in life. This can help guide clinical management of preterm infants, interventions, and provide information to parents.

### 6.2 Aims

This chapter investigates if there are preterm individuals whose performance on the cognitive tests is at a suboptimal level. If this is the case, the second aim is to discover which brain structure alterations are associated with these cognitive
difficulties and if there are neonatal characteristics that predict suboptimal performance in adulthood.

6.3 Hypotheses

Cognition

- There is a subgroup of preterm individuals who perform suboptimally (SP); i.e. at a significantly lower level than what would be expected considering their background (maternal education).

Neonatal predictors

- Neonatal factors predict being in the SP group.
  - Male sex, low GA, low birthweight, SGA, low maternal education, mechanical ventilation, infection/NEC, receiving the standard nutrient (as opposed to the high nutrient) diet and the proportion of human milk in the diet.

Grey matter

- The SP subjects have lower grey matter volumes compared to the control (C) group and, although to a lesser extent, to the normally performing preterm subjects (NP).
  - Thalamus, cerebellum, temporal lobe, caudate, and hippocampus.
White matter

- The SP group has lower white matter integrity compared to the NP and C groups.
  - Increased mean diffusivity (MD), increased diffusivity perpendicular to the white matter fibres (RD), and a resulting reduction of fractional anisotropy (FA).
  - Decreased MT values.
- The SP subjects have reduced white matter capacity and organisation to the C group and, although to a lesser extent, to the NP subjects.
  - Reduced white matter volume.
  - Reduced number of connections (edge density).
  - Reduced fibre coherence/orientation density entropy (ODE).
  - Global network organization (global efficiency) is preserved, but local connectivity (local efficiency) is reduced.
- White matter alterations are associated with lower performance on cognitive tests.
6.4 Methods

6.4.1 Data collection

Neonatal Study population descriptions are outlined in chapter 3 and 4. Neonatal nutritional intake in mL was recorded daily. The ‘nutritional plane’ category divides participants into a high nutrient group (PTF) and a standard nutrient group (unfortified BBM or TF). Administration of mechanical ventilation and episodes of NEC and infection were recorded prospectively by research staff during the hospital stay. NEC was classified using the British Association for Perinatal Pediatrics classification (British Association for Perinatal Paediatrics, 1983). Infection was classified by a positive blood culture and increased white blood cell count. NEC was classified using the British Association for Perinatal Pediatrics classification. Diagnostic criteria included at least two features of NEC; pneumatosis intestinalis, or free air in the abdomen or frothy appearance of bowel lumen as seen on a radiograph, lethargy, hypotonia, blood in the stool, and/or apneic episodes. Severe cases additionally showed gas in the portal vein or free air in the abdomen on a radiograph, abdominal tenderness or rigidity, tissue in the stool, bleeding, low white blood cell count (< 6*10^9/l) or platelet count (< 100*10^9/l) at the time of illness (Lucas and Cole, 1990). Infection was diagnosed by a positive blood culture and/or increased white blood cell count.
Childhood follow-up

At age 7.5-8, 768 children of the original cohort completed the abbreviated IQ test (Wechsler, 1974).

Adult follow-up

The MRI acquisition details, computation of brain volumes, and voxel-based imaging analysis techniques TBSS, VBM, VBQ, and connectivity are outlined in chapter 4.

6.4.2 Statistical analyses

Group differences in demographic characteristics were examined with a t-test or Wilcoxon test for continuous variables, and chi-square test for binary variables. A one-way ANCOVA F-test with three groups (SP/NP/C groups) was used to test a main group effect in cognitive test outcomes, brain volumes, and average properties of white matter. Age, sex, and maternal education were taken as covariates in these analyses as well as in the voxel-based imaging analyses. For VBM and brain volume analyses, intracranial volume (ICV) was added as an additional covariate. If there was a significant main effect, post-hoc comparisons were done to examine differences between all groups. These were first adjusted for multiple comparisons across the group comparisons within the Tukey’s HSD test. Subsequently, p-values adjusted for multiple comparisons were calculated with the Benjamini-Hochberg method across metrics of the same subtopic (Benjamini and Hochberg, 1995).
Partial correlation analyses between diffusion metrics and cognitive test scores were done, adjusting for age, sex, and maternal education.

6.4.3 Suboptimal performance group classification

The mean and standard deviation of the control group were used to calculate z-scores of each test for all participants. Z-scores lower than -2, meaning at least two standard deviations below the mean of the control group, were classified as “fail”. This resulted a binary “fail” variable for each subject per test. Then, the number of tests failed were summed. Some participants did not complete all tests, which affects the total number of tests failed. In order to ensure that the total number of tests failed is not affected by the number of tests completed, the number of tests failed was divided by the number of test measures completed and multiplied by the total number of test measures. Since FSIQ is a composite of VCI and PRI scores, this outcome measure was not included in the calculation of the total number of tests failed.

In order to remove the effect of maternal education, the number of tests failed were put in a regression model with maternal education as predictor. Subjects with standard residuals > 2, i.e. those who failed more tests than expected based on their maternal education, were classified as “suboptimally performing”.
This resulted in three groups; preterm subjects performing suboptimally (SP), preterm subjects performing in the normal range (NP), and control subjects (C). In order to ensure that the classification was not affected by one outlier test within an individual, the lowest two Z-scores were replaced by the mean of the two scores prior to running the regression model. The same subjects were identified as performing suboptimally.

*Z-scores per test*

The difference in performance between preterm and control subjects was largest for the RCFT, with 25% of preterm subjects failing the delayed recall measure compared to only 3% of control subjects (Figure 16). Interestingly, even though on average preterm subjects were slower to complete the zoo map test, more controls than preterm subjects failed the time to complete the zoo map measure.
Since there are some tests with little difference between the preterm and control group, such as the pegboard assembly test, it is unsurprising that the max z-scores are similar between the groups (Figure 17A). There is much more variability in the minimum Z-scores (Figure 17B), with a few individuals in the preterm group with a minimum Z-score below that of any of the control subjects. The difference between the highest and lowest Z-score and the standard deviation of Z-scores across the different test measures is larger in some preterm individuals than in any of the control subjects (Figure 17C).
Figure 17: Minimum, maximum and range of Z-scores across cognitive test measures for preterm and control subjects

**Fail scores**

As depicted in figure 18, all subjects who failed more than 25% of the test measures were born preterm. Seven preterm subjects and no controls performed worse than would be expected taking their maternal education into account.
6.5 Results

6.5.1 Demographics and neonatal characteristics

As was previously reported in chapter 5, table 17 shows that control subjects were significantly younger than preterm subjects. There was no significant difference in age between the suboptimally performing subjects and the normally performing preterm subjects. Characteristics at birth, i.e. sex, GA, birthweight and maternal education did not significantly predict which participants ended up in the SP versus
the NP groups. Birthweight z-score is significantly different between SP and NP groups, but not after correcting for multiple comparisons.

There was a higher proportion of SP subjects with neonatal infection (four out of seven subjects, 54%) compared to NP subjects (seven out of 64, 11%). Although mechanical ventilation rate was slightly higher in the SP group, this was not significantly different from the NP group. There was no significant effect of randomisation to a high or standard nutrient diet or the percentage human milk on performance group membership in adulthood. FSIQ at age 7 was predictive of suboptimal performance in adulthood.
Table 17: Characteristics of the suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects

<table>
<thead>
<tr>
<th></th>
<th>SP (n=7)</th>
<th>NP (n=64)</th>
<th>C (n=72)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (SD), years</td>
<td>33.3 (1.0)</td>
<td>33.6 (1.0)</td>
<td>30.9 (4.0)</td>
<td>0.74/141/397^c</td>
</tr>
<tr>
<td>Sex male, %</td>
<td>43</td>
<td>55</td>
<td>47</td>
<td>0.04/0.01^b/0.01^b</td>
</tr>
<tr>
<td>Maternal education &lt; A-levels, %</td>
<td>57</td>
<td>33</td>
<td>44</td>
<td>0.64^a/0.07^b/0.13^b</td>
</tr>
<tr>
<td>Gestational age mean (SD)</td>
<td>31.4 (3.5)</td>
<td>30 (2.2)</td>
<td>-</td>
<td>-1.09/^-/-</td>
</tr>
<tr>
<td>Birthweight mean (SD)</td>
<td>1353 (315)</td>
<td>1302 (302)</td>
<td>-</td>
<td>-0.40/-/-</td>
</tr>
<tr>
<td>Birthweight z-score mean (SD)</td>
<td>-1.3 (0.8)</td>
<td>-0.6 (1.2)</td>
<td>-</td>
<td>2.04/-/-</td>
</tr>
<tr>
<td>Small for gestational age, %</td>
<td>57</td>
<td>25</td>
<td>-</td>
<td>1.83/-/-</td>
</tr>
<tr>
<td>Neonatal infection, %</td>
<td>54</td>
<td>11</td>
<td>-</td>
<td>7.06/-/-</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>57/14/29</td>
<td>44/33/23</td>
<td>-</td>
<td>0.08/-/-</td>
</tr>
<tr>
<td>Standard nutrient group, %</td>
<td>43</td>
<td>47</td>
<td>-</td>
<td>&lt;0.01/-/-</td>
</tr>
<tr>
<td>Human milk, %</td>
<td>44.3 (45.5)</td>
<td>51.9 (39.4)</td>
<td>-</td>
<td>0.42/-/-</td>
</tr>
<tr>
<td>FSIQ at age 7</td>
<td>83.4 (9.5)</td>
<td>110.6 (11.9)</td>
<td>-</td>
<td>6.96/-/-</td>
</tr>
</tbody>
</table>

SP=suboptimally performing preterm subjects, NP=normally performing preterm subjects, C=control subjects

^a t-test, ^b Chi-squared test, ^c Wilcoxon test. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

6.5.2 Cognition

There was a group effect for all tests (p<0.05) except for the switch computer test, the pegboard test, and the zoo map.
Table 18: Post-hoc comparisons of suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects in cognitive test scores

<table>
<thead>
<tr>
<th></th>
<th>SP-NP</th>
<th></th>
<th>SP-C</th>
<th></th>
<th>NP-C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean difference* (95% CI)</td>
<td>p</td>
<td>mean difference* (95% CI)</td>
<td>p</td>
<td>mean difference* (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>prepare^b (s)</td>
<td>-703 (-181, -1224)</td>
<td>0.005</td>
<td>-803 (-284, -1322)</td>
<td>0.001</td>
<td>-100 (95, -294)</td>
<td>0.44</td>
</tr>
<tr>
<td>inhibit^b (s)</td>
<td>-1 (0, -3)</td>
<td>0.04</td>
<td>-2 (0, -3)</td>
<td>0.02</td>
<td>0 (0, -1)</td>
<td>0.74</td>
</tr>
<tr>
<td>Verbal list trial A6 (correct items)</td>
<td>-4 (-2, -6)</td>
<td>&lt;0.001</td>
<td>-5 (-3, -8)</td>
<td>&lt;0.001</td>
<td>-1 (0, -2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Verbal list trial B (correct items)</td>
<td>-3 (-1, -5)</td>
<td>0.01</td>
<td>-4 (-1, -6)</td>
<td>&lt;0.001</td>
<td>-1 (0, -2)</td>
<td>0.03</td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td>-6 (-4, -8)</td>
<td>&lt;0.001</td>
<td>-7 (-5, -9)</td>
<td>&lt;0.001</td>
<td>-1 (0, -2)</td>
<td>0.09</td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td>-7 (-2, -13)</td>
<td>0.004</td>
<td>-11 (-6, -16)</td>
<td>&lt;0.001</td>
<td>-4 (-1, -6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HSTC 1 (time)</td>
<td>-12 (-3, -22)</td>
<td>0.008</td>
<td>-12 (-2, -21)</td>
<td>0.01</td>
<td>1 (5, -4)</td>
<td>0.95</td>
</tr>
<tr>
<td>HSTC 2 (time)</td>
<td>-58 (-32, -84)</td>
<td>&lt;0.001</td>
<td>-65 (-39, -91)</td>
<td>&lt;0.001</td>
<td>-7 (5, -18)</td>
<td>0.34</td>
</tr>
<tr>
<td>HSTC 2^ (error)</td>
<td>-2 (0, -4)</td>
<td>0.01</td>
<td>-2 (-1, -4)</td>
<td>0.001</td>
<td>0 (0, -1)</td>
<td>0.30</td>
</tr>
<tr>
<td>arithmetic (score)</td>
<td>-12 (-8, -16)</td>
<td>&lt;0.001</td>
<td>-14 (-10, -19)</td>
<td>&lt;0.001</td>
<td>-2 (-1, -4)</td>
<td>0.007</td>
</tr>
<tr>
<td>verbal fluency (correct items)</td>
<td>-27 (-13, -41)</td>
<td>&lt;0.001</td>
<td>-35 (-20, -49)</td>
<td>&lt;0.001</td>
<td>-8 (-1, -14)</td>
<td>0.01</td>
</tr>
<tr>
<td>VCI^c</td>
<td>-31 (-19, -43)</td>
<td>&lt;0.001</td>
<td>-35 (-23, -47)</td>
<td>&lt;0.001</td>
<td>-4 (1, -10)</td>
<td>0.13</td>
</tr>
<tr>
<td>PRI^c</td>
<td>-19 (-7, -31)</td>
<td>&lt;0.001</td>
<td>-24 (-12, -36)</td>
<td>&lt;0.001</td>
<td>-5 (0, -10)</td>
<td>0.05</td>
</tr>
<tr>
<td>FSIQ^c</td>
<td>-28 (-17, -38)</td>
<td>&lt;0.001</td>
<td>-33 (-23, -44)</td>
<td>&lt;0.001</td>
<td>-6 (-1, -11)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

SP=suboptimally performing preterm subjects (n=7), NP=normally performing preterm subjects (n=64), C=control subjects (n=72) CI=confidence interval
RCFT=Rey Complex Figure Test; HSCT=Hayling Sentence Completion Test, VCI=Verbal Comprehension Index, PRI=Perceptual Reasoning Index, FSIQ=Full Scale Intelligence Quotient.

ANOVA Tukey post-hoc comparisons for group differences, adjusted for age, sex, maternal education. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.
* Negative mean difference indicates SP<NP/SP<C/NP<C
^ Values multiplied by -1, higher scores indicate better performance. ^ degrees of freedom may vary due to missing data points.
^ Age not included as covariate, adjusted for age at scoring

The post-hoc test results are shown in table 18. The SP group performed significantly worse compared to NP and C groups on all of the measures analysed.
with post-hoc tests. The NP group scores lower on the verbal list trial B, the RCFT delayed recall, arithmetic, verbal fluency scores, and FSIQ than the C group.

6.5.3 Brain structure

*Grey matter volume – voxel-based*

Suboptimally performing preterm subjects had significant reductions in grey matter volume in the cerebellum compared to normally performing preterm subjects, extending into the lingual, cingulate, and fusiform gyri compared to the controls (Figure 19). There were bilateral grey matter volume reductions in the thalamus, caudate, the parahippocampal gyrus as well as the orbitofrontal cortex, cerebellum, and middle temporal lobe extending into the central opercular cortex, the fusiform gyrus, and the parahippocampal gyrus, and the right amygdala in the NP compared to the C group.
There was an overall significant effect of group on total intracranial volume 
(p=0.018), cortical white matter volume (left: p<0.001; p=0.001), left ventricular 
volume (p=0.038), bilateral thalamus (left: p<0.001; right: p<0.001), bilateral caudate
(left: p<0.001; right: p=0.002), bilateral putamen (left: p<0.001; right: p=0.002), bilateral hippocampus (left: p<0.001; right: p=0.001), and bilateral accumbens (p=0.041; right: p=0.006).

Table 19: Post-hoc comparisons of suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects in brain volumes

<table>
<thead>
<tr>
<th>Brain volumes (mm³)</th>
<th>SP-NP</th>
<th>SP-C</th>
<th>NP-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean difference*</td>
<td>(95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Cortical white matter left</td>
<td>-39289 (23577,-55001)</td>
<td>&lt;0.001</td>
<td>-1536 (-25938,-57133)</td>
</tr>
<tr>
<td>Cortical white matter right</td>
<td>-926 (4037,-5890)</td>
<td>-926 (4037,-5890)</td>
<td>0.898</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>-895 (2867,-4657)</td>
<td>0.839</td>
<td>1244 (4979,-2491)</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>-738 (-90,1377)</td>
<td>0.019</td>
<td>-1268 (-634,-1903)</td>
</tr>
<tr>
<td>Thalamus left</td>
<td>-443 (-70,815)</td>
<td>0.015</td>
<td>-662 (-292,-1032)</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>-247 (108,602)</td>
<td>0.228</td>
<td>-509 (-156,-861)</td>
</tr>
<tr>
<td>Caudate left</td>
<td>-883 (-273,1092)</td>
<td>&lt;0.001</td>
<td>-860 (-453,-1267)</td>
</tr>
<tr>
<td>Caudate right</td>
<td>-152485 (23208,-281762)</td>
<td>0.016</td>
<td>-219028 (-90690,-347837)</td>
</tr>
</tbody>
</table>

SP=suboptimally performing preterm subjects (n=7), NP=normally performing preterm subjects (n=61), C=control subjects (n=71)
CI=confidence interval
ANOVA Tukey post-hoc comparisons for group differences, adjusted for age, sex, maternal education, and intracranial volume (ICV). Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.
* Negative mean difference indicates SP <NP/SP <C/NP < C
b not adjusted for ICV

Post-hoc comparisons showed that the left cortical white matter, the left thalamus, the left putamen, the left hippocampus and total intracranial volume were significantly smaller in the preterm subjects who perform suboptimally compared to normally performing preterm subjects. Compared to control subjects, suboptimally
performing preterm subjects additionally have smaller right hippocampus (Table 19).
In contrast with suboptimally performing preterm subjects, normally performing preterm subjects had larger ventricles, smaller right hippocampus, and no significant differences in white matter volumes compared to control subjects.

*White matter: MT – voxel–based*

There were no group differences in MT values.

*White matter: Diffusion – voxel-based*

Compared to control subjects and preterm subjects who performed normally, preterm subjects who perform suboptimally showed widespread decreases in tensor and SMT FA as well as increases in tensor and SMT RD. While tensor FA and RD differences were somewhat more restricted to the central and left hemispheric white matter, SMT measures differ bilaterally including the corpus callosum, the superior longitudinal fasciculi, cingulum, fornix, external capsule, and cerebellum (Figure 20).

Tensor FA was increased where the right inferior fronto-occipital fasciculus, the anterior corona radiata, and the anterior thalamic radiations cross. Tensor AD was higher and the mode of the tensor more planar in the right parietal lobe in the NP compared to the C group. Here, there was an increase in FA in the same right parietal region. SMT FA and AD, and MD measures were not increased in these regions. There was however, a widespread decrease in fibre orientation dispersion in
the NP compared to control subjects. SP individuals exhibited widespread decreased intra-neurite volume fraction, as well as increased extra-neurite transverse microscopic diffusivity.
Figure 20: TBSS results showing group differences between suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects

(Figure continues on next page)
<table>
<thead>
<tr>
<th>Tensor</th>
<th>SMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial Diffusivity (AD)</td>
<td></td>
</tr>
<tr>
<td>NP-C</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mean Diffusivity (MD)</td>
<td></td>
</tr>
<tr>
<td>SP-NP</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mode of anisotropy (MO)</td>
<td></td>
</tr>
<tr>
<td>SP-C</td>
<td>N/A</td>
</tr>
<tr>
<td>NP-C</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Figure 20:** TBSS results showing group differences between suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects

*(Figure continues on next page)*
### Figure 20: TBSS results showing group differences between suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Tensor</th>
<th>SMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-neurite volume fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-NP</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>SP-C</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Orientation Dispersion Entropy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP-C</td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>

SP=suboptimally performing preterm subjects, NP=normally performing preterm subjects, C=control subjects, N.S.=non-significant, N/A=not applicable: no equivalent metric in this model.

Statistical brain maps showing significant differences between subjects in diffusion metrics within the white matter skeleton between preterm and control subjects (p<0.05 TFCE corrected). Red: SP<NP/SP<C/NP<C, blue: SP>NP/SP>C/NP>C. Boxplots of diffusion metric values in voxels with significant group differences, adjusted for sex, age, and maternal education.

Left: diffusion tensor model metrics, right: spherical mean technique (SMT) model metrics.

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Diffusion metrics and cognitive test scores

The differences in SMT FA in the SP group compared to the NP and C groups appear to be driven by changes in RD and intra-neurite volume fraction. Therefore, the relationship between this measure and cognitive outcome was examined. In the voxels that showed a significant group difference between SP and NP or C groups in SMT FA, there was a significant relationship between FA and VCI ($\beta=349$, $p=<0.001$), and FSIQ ($\beta=323$, $p=<0.001$) scores. Additionally, there was a relationship with HSCT time 2 ($\beta=1461$, $p=<0.001$) and arithmetic ($\beta=148$, $p=0.007$) scores in preterm subjects only (Figure 21). However, the latter two relationships within the preterm group were no longer significant after removing the outlier with very low HSCT time 2, and arithmetic score from the analysis.

![Figure 21: Associations between SMT FA and cognitive test scores in voxels with group differences in SMT FA](image)

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For the SMT intra-neurite volume fraction, there was a significant relationship with HSCT time 2 ($\beta=348$, $p=<0.001$), zoo map 1 error score ($\beta=-19$, $p=0.009$), VCI ($\beta=119$, $p=0.004$), and FSIQ ($\beta=121$, $p=0.001$), and in preterm subjects with PRI ($\beta=160$, $p=0.004$; Figure 22). After removing the outlier on the HSCT time 2 variable, the association was no longer significant.

Figure 22: Associations between SMT intra-neurite volume fraction and cognitive test scores in voxels with significant group differences in SMT intra-neurite volume fraction
White matter – Diffusion per tract

There was a main effect of FA in the arcuate fasciculus (left: p=0.018, right: p=0.040), bilateral cingulum (left: p=0.019, right: p=0.024), left uncinate fasciculus (p=0.010), the left corticospinal tract (p=0.019), and the splenium (F(125,2)=5.2, p=0.008).

FA was reduced in the splenium and left uncinate fasciculus in the NP compared to the C group (Table 20).

Table 20: Post-hoc comparisons of suboptimally performing preterm subjects, normally performing preterm subjects, and control subjects in white matter tract FA

<table>
<thead>
<tr>
<th>Tract</th>
<th>SP-NP*</th>
<th>SP-C*</th>
<th>NP-C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcuate fasciculus left</td>
<td>-0.03(0.0-0.06)</td>
<td>0.04</td>
<td>-0.04(0.0-0.07)</td>
</tr>
<tr>
<td>Arcuate fasciculus right</td>
<td>-0.02(0.02-0.05)</td>
<td>0.46</td>
<td>-0.03(0.0-0.06)</td>
</tr>
<tr>
<td>Cingulum left</td>
<td>-0.01(0.04-0.06)</td>
<td>0.90</td>
<td>-0.03(0.02-0.08)</td>
</tr>
<tr>
<td>Cingulum right</td>
<td>-0.02(0.03-0.06)</td>
<td>0.68</td>
<td>-0.04(0.01-0.09)</td>
</tr>
<tr>
<td>Corticospinal tract left</td>
<td>-0.03(0.01-0.08)</td>
<td>0.18</td>
<td>-0.01(0.03-0.06)</td>
</tr>
<tr>
<td>Splenium</td>
<td>0.05(0.11,0)</td>
<td>0.08</td>
<td>0.02(0.07-0.04)</td>
</tr>
<tr>
<td>Uncinate fasciculus left</td>
<td>-0.01(0.03-0.04)</td>
<td>0.89</td>
<td>-0.03(0.0-0.06)</td>
</tr>
</tbody>
</table>

SP= suboptimally performing preterm subjects (n=7), NP= normally performing preterm subjects (n=60), C= control subjects (n=69) CI= confidence interval

ANOVA Tukey post-hoc comparisons for group differences, adjusted for age, sex, maternal education. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

* Negative mean difference indicates SP<NP or SP<C or NP<C
There was no significant main group effect on network measures.
6.6 Results summary

Cognition

- Results supporting hypotheses:
  - There is a subgroup of preterm individuals who perform at a level below what would be expected considering their background (maternal education; SP).

Neonatal predictors

- Results supporting hypotheses:
  - Neonatal infection/ NEC was associated with an increased chance of being in the SP group in adulthood.

- Results not supporting hypotheses:
  - Male sex, low GA, low birthweight, SGA, low maternal education, mechanical ventilation, infection/NEC, receiving a standard nutrient diet and less human milk did not significantly increase the chance of being in the SP group.

Grey matter

- Results supporting hypotheses:
• The SP group compared to the NP group showed reduced grey matter volume in the cerebellum as well as smaller left thalamus, hippocampus and total brain volume.

• Compared to the C group, the SP group showed additional reductions in grey matter in the cerebellum and a reduction in right hippocampus size.

• The NP and C group differences were similar to those reported in chapter 5, including for NP larger ventricles, smaller total brain volume, left thalamus and bilateral hippocampus volumes, as well as reduced grey matter volume in the caudate, right amygdala, middle temporal lobe extending into the central opercular cortex, the fusiform gyrus, and the parahippocampal gyrus as well as the orbitofrontal cortex, the cerebellum, and occipital cortex.

• Results not supporting the hypotheses:

  o There were no significant differences in the right thalamus, right putamen, and caudate volumes between the groups.

  o The voxel-based morphometry analysis did not show significant grey matter differences between the SP and C group in the caudate, right amygdala, middle temporal lobe extending into the central opercular cortex, the fusiform gyrus, and the parahippocampal gyrus, the orbitofrontal cortex, the cerebellum, and occipital cortex.
White matter

- Results supporting the hypotheses:
  - Compared to NP and C groups, the SP group showed decreased white matter integrity; decreased FA and increased RD and MD.
  - Compared to NP and C groups, the SP group showed decreased white matter capacity; decreased left white matter volume and intra-neurite volume fraction.
  - Differences between NP and C groups were consistent with those reported in chapter 5; the normally performing preterm group showed increased tensor FA in the anterior corona radiata and increased AD in the right parietal lobe. Tensor FA was increased in the parietal lobe and there were widespread decreases in fibre coherence (ODE). Tract averaged reductions in FA were found in the corpus callosum splenium and left uncinate fasciculus in the NP compared to the C group.
  - Reductions in SMT FA were associated with cognitive test scores.

- Results not supporting the hypotheses:
  - There were no significant reductions in right white matter volume in the SP or NP groups compared to the C group.
  - There were no significant differences in MT values.
  - There were no significant differences in network measures.
6.7 Discussion

6.7.1 Cognition

Upon closer examination of the distribution of performance across all cognitive tests, a clear tail can be observed in the preterm group of subjects who perform at a lower level than would be expected given their maternal education. Approximately 10% of the preterm subject perform below two standard deviations of the control mean for more tests than predicted, while none of the term born subjects in this cohort falls into the SP category. The mean IQ of the control group was 115, which is considerably higher than the standardised norm of 100. This indicates that the control population was relatively high performing, and therefore preterm participants appear to perform worse compared to this control group than compared to a sample that is truly representative of the population mean. This might have resulted in inclusion of more preterm participants in the suboptimally performing group. It is for this reason that the relatively conservative threshold of two standard deviations below the control mean was chosen.

Considering potentially selective attrition, there may be a much larger number of preterms with worrying cognitive suboptimal performance out there than the, roughly, 10% indicated here. The economic and social impact of even 1 in 10 on the NHS and society in general will be profound.
When removing the seven suboptimally performing individuals out of the group of 71 preterm subjects, the preterm-control group differences between NP preterm and control subjects in the verbal list trial A6, the RCFT copy score, VCI and PRI scores as was shown in chapter 5 were no longer significant. The verbal list trial B, the RCFT delayed recall, arithmetic, verbal fluency scores, and FSIQ remained significantly different between the normally performing preterm subjects and the controls. This suggests that some cognitive processes are indeed affected in some but not all people born preterm. Alternatively, some of the tests may simply be more sensitive than others.

Cognitive tests have their limitations and arbitrary factors such as fatigue, inattentiveness, and motivation can significantly affect performance on a test. To reduce false positives, the relatively strict norm was set on two standard deviations below the control subjects’ mean scores. Classification was based on a large test battery with cognitive measures that had previously been shown to be impaired in preterm populations; processing speed, attention shifting, executive functioning, memory, fine motor skills, arithmetic, and language skills. Only subjects who performed below the norm on multiple test measures were classified as performing suboptimally.

Most of the tests capture abilities on multiple cognitive domains. A potential downside of this approach is that it does not identify subjects with a specific deficit that is only captured by one test. Since the test battery was designed to pick up on
the cognitive problems that frequently occur in preterm born individuals, participants with deficits in domains that were not tested, such as episodic memory or emotion recognition, might have been wrongly classified as normally performing. Secondly, classification into the SP category most often required failing at least two tests (depending on maternal education and number of tests completed). If a subject performed very badly on only one test, they would not be classified as suboptimally performing, but may still have some issues in daily functioning.

Nevertheless, with this method a group of subjects with problems in multiple systems could be identified. This allowed investigation of i) the potential long-term consequences of preterm birth and its sequelae on the brain, ii) what neonatal characteristics are risk factors for later suboptimal performance in preterm individuals, iii) what structural brain differences are associated with suboptimal performance in preterm individuals.

6.7.2 Quality of Life

Self-report quality of life questionnaire responses could give an idea of the ecological validity of this classification (see supplementary material on p.440 for the questionnaire). This was not formally analysed, but figure 23 and figure 24 show that although the SP individuals were more often in the lower education and income brackets and they were less satisfied with their economic situation, their subjective
wellbeing graded on a likert scale is on par or sometimes even higher than the NP and C groups.

This has been previously reported in studies that compared preterm and control subjects on socioeconomic and quality of life outcomes. Young adults born preterm are less likely to be in school or employed (Hille et al., 2007), but self-reported quality of life of adults born preterm is similar and sometimes even higher than full-term adults (Vieira and Linhares, 2016).
**Figure 23: Responses to self-report questions on socioeconomic circumstances**

SP = suboptimally performing preterm, NP = normally performing preterm, C = control
Figure 24: Subjective self-report questions on quality of life on a likert scale
6.7.3 Neonatal factors

Perinatal risk factors such as GA, low birthweight and foetal growth restriction, do not significantly predict classification as suboptimally or normally performing in adulthood. Although some individual studies have reported an association between these factors and later outcome, a systematic review showed that the effect of male sex, birthweight and GA was not sustained past childhood (Linsell et al., 2015). This suggests that the effect of neonatal risk factors reduces over time, either because other factors such as schooling, diet, and exercise become more important or because adaptation has taken place.

However, investigating risk factors separately probably does not tell the whole story. An infant who was born early and received optimal nutrition might have done as well, as infants born at a higher GA who received suboptimal nutrition. Those at a low GA who received suboptimal nutrition, on the other hand, could have done much worse. One study reported that preterm infants born after 28 weeks with appropriate birth weight and uncomplicated neonatal history performed in the normal range as adolescents, whilst those who had been born SGA and prior to 28 weeks GA showed significant cognitive impairment (Lundequist et al., 2015). It is likely that risk factors have a cumulative effect and it is the combination of two or more risk factors that could determine if a preterm infant will develop cognitive difficulties. Neonatal infection/NEC on its own did increase chances of being in the suboptimally performing group from 11% to 54%.
6.7.4 **Brain structure**

*Grey matter*

Brain volume analysis showed a stepwise decrease in volumes between C, NP, and SP groups in the left thalamus, hippocampus, and putamen volumes. The voxel-based analysis showed particular reductions in the SP group in the cerebellum, but it did not show as many differences as the NP compared to the C group. This could be due to the lower statistical power related to the lower sample size of the SP compared to the NP group. Voxel-based analyses have to correct for the very large number of comparisons that is required to compare brain images of subjects on a voxel-by-voxel basis, which can result in false negatives in analyses with small samples.

There were also results that did not show any differences between SP and C groups, while there are differences between the NP and C groups, for example, the right hippocampus and both ventricles. It has been reported before that ventricular dilation is relatively common in preterm infants, but without additional presence of germinal matrix or intraventricular haemorrhage, it was not associated with IQ scores (Vollmer *et al.*, 2006).
White matter

White matter showed a different pattern, with many of the measures showing differences between the SP group and both NP and C groups, while there was little difference between the latter two groups. This suggests that preterm birth is associated with white matter alterations in some, but not all individuals, and that these are a predictor of suboptimal cognitive performance.

For example, left white matter volume was decreased in the SP group, while the majority of subjects in the NP did not have significantly different left grey matter volume compared to the C subjects. Results of the voxel-wise TBSS analyses in the diffusion scans follow a similar pattern. Diffusion metrics indicated widespread compromised white matter integrity in the SP individuals compared to the NP and C groups. Increased diffusivity perpendicular to the principal diffusion direction (Tensor and SMT RD) are thought to reflect reduced integrity and/or coherence of the white matter. Increased transverse diffusivity with stable diffusivity in the principal direction (tensor and SMT AD), results in the observed decreases in FA. It has consistently been reported that a decrease in FA was caused by elevated RD in preterm populations of varying ages (Vangberg et al., 2006; Anjari et al., 2007; Allin et al., 2011; Eikenes et al., 2011). Reductions in intra-neurite volume fraction in combination with the increase in RD suggest that neurites are less densely packed, or neurites are less well myelinated (Kaden et al., 2016), but this is difficult to distinguish with DWI alone. The VBQ analysis did not suggest that there are any
group differences in myelination. Multi-modal analyses, where all brain maps are combined in one statistical model, should be used to further inform what microstructural properties most likely underlie the changes in MR parameters. Increased SMT FA and intra-neurite volume fraction are associated with higher cognitive scores. This further supports the theory that these represent white matter microstructure properties that are required for efficient information transfer in the brain, and that compromised white matter is responsible for the suboptimal performance.

The differences between SP and C groups are more in line with the literature than those of the preterm-control comparisons in chapter 5, which demonstrated that the preterm group as a whole did not show any signs of reduced white matter integrity. Similar to the findings in the SP group presented here, studies have reported reduced FA and increased RD in the corpus callosum and the left superior longitudinal fasciculus (Allin et al., 2011; Eikenes et al., 2011), cingulum, fornix, left external capsule (Eikenes et al., 2011) in preterm adults. Eikenes et al. additionally showed significant differences in FA the right superior longitudinal fasciculus and the right external capsule (Eikenes et al., 2011). The previous studies did not report group differences in the cerebellar grey matter as shown here.
Result patterns

Three patterns of results can be discerned (Table 21). Generally, NP adults have subcortical volumes that are larger than those of SP subjects, and smaller than normal controls.

Table 21: Overview of brain structure differences between suboptimally performing preterm subjects, normally performing preterm subjects, and term born controls.

<table>
<thead>
<tr>
<th></th>
<th>SP&lt;NP&lt;C</th>
<th>SP=NP&lt;C</th>
<th>SP&lt;NP=C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey matter structures</td>
<td>Left thalamus</td>
<td>Ventricular volume</td>
<td>Left putamen</td>
</tr>
<tr>
<td></td>
<td>Left hippocampus</td>
<td>Right hippocampus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey matter voxel-based</td>
<td>Cerebellum</td>
<td>Thalamus, caudate, the</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>parahippocampal gyrus,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>orbitofrontal cortex,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cerebellum, middle temporal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>lobe, central opercular</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cortex, fusiform gyrus,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>parahippocampal gyrus,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>right amygdala</td>
<td></td>
</tr>
<tr>
<td>White matter structures</td>
<td>Splenium FA</td>
<td>Left white matter volume</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uncinate FA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter voxel-based</td>
<td>MO</td>
<td>ODE</td>
<td>FA (tensor +SMT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RD* (tensor +SMT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intra</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MD*</td>
</tr>
</tbody>
</table>

MO=mode of anisotropy, ODE=orientation dispersion entropy, FA=fractional anisotropy, RD=radial diffusivity, Intra=intracellular volume fraction, MD=mean diffusivity, ICV=intracranial volume

* Higher values in the SP group (SP>NP=C)
White matter integrity seems to be a good predictor of suboptimal performance in preterm subjects while brain and ventricular volumes differentiate preterm from term born individuals. SMT FA, RD, and intra-neurite volume fraction parameters in particular could be valuable biomarkers of cognitive performance in people born preterm, since these show widespread differences between the SP and the NP and C groups.

This could also apply to the preterm born adolescents of the Stockholm Neonatal Project, where some performed in the same range as term born controls on tests of general intelligence and executive functioning, while others performed suboptimally (Lundequist et al., 2015). Executive functioning was related to FA, but not to grey matter volumes. Furthermore, FA was strongly correlated with cognitive functions in preterm adolescents, but not in their term born peers (Vollmer et al., 2017).

The tests that were used to determine cognitive outcome groups tend to rely on communication between different brain regions. It is therefore possible that these reflect the effect of executive functioning skills, which is not specific to preterm subjects. However, there is no significant relationship between FA in those voxels showing group differences and cognitive outcome in control subjects. There is a significant correlation between FA in those voxels showing group differences and the HSCT error 2, arithmetic, verbal fluency, and IQ scores in preterm subjects. This further supports the conclusion that preterm birth can have a negative impact on white matter development, which is associated with poorer cognitive outcome.
Diffuse white matter injury is associated with reduced thalamic volumes (Boardman et al., 2006), which are both in turn associated with lower cognitive outcome in childhood (Boardman et al., 2010). As was discussed in chapter 1, reductions in grey matter volumes in people born preterm might be secondary to white matter injury (Back and Miller, 2014). It is possible that white matter injury and the resulting alterations in connectivity is the primary cause of suboptimal performance. While grey matter development is dependent on healthy white matter, and those with white matter injury are therefore more likely to have smaller grey matter volumes, the data presented here indeed suggest that the alterations in white matter are more closely linked to suboptimal performance than the grey matter. Those individuals with the poorest general cognitive outcome do not necessarily have the lowest grey matter volumes. Nevertheless, lower grey matter volumes associated with preterm status might still have a negative impact on cognitive scores.

It is possible that these white matter differences are caused by early white matter injury. Neonatal infection/NEC increased the odds of being in the SP group for preterm subjects. This is an indication that prevention of infection and/or controlling the inflammatory response in preterm infants is of critical importance for healthy brain development. As breast milk has been shown to be effective in preventing infection and moderating the inflammatory response, the next two chapters will examine the relationships between neonatal human milk intake, infection and outcome.
6.8 Conclusion

In conclusion, there is a subset of preterm individuals who perform significantly below the norm. While grey matter volumes of some structures were smaller in the suboptimally performing preterm group, it was the white matter that really set this group apart from the rest. Longitudinal imaging data would be required to investigate when these white matter differences developed, and which factors might have driven these alterations.

MRI parameters of white matter integrity, in particular SMT FA, RD, and intra-neurite volume fraction, could be novel biomarkers of cognitive suboptimal performance in people born preterm.
7 The effect of neonatal human milk intake on outcome in adults born preterm

7.1 Background

The short-term benefits of breast milk for the growing infant are undisputed; it is strongly associated with decreases in infant mortality, and the incidence of infectious disease, and promotes brain development both in term born (Victora et al., 2016) and preterm infants (Lucas et al., 1994; Keunen et al., 2014). In addition to the simple macro and micro nutrients that are also present in formula, there are important bioactive factors in human milk, including cells, anti-infectious and anti-inflammatory agents, growth factors, and prebiotics (Ballard and Morrow, 2013). These might protect the brain from injury and influence brain development via the microbiome-gut-brain axis (Keunen et al., 2014).

Human milk promotes growth of brain tissue through its unique macronutrient composition (see section 1.6.2), growth factors, and ‘prebiotic’ oligosacharides that selectively encourage the growth of beneficial (probiotic) organisms. However, it was not yet common practice to fortify maternal milk for preterm infants at the time of this study and the preterm formula was higher in energy and protein content. Therefore,
there might not be a noticeable advantage in absolute brain volume growth when comparing relative proportions of human milk and formula.

Given this protective effect against injury due to bioactive components, human milk might play an important role in moderating the white matter damage preterm birth (see section 1.1.2). This would affect both the quality as well as the quantity of white matter, which in turn could have a positive effect on cognitive outcomes.

Maternal education is an important confounder in the relationship between breast milk feeding and later academic attainment (Horta and Victoria, 2013), since women of higher socioeconomic status are more likely to breast feed (Thulier and Mercer, 2009). It is difficult to eliminate its influence since the short-term benefits of breast milk are widely accepted. It is currently neither feasible nor ethical to randomise infants to a diet without human milk. In contrast, at the start of the trial studied here, this was not yet established and preterm infants were randomly allocated to BBM or formula diets. The 18 month follow-up indicated an advantage on developmental scores of human milk over TF, notwithstanding its levels of macronutrients (Lucas et al., 1994). Furthermore, at age 7, children whose mothers chose to provide breast milk had higher scores than those whose mothers decided not to do so. This effect did not appear to be driven by confounding socioeconomic factors. Children whose mothers elected to provide breast milk but did not manage to do so, had similar IQ scores compared to the other children who did not receive any maternal milk (Lucas et al., 1992). Other studies that adjust for maternal IQ also still report a benefit of
breastfeeding on IQ outcomes (Horta and Victoria, 2013; Horta, Loret de Mola and Victora, 2015).

There is thus a strong case to be made for encouraging human milk feeding for preterm infants. Short-term benefits are well established in both preterm and term born infants (Hennet and Borsig, 2016), but these do not necessarily always translate into long-term benefits (Kerkhof et al., 2012; Horta and Victoria, 2013). The long-term benefits of breast milk feeding and duration on IQ and educational attainment are well established in term born infants (Horta, Loret de Mola and Victora, 2015), and a previous publication on this trial demonstrated a positive correlation between the percentage of maternal breast milk intake and IQ scores in adolescents. The effect was found strongest for VIQ (Isaacs et al., 2010). It was suggested that this might be due to the timing of the intervention, as early nutrition has been found to particularly effect verbal ability (Horwood, Mogridge and Darlow, 1998), whereas the implications of dietary changes later in development may selectively involve PIQ. This again is in line with the neuroconstructivist idea that developmental timing is crucial in understanding differences in cognitive outcomes (Karmiloff-Smith, 1998). However, another study on nutritional intake in infancy and later cognitive outcomes painted a contrasting picture, where energy received from human milk in the first three weeks of life affected PIQ, while human milk intake after 6 weeks affected VIQ (Sammallahti et al., 2017).
There is evidence for altered developmental trajectories of preterm children compared to their term born peers (Allin et al., 2008; Parker et al., 2008), which could change the relationship between neonatal nutrition and outcome over time.

To date, very few studies have reported on the effect of breast milk on adult cognitive outcome in preterm individuals. One study in preterm adults reported no significant IQ differences between participants who received maternal milk and those who did not (Sammallahti et al., 2017). However, it is difficult to draw conclusions about the relationship between human milk and cognitive outcome from this study, since there were only a few participants who did not receive any maternal milk and the dose-response relationship was not assessed.

7.2 Aims

This chapter aims to investigate if neonatal human milk intake is associated with improved cognitive and brain outcomes in adulthood.

7.3 Hypotheses

Human milk hypothesis: Neonatal human milk has a beneficial impact on cognitive and brain structure outcomes in people born preterm.
Cognition

• Neonatal human milk intake is associated with higher cognitive scores
  o In particular on cognitive measures that reflect verbal ability; verbal list
    learning, verbal fluency, HSTC time 1, and VCI.

White matter

• Neonatal human milk intake is associated with higher white matter quality
  o Increased fractional anisotropy (FA), reduced radial diffusivity (RD),
    and increased intra-neurite volume fraction (intra).
  o Higher MT values in the white matter.

• Neonatal human milk intake is related to increased white matter capacity
  o Increased white matter volume.
  o Increased number of connections (edge density) and increased local
    connectivity.

• White matter alterations are associated with lower performance on cognitive
  tests.
7.4 Methods

7.4.1 Data collection

Neonatal

As is described in more detail in chapter 3, all mothers were given the choice to provide breast milk. Infants in the three centres with a breast milk bank had a 50% chance to be randomised to the banked donor breast milk diet. Nutritional intake in mL was recorded daily and samples of human milk were collected from each pooled 24-hour milk collection. The percentage human milk intake was calculated by adding the percentage maternal milk and BBM intake of the total diet.

Episodes of NEC and infection were recorded prospectively by research staff during the hospital stay. NEC was classified using the British Association for Perinatal Paediatrics classification (British Association for Perinatal Paediatrics, 1983). Diagnosis criteria included at least two features of NEC; pneumatosis intestinalis, or free air in the abdomen or frothy appearance of bowel lumen as seen on a radiograph, lethargy, hypotonia, blood the stool and/or apnoeic episodes, gas in the portal vein or free air in the abdomen on a radiograph, abdominal tenderness or rigidity, tissue in the stool, bleeding, low white blood cell count (< $6 \times 10^9/l$) or platelet count (< $100 \times 10^9/l$) at the time of illness (Lucas and Cole, 1990). Infection was detected by a positive blood culture and/or increased white blood cell count.
**Childhood follow-up**

At age 7.5-8, 768 children of the original cohort completed the abbreviated IQ test (Wechsler, 1974).

**Adult follow-up**

The MRI acquisition details, the study population descriptions, computation of brain volumes, and voxel-based imaging analysis techniques TBSS, VBM, VBQ, and connectivity are described in chapter 4.

7.4.2 **Statistical analysis**

Correlations between human milk intake and age at the time of the study visit were examined with a Pearson’s correlation test and differences in human milk intake between males and females as well as low and high maternal education groups were examined with a t-test. A multiple linear regression was used to calculate the relationship between human milk and cognitive test measures, brain volumes, tract-averaged FA, and graph theory measures. Age, sex, maternal education, GA, SGA, and duration of ventilation were included as covariates. Intracranial volume (ICV) was added as an additional covariate for analyses with VBM and brain volumes as outcomes.
Subjects included in the analysis with childhood IQ data were all part of the childhood follow-up, but not all subjects had IQ scores at the adult follow-up. A mixed model was used to test a change in the effect of the proportion human milk intake on IQ scores over time for those with IQ data at both time points.

Results were adjusted for multiple comparisons using the Benjamini Hochberg method (Benjamini and Hochberg, 1995). When effect sizes were examined, the multiple regression was run with the natural logarithm of the outcome measures to estimate the change of the predictor in relationship to the outcome variable in percentages.

Partial correlation analyses between diffusion metrics and cognitive test scores were done, adjusting for age, sex, and maternal education.

7.5 Results

7.5.1 Demographics and neonatal characteristics

Population characteristics of the preterm samples in the childhood follow-up and this adult study are summarised in Table 6. Table 22 shows there were no significant associations between the covariates and the percentage human milk intake.
Table 22: Associations between the proportion human milk in the neonatal diet and covariates

<table>
<thead>
<tr>
<th>%Human milk</th>
<th>df</th>
<th>$r^2$/$t^2$/F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (SD), years</td>
<td>-</td>
<td>69</td>
<td>0.07a</td>
</tr>
<tr>
<td>Male/female, mean (SD)</td>
<td>55(38)/48(42)</td>
<td>69</td>
<td>0.80b</td>
</tr>
<tr>
<td>Maternal education &lt; A-levels, %</td>
<td>47(41)/54(39)</td>
<td>48</td>
<td>-0.66b</td>
</tr>
<tr>
<td>Gestational age</td>
<td>-</td>
<td>69</td>
<td>0.96a</td>
</tr>
<tr>
<td>Birthweight</td>
<td>-</td>
<td>69</td>
<td>0.15a</td>
</tr>
<tr>
<td>Birthweight z-score</td>
<td>-</td>
<td>69</td>
<td>0.02a</td>
</tr>
<tr>
<td>Small for gestational age, mean (SD)</td>
<td>50(40)/54(39)</td>
<td>36</td>
<td>-0.39b</td>
</tr>
<tr>
<td>Mechanical ventilation mean (SD) (no/1-5 days/&gt;6 days)</td>
<td>59(39)/50(40)/38(39)</td>
<td>1,69</td>
<td>-10.42c</td>
</tr>
<tr>
<td>Standard/high nutrient group</td>
<td>51(42)/51(38)</td>
<td>65</td>
<td>-0.06b</td>
</tr>
</tbody>
</table>

* Pearson’s correlation test *t*-test, *c* One way ANOVA

7.5.2 Cognition

Table 23 shows the relationships between the proportion of human milk intake and cognitive test scores. Higher human milk intake was associated with higher RCFT delayed recall scores in males ($\beta=0.11$, p<0.001).
Table 23: Associations between the proportion human milk in the neonatal diet and cognitive test scores

<table>
<thead>
<tr>
<th>Cognitive test scores</th>
<th>% human milk</th>
<th>R²</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computer preparea (s)</td>
<td></td>
<td>0.31</td>
<td>-0.842</td>
<td>0.67</td>
</tr>
<tr>
<td>Computer switcha (s)</td>
<td></td>
<td>0.15</td>
<td>0.612</td>
<td>0.13</td>
</tr>
<tr>
<td>Computer inhibita (error)</td>
<td></td>
<td>0.1</td>
<td>0.009</td>
<td>0.18</td>
</tr>
<tr>
<td>CVLT trial A6 (correct items)</td>
<td></td>
<td>0.18</td>
<td>0.003</td>
<td>0.76</td>
</tr>
<tr>
<td>CVLT trial B (correct items)</td>
<td></td>
<td>0.07</td>
<td>0.002</td>
<td>0.72</td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td></td>
<td>0.09</td>
<td>0.006</td>
<td>0.53</td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td></td>
<td>0.2</td>
<td>0.058</td>
<td>0.004 ±</td>
</tr>
<tr>
<td>HSCT 1 timea (s)</td>
<td></td>
<td>0.13</td>
<td>0.017</td>
<td>0.63</td>
</tr>
<tr>
<td>HSCT 2 timea (s)</td>
<td></td>
<td>0.11</td>
<td>-0.079</td>
<td>0.5</td>
</tr>
<tr>
<td>HSCT 2a error</td>
<td></td>
<td>0.11</td>
<td>0.002</td>
<td>0.79</td>
</tr>
<tr>
<td>Arithmetic (correct items)</td>
<td></td>
<td>0.15</td>
<td>0.021</td>
<td>0.28</td>
</tr>
<tr>
<td>Pegboard both hands (score)</td>
<td></td>
<td>0.06</td>
<td>-0.007</td>
<td>0.44</td>
</tr>
<tr>
<td>Pegboard assembly (score)</td>
<td></td>
<td>0.2</td>
<td>0.037</td>
<td>0.14</td>
</tr>
<tr>
<td>Zoo map errorab</td>
<td></td>
<td>0.06</td>
<td>0.004</td>
<td>0.56</td>
</tr>
<tr>
<td>Zoo map 1 timea (s)</td>
<td></td>
<td>0.16</td>
<td>0.135</td>
<td>0.68</td>
</tr>
<tr>
<td>Verbal fluency (correct items)</td>
<td></td>
<td>0.14</td>
<td>0.074</td>
<td>0.14</td>
</tr>
<tr>
<td>VCI</td>
<td></td>
<td>0.24</td>
<td>0.022c</td>
<td>0.64</td>
</tr>
<tr>
<td>PRI</td>
<td></td>
<td>0.28</td>
<td>0.089f</td>
<td>0.03</td>
</tr>
<tr>
<td>FSIQ</td>
<td></td>
<td>0.31</td>
<td>0.065c</td>
<td>0.13</td>
</tr>
<tr>
<td>VIQ age7</td>
<td></td>
<td>0.14</td>
<td>&lt;0.001c,d</td>
<td>0.99</td>
</tr>
<tr>
<td>PIQ age7</td>
<td></td>
<td>0.10</td>
<td>0.027c,d</td>
<td>0.05</td>
</tr>
<tr>
<td>FSIQ age7</td>
<td></td>
<td>0.15</td>
<td>0.012c,d</td>
<td>0.33</td>
</tr>
</tbody>
</table>

RCFT=Rey Complex Figure Test; HSCT=Hayling Sentence Completion Test, VCI=Verbal Comprehension Index, PRI=Perceptual Reasoning Index, FSIQ=Full Scale Intelligence Quotient.

Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

± relationship differs between males and females

β coefficients indicate a change in score per 1% increase in human milk

a Values multiplied by -1, higher scores indicate better performance.
b Degrees of freedom may vary due to missing data
c Age not included as covariate, adjusted for age at scoring.
d Across entire childhood sample (n=768)
**Longitudinal IQ**

There was no significant change in the effect of human milk on IQ scores over time.

### 7.5.3 Brain structure

**Grey matter volume- voxel-based**

There were no significant clusters of grey matter volume correlations with percentage human milk intake.

**Volume – per structure**

There were no significant relationships between human milk intake and brain volumes in the complete sample after correction for multiple comparisons (Table 24). In males, human milk intake was positively associated with left white matter volume ($\beta = 285.3$, $p=0.008$). A ten percent increased intake in human milk was associated with an increase of $0.13 \text{ cm}^3$ in left white matter volume, which equates to a 0.16% change.
Table 24: Associations between the proportion human milk in the neonatal diet and brain volumes

<table>
<thead>
<tr>
<th>Brain volumes (mm³)</th>
<th>% human milk</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Cortical white matter left</td>
<td>0.76</td>
<td>127.5</td>
<td>0.04 ±</td>
</tr>
<tr>
<td>Cortical white matter right</td>
<td>0.71</td>
<td>126.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>0.22</td>
<td>-3.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.36</td>
<td>-8.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Thalamus left</td>
<td>0.47</td>
<td>2.3</td>
<td>0.34</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>0.49</td>
<td>3.0</td>
<td>0.19</td>
</tr>
<tr>
<td>Caudate left</td>
<td>0.4</td>
<td>0.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Caudate right</td>
<td>0.46</td>
<td>0.9</td>
<td>0.55</td>
</tr>
<tr>
<td>Hippocampus left</td>
<td>0.18</td>
<td>-0.4</td>
<td>0.79</td>
</tr>
<tr>
<td>Hippocampus right</td>
<td>0.28</td>
<td>-0.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Putamen left</td>
<td>0.47</td>
<td>0.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Putamen right</td>
<td>0.59</td>
<td>0.2</td>
<td>0.87</td>
</tr>
<tr>
<td>ICV</td>
<td>0.5</td>
<td>852.4a</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation ± relationship differs between males and females β coefficients indicate a change in score per 1% increase in human milk a not adjusted for ICV

There were no significant clusters of MT value correlations with percentage human milk intake.
White matter – Diffusion voxel-based

There were no significant relationships between diffusion metric values and human milk intake.

White matter – Diffusion per tract

There were no significant relationships between FA in the white matter tracts and human milk intake (Table 25).
Table 25: Associations between the proportion human milk in the neonatal diet and white matter tract FA.

<table>
<thead>
<tr>
<th>Tract averaged FA</th>
<th>% human milk</th>
<th>R²</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcuate fasciculus left</td>
<td></td>
<td>0.13</td>
<td>-0.00009</td>
<td>0.46</td>
</tr>
<tr>
<td>Arcuate fasciculus right</td>
<td></td>
<td>0.09</td>
<td>0.00019</td>
<td>0.11</td>
</tr>
<tr>
<td>Anterior thalamic radiations left</td>
<td></td>
<td>0.06</td>
<td>-0.00003</td>
<td>0.76</td>
</tr>
<tr>
<td>Anterior thalamic radiations right</td>
<td></td>
<td>0.07</td>
<td>0.00006</td>
<td>0.60</td>
</tr>
<tr>
<td>Cingulum left</td>
<td></td>
<td>0.22</td>
<td>0.00008</td>
<td>0.63</td>
</tr>
<tr>
<td>Cingulum right</td>
<td></td>
<td>0.14</td>
<td>0.00001</td>
<td>0.97</td>
</tr>
<tr>
<td>Corticospinal tract left</td>
<td></td>
<td>0.16</td>
<td>0.00021</td>
<td>0.18</td>
</tr>
<tr>
<td>Corticospinal tract right</td>
<td></td>
<td>0.08</td>
<td>-0.00001</td>
<td>0.95</td>
</tr>
<tr>
<td>Corpus Callosum genu</td>
<td></td>
<td>0.07</td>
<td>-0.00023</td>
<td>0.30</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus left</td>
<td></td>
<td>0.10</td>
<td>-0.00022</td>
<td>0.40</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus right</td>
<td></td>
<td>0.08</td>
<td>0.00002</td>
<td>0.89</td>
</tr>
<tr>
<td>Corpus callosum splenium</td>
<td></td>
<td>0.03</td>
<td>-0.00015</td>
<td>0.24</td>
</tr>
<tr>
<td>Uncinate fasciculus left</td>
<td></td>
<td>0.13</td>
<td>-0.00017</td>
<td>0.18</td>
</tr>
<tr>
<td>Uncinate fasciculus right</td>
<td></td>
<td>0.15</td>
<td>0.00002</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation. β coefficients indicate a change in score per 1% increase in human milk.

Network

The relationship between human milk intake and graph theory measures was only significant in females (edge density: β =-0.03354, p=0.010, shortest path length: β =0.00064, p=0.014, global efficiency: β =-0.00018, p=0.009, local efficiency: β =-0.0009, p=0.010, cluster coefficient: β =-0.00017, p=0.008; table 26 and figure 25).
However, the effect sizes between human milk and network measures were very small. Regressing against the natural logarithm of the network outcome measures indicated that for every 10% increase, the edge density reduced 0.9%, the shortest path length increased by 0.5%, global efficiency decreased by 0.4%, local efficiency decreased by 0.2% and cluster coefficient by 0.4%.

Table 26: Associations between the proportion human milk in the neonatal diet and network measures

<table>
<thead>
<tr>
<th>Network measures</th>
<th>% human milk</th>
<th>$R^2$</th>
<th>$\beta$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge density</td>
<td></td>
<td>0.21</td>
<td>-0.02394</td>
<td>0.02 ±</td>
</tr>
<tr>
<td>Shortest path length</td>
<td></td>
<td>0.17</td>
<td>0.00045</td>
<td>0.03 ±</td>
</tr>
<tr>
<td>Global efficiency</td>
<td></td>
<td>0.25</td>
<td>-0.00014</td>
<td>0.01 ±</td>
</tr>
<tr>
<td>Local efficiency</td>
<td></td>
<td>0.25</td>
<td>-0.00007</td>
<td>0.009 ±</td>
</tr>
<tr>
<td>Cluster coefficient</td>
<td></td>
<td>0.21</td>
<td>-0.00012</td>
<td>0.01 ±</td>
</tr>
</tbody>
</table>

Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation. Results that are significant after correcting for multiple comparisons (FDR method, $p<0.05$) are printed in bold.

$\beta$ coefficients indicate a change in score per 1% increase in human milk

± relationship differs between males and females
Figure 25: Associations between human milk intake and network measures for males and females
Network measures and cognitive test scores

Measures of edge density, shortest path length, global efficiency, local efficiency, and cluster coefficient in females were not significantly related to cognitive test scores.

7.5.4 Human milk without BBM

When repeating the same analyses excluding subjects who received BBM, human milk intake was still positively associated with RCFT delayed recall ($\beta=0.118$, $p<0.001$) in males.

In addition, human milk intake in male subjects who did not receive BBM was associated with higher adult FSIQ ($\beta=0.197$, $p=0.003$), childhood PIQ ($\beta=0.085$, $p=0.002$), and childhood FSIQ ($\beta=0.070$, $p=0.004$), while this relationship was not significant in the complete preterm sample.

Another difference in results with the analysis excluding participants who did receive BBM, was that there was no significant interaction effect with sex and PRI or left cortical white matter. Human milk intake was significantly associated with PRI scores ($\beta=0.215$, $p<0.001$), but the relationship with left cortical white matter was not significant after multiple comparisons. There are also no significant relationships with network measures.
7.6 Results summary

*Cognition*

- Results supporting the hypotheses:
  - Neonatal human milk intake was associated with better visuospatial memory performance (RCFT delayed recall) in males.

- Results not supporting the hypotheses:
  - There were no differences in any of the measures of verbal ability.

*White matter*

- Results supporting the hypotheses:
  - Neonatal human milk intake was positively associated with left white matter volume in males.

- Results not supporting the hypotheses
  - There were no significant increases in white matter integrity measures (FA, RD, intra).
  - There was a very small reduction in network efficiency in females associated with higher HM intake.

7.7 Discussion

The association with delayed RCFT recall reached statistical significance in males, indicating that human milk intake is associated with better visuospatial memory.
Visuospatial memory has not previously been reported on in relation to neonatal human milk intake. The beta coefficient direction of the majority of results indicate a positive relationship between neonatal human milk intake and cognitive test outcomes and larger brain volumes, but these relationships did not reach statistical significance. This is an indication that the effect of human milk on other cognitive outcomes is in the same direction, yet not sufficiently strong to reach statistical significance. Previous studies in this cohort have suggested that neonatal diet has a greater effect on males compared to females, although this effect was observed in verbal IQ and FSIQ scores (Lucas, Morley and Cole, 1998; Isaacs et al., 2010). The increased sensitivity of male infants to diet could be due to the fact that preterm born males are generally more vulnerable. Indeed, similar to the original sample of this cohort (Lucas, Morley and Cole, 1998), the requirement of extended mechanical ventilation (> 5 days) was higher in males (33%) compared to females (16%).

There was a significant relationship between human milk intake and PRI scores, and at trend level in the childhood PIQ scores, but these were not significant after correcting for multiple comparisons. One previous observational study on long-term effects of neonatal nutrition showed a positive relationship between energy intake from human milk during the first six weeks and PIQ scores. This study also showed that energy intake from human milk during weeks 3-6 was related to FSIQ scores, and intake during weeks 6-9 was associated to FSIQ and VIQ outcomes (Sammallahti et al., 2017). The comparison between the results of the study by Sammallahti and colleagues and the results presented here is difficult for multiple
reasons. First, they measured the energy intake from milk rather than the relative proportion of human milk intake. The effects could therefore reflect an increased energy intake in infants with a human milk diet. The significant relationship between fat intake from any diet at 3–6 weeks and FSIQ and VIQ indeed suggests that those cognitive measures are more sensitive to the absolute energy intake, while PIQ might specifically be related to human milk intake. However, a previous study in this cohort reported a dose response relationship between maternal milk (MBM) and VIQ but not with PIQ (Lucas et al., 1992). Chapter 9 will discuss the relationship between differences in nutrient content of the diet and IQ outcomes. Second, our data includes nutritional intake averaged over the entire hospital stay, the length of which varied between infants (mean: 39, range: 4-100 days). Third, adjustment for confounding factors was performed differently. Nutrition no longer predicted IQ outcomes after adjusting for neonatal complications, which included one of the covariates in our analysis; duration of ventilation. The authors suggest that nutritional differences reflected, or possibly mediated, the severity of neonatal illness and its effect on neurodevelopment (Sammallahti et al., 2017).

The association between human milk intake and left white matter volume is in line with results published on this cohort in adolescence, when the percentage MBM was positively related to left white matter volume in boys (Isaacs et al., 2010).

In summary, there are some significant but small positive relationships between human milk intake and cognition and white matter volume. Longitudinal analysis with
IQ data did not show an attenuation in the effect of human milk intake on outcomes over time. This may suggest that neonatal nutrition can affect brain development in a way that determines later cognitive ability. Human milk intake was also associated with changes in network measures in females, suggesting fewer connections and a less efficient network. However, the changes were extremely small (between 0.2% and 0.9% for every 10% increase in human milk intake), and not associated with cognitive test scores. Therefore, this finding should not be interpreted as strong evidence for a negative effect of human milk on white matter structure development.

There are some limitations inherent to this study which may have masked potential effects of human milk on outcomes. First, maternal milk intake cannot be randomized, since mothers must always be encouraged to provide breast milk for ethical reasons. Infants were randomly allocated to receive donor milk or formula, but sample sizes did not allow for direct comparison of these two groups. This could have introduced a potential bias with regards to socioeconomic background. As was mentioned in chapter 1, mothers with a higher socioeconomic status are more likely to provide breastmilk. Furthermore, since lactation can be difficult for mothers of preterm infants and the infants were at the NICU and could not be breastfed directly, providing breastmilk required even more effort than usual. The mothers who provided breastmilk might have been more involved and persistent with child rearing, which could have also shaped the post-discharge nutrition, and home environment during childhood. Second, breast milk itself varies in composition depending on maternal diet and health, length of gestation, time after delivery, and length of each
lactation episode (Tudehope, 2013). Therefore, even infants who receive the same amount of breast milk receive different quantities of nutrients. Third, MBM and BBM were combined into one human milk variable, but BBM had a lower nutrient content and fewer bioactive substances than MBM. The low nutrient content could have attenuated the results. It was shown that increased MBM intake was associated with higher PIQ, VIQ, and FSIQ scores during childhood (Lucas et al., 1992), and longitudinal analysis did not suggest a significant change in the relationship between human milk intake and outcome. This supports a positive relationship between human milk and IQ outcome despite a weaker association in this adult follow-up. Fourth, the human milk intake variable was not normally distributed since the infants in the BBM arm of the trial all received 100% milk.

To address the latter two issues, an exploratory analysis was performed whereby participants who had received BBM were excluded. The relationship with delayed recall on the RCFT in males remained significant, and there were associations with adult FSIQ, and childhood PIQ and FSIQ scores, but the relationships with left white matter volume and graph theory measures did not remain significant.

Human milk affects development in a myriad of ways. While some of these effects become less prominent over time, others permanently change the developmental trajectory. One candidate for such a process is neonatal infection/NEC. Chapter 6 showed that neonatal infection/NEC was significantly higher in preterm subjects who
had developed cognitive difficulties. The next chapter will therefore examine the direct relationship between infection/NEC and outcome.

### 7.8 Conclusion

There is limited evidence for a direct beneficial relationship between human milk intake and outcome in adulthood in this cohort. The relationship between human milk and IQ in childhood and adulthood was stronger when infants who had received BBM were excluded from the analysis, suggesting that some properties of BBM, such as its low nutrient content, might have attenuated the association between human milk and outcomes.
8 The effect of neonatal infection/NEC on outcomes in adults born preterm

8.1 Background

A recent study in preterm adults suggested that the relationship between human milk intake and cognitive outcome was largely explained by neonatal complications (Sammallahti et al., 2017). It is possible that over time, the direct beneficial effects of human milk on brain development become more difficult to detect, but the destructive consequences of neonatal complications remain.

The positive effect of human milk on cognitive outcome could potentially in part be explained by its protective effect against infection or its moderating effect on inflammation. Infection and necrotizing enterocolitis (NEC) are common amongst preterm infants (Stoll et al., 2004). Intake of breast milk during the first days of life has been shown to be associated with reduced incidence of infection and NEC in very low birthweight infants (Lucas and Cole, 1990; Sisk et al., 2007; Meinzen-Derr et al., 2009; Corpeleijn et al., 2012; Embleton and Cleminson, 2017). This can have a long-term impact on the brain, since the systemic inflammatory response to infection and NEC can be harmful for the developing brain of the preterm infant (Martin and Walker, 2008). Inflammation activates microglia, which release free
radicals that damage pre-oligodendrocytes during the preterm period. This results in disturbances in white matter development and white matter injury (Volpe, 2009b).

Infection and/or NEC could therefore in some cases be responsible for some of the matter abnormalities that are often observed in people born preterm. Indeed, white matter abnormalities at term equivalent age have been suggested to mediate the relationship between neonatal sepsis/NEC and lower neurodevelopmental scores in infancy (Shah et al., 2008) and childhood (Rand et al., 2015). In newborns, infection has been related to lower FA (Chau et al., 2012), higher MD (Lee et al., 2014), and slower FA increase (Adams et al., 2010). However, one study found no association between NEC and FA in a TBSS analysis in preterm infants (Alexandrou et al., 2014).

Providing breast milk can be challenging for mothers after preterm delivery. Banked donor breast milk (BBM) is a human milk alternative in case maternal breast milk (MBM) is unavailable or insufficient to meet requirements. Despite potential loss of nutrients and anti-infective agents during the pasteurization and freezing processes of BBM (Leaf and Winterson, 2009; Vieira et al., 2011), it has been found to be equally protective against NEC as MBM (Lucas and Cole, 1990).

Chapter 7 showed a significant relationship between increased proportion of human milk in the neonatal diet and left white matter volume and memory performance (RCFT delayed recall) in males. Therefore, it would only be appropriate to perform a
mediation analyses to assess the mediatory role of infection/NEC in the relationship between human milk and these outcomes if these were to have a direct relationship with infection/NEC. It is also possible to examine the relationship between human milk and neonatal infection/NEC and between infection/NEC and outcomes. Additionally, it can be investigated whether human milk reduced any negative outcomes in infants who developed neonatal infection/NEC. In other words, to examine if human milk acted as a moderator on the effects of infection/NEC on the brain.

8.2 Aims

This chapter aims to investigate if infection/NEC is associated with adult cognitive and brain outcomes and if it plays a role in the relationship between human milk and these outcomes.

8.3 Hypotheses

- Human milk intake is associated with lower infection/NEC rate.

  Cognition

- Participants who had neonatal infection/NEC have lower cognitive outcomes.
White matter

- Participants who had neonatal infection/NEC have compromised white matter microstructure.
  - Lower fractional anisotropy (FA), higher radial diffusivity (RD), and lower intra-neurite volume fraction (intra).
  - Lower MT values.
- Participants who had neonatal infection/NEC have lower white matter capacity.
  - Lower white matter volume.
  - Lower number of connections (edge density) and lower local connectivity.
- White matter alterations are associated with lower performance on cognitive tests.

Human milk moderates the negative effect of infection/NEC on outcomes.

- There is an interaction effect between human milk and infection in relationship to outcomes. A higher proportion of human milk in the neonatal diet is associated with improved outcome in those with neonatal infection/NEC.
8.4 Methods

8.4.1 Data collection

Neonatal

Episodes of NEC and infection were recorded prospectively by research staff during the hospital stay. NEC was classified using the British Association for Perinatal Paediatrics classification (British Association for Perinatal Paediatrics, 1983). Diagnosis criteria included at least two features of NEC; pneumatosis intestinalis, or free air in the abdomen or frothy appearance of bowel lumen as seen on a radiograph, lethargy, hypotonia, blood the stool and/or apnoeic episodes, gas in the portal vein or free air in the abdomen on a radiograph, abdominal tenderness or rigidity, tissue in the stool, bleeding, low white blood cell count (< 6*10⁹/l) or platelet count (< 100*10⁹/l ) at the time of illness (Lucas and Cole, 1990). Infection was detected by a positive blood culture and/or increased white blood cell count.

Since there were only 11 cases of infection/NEC in this sample, there was not enough power to investigate the relationship between human milk and infection in the current cohort. Instead, data from the original cohort of 926 infants was used. For this analysis, age at the time of the study visit was not relevant as a confounding factor. Sex, maternal education, GA, SGA, and mechanical ventilation were included as covariates.
Childhood follow-up

At age 7.5-8 years, 768 children of the original cohort completed the abbreviated IQ test (Wechsler, 1974).

Adult follow-up

The MRI acquisition details as well as the study population descriptions, computation of brain volumes, and voxel-based imaging analysis techniques TBSS, VBM, VBQ, and connectivity are described in chapter 4.

8.4.2 Statistical analyses

An ANCOVA was used to calculate differences between those with and without neonatal infection in cognitive test measures, brain volumes, tract-averaged FA, and graph theory measures. Age, sex, maternal education, GA, SGA, and duration of ventilation were included as covariates in group comparisons with cognitive tests and voxel-based imaging. Intracranial volume (ICV) was added as a covariate for VBM and brain volume analysis.

The IQ data (VIQ, PIQ, and FSIQ scores) from the childhood follow-up was used to examine the relationship between infection on cognition with more statistical power due to the larger sample size. Subjects included in the analysis were all part of the childhood follow-up, but not all subjects had IQ scores at the adult follow-up. A mixed
model was used to test a change in the effect of infection/NEC on IQ scores over time for those with IQ data at both time points.

Results are adjusted for multiple comparisons using the Benjamini Hochberg method (Benjamini and Hochberg, 1995).

8.5 Results

8.5.1 Demographics and neonatal characteristics

Due to attrition, the 11 people with neonatal infection/NEC included here comprise 8% of those with infection/NEC in the original cohort. The proportion of people with infection/NEC relative to the total sample studied here (15%), is not significantly different of the proportion of infants with infection/NEC in the original cohort (14%; see table 6). Infants who suffered neonatal infection/NEC had significantly lower birthweight and lower GA compared to those who did not have neonatal infection/NEC (Table 27).
8.5.2 Human milk and infection/NEC

There was no significant interaction effect between banked donor breast milk and maternal milk intake and infection/NEC. Therefore, the human milk variable was used, indicating the proportion of human milk (BBM and MBM) in the neonatal diet. In the original cohort, a 10% increase in human milk was associated with a 12% reduction in the odds of infection/NEC (p<0.001). This relationship was still in the same direction, but no longer significant in the subsample of the current cohort with 11 cases of infection/NEC.
8.5.3 Cognition

The neonatal infection/NEC group scored lower on the majority of tests (computer prepare: $d=0.38$, CVLT A6: $d=0.39$, RFCT copy: $d=0.34$, RFCT delay: $d=0.82$, HSCT 2 time: $d=0.26$, HSCT 2 error: $d=0.9$, arithmetic: $d=0.22$, pegboard assembly: $d=0.34$, verbal fluency: $d=0.29$, VCI=0.18, PRI=0.36, FSIQ=0.28), but only a difference in HSCT scores in females remained significant after correction for multiple comparisons. In addition, infection/NEC was also associated with reduced childhood VIQ and FSIQ scores (Table 28).
### Table 28: Comparisons of neonatal infection/NEC groups in cognitive test measures

<table>
<thead>
<tr>
<th>Cognitive test scores</th>
<th>Infection/NEC (n=11; 8% of original cohort)</th>
<th>No infection/NEC (n=60; 8% of original cohort)</th>
<th>Group difference</th>
<th>df^b</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computer prepare^a (s)</td>
<td>-690 (982)</td>
<td>-479 (420)</td>
<td>1.46</td>
<td>0.44</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Computer switch^a (s)</td>
<td>-61 (105)</td>
<td>-116 (112)</td>
<td>1.48</td>
<td>5.27</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Computer inhibit^b (error)</td>
<td>-1 (1)</td>
<td>-1 (2)</td>
<td>1.46</td>
<td>0.16</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>CVLT trial A6 (correct items)</td>
<td>10 (3)</td>
<td>11 (3)</td>
<td>1.61</td>
<td>0.05</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>CVLT trial B (correct items)</td>
<td>7 (2)</td>
<td>6 (2)</td>
<td>1.60</td>
<td>1.11</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td>32 (4)</td>
<td>33 (3)</td>
<td>1.61</td>
<td>1.94</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td>15 (7)</td>
<td>20 (6)</td>
<td>1.61</td>
<td>5.22</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>HSCT 1 time^c (s)</td>
<td>-5 (4)</td>
<td>-10 (12)</td>
<td>1.61</td>
<td>3.68</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>HSCT 2 time^d (s)</td>
<td>-41 (33)</td>
<td>-32 (37)</td>
<td>1.61</td>
<td>0.51</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>HSCT 2* error</td>
<td>-3 (3)</td>
<td>-2 (2)</td>
<td>1.60</td>
<td>8.69</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Arithmetic (correct items)</td>
<td>14 (8)</td>
<td>16 (6)</td>
<td>1.61</td>
<td>1.36</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Pegboard both hands (score)</td>
<td>5 (4)</td>
<td>4 (3)</td>
<td>1.61</td>
<td>1.37</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Pegboard assembly (score)</td>
<td>14 (10)</td>
<td>16 (8)</td>
<td>1.61</td>
<td>0.01</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Zoo map error^a</td>
<td>-1 (2)</td>
<td>-1 (2)</td>
<td>1.61</td>
<td>0.07</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Zoo map 1 time^e (s)</td>
<td>-196 (132)</td>
<td>-201 (94)</td>
<td>1.59</td>
<td>0.04</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Verbal fluency (correct items)</td>
<td>59 (18)</td>
<td>64 (16)</td>
<td>1.61</td>
<td>0.99</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>VCI</td>
<td>104 (18)</td>
<td>107 (16)</td>
<td>1.61</td>
<td>0.43</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>PRI</td>
<td>102 (14)</td>
<td>108 (14)</td>
<td>1.60</td>
<td>3.97</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>FSIQ</td>
<td>104 (18)</td>
<td>108 (15)</td>
<td>1.60</td>
<td>2.01</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Infection/NEC (n=89; 67% of original cohort)</th>
<th>No infection/NEC (n=679; 85% of original cohort)</th>
<th>Group difference</th>
<th>df^c</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIQ age 7^c,d</td>
<td>95 (20)</td>
<td>101 (15)</td>
<td>1,710</td>
<td>5.56</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>PIQ age 7^c,d</td>
<td>95 (23)</td>
<td>100 (18)</td>
<td>1,719</td>
<td>1.28</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>FSIQ age 7^c,d</td>
<td>95 (19)</td>
<td>102 (16)</td>
<td>1,712</td>
<td>8.68</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

CVLT=California Verbal Learning Test; RCFT=Rey Complex Figure Test; HSCT=Hayling Sentence Completion Test, VCI=Verbal Comprehension Index, PRI=Perceptual Reasoning Index, FSIQ=Full Scale Intelligence Quotient.

ANCOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, and duration of ventilation. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

± relationship differs between males and females

^a Values multiplied by -1, higher scores indicate better performance. ^b degrees of freedom may vary due to missing data points. ^c Age not included as covariate, adjusted for age at scoring. ^d Across entire childhood sample (n=768)
Females with neonatal infection scored significantly worse compared to females without neonatal infection/NEC on the Hayling sentence completion task trial 2 (F(1,31)=13.26, p=0.001), while there was no such difference in males (F(1,23)=0.35, p=0.56; Figure 26).

Figure 26: Interaction between sex and infection/NEC on HSCT trial 2 error scores adjusted for maternal education, age, GA, foetal growth restriction, and duration of ventilation

Longitudinal IQ

There was no significant effect of time on the relationship between infection and FSIQ, VIQ or PIQ scores. Of the subjects with neonatal infection/NEC, those who also participated in the adult follow-up had higher FSIQ scores at age 7 (mean=100,
SD=19) than those who did not participate in the adult follow-up (mean=94, SD=19), although the difference was only significant at trend level (t=1.82, p=0.07).

8.5.4 Brain structure

Grey matter – volume voxel-based

There were no significant differences in grey matter structure between preterm individuals with and without neonatal infection.

Grey matter – volume per structure

ICV was larger on average within the no infection/NEC group, all subcortical volumes measured as well as the white matter volumes were smaller in the infection/NEC group. None of these differences reached statistical significance (Table 29).
### Table 29: Comparisons of neonatal infection/NEC groups in brain volumes

<table>
<thead>
<tr>
<th>Brain volumes (mm³)</th>
<th>Infection/NEC (n=11)</th>
<th>No infection/NEC (n=57)</th>
<th>Group difference</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical white matter left</td>
<td>218517 (43730)</td>
<td>228728 (32516)</td>
<td>1.56</td>
<td>1</td>
<td>2.61</td>
<td>0.11</td>
</tr>
<tr>
<td>Cortical white matter right</td>
<td>221258 (33067)</td>
<td>230554 (33347)</td>
<td>1.56</td>
<td>1</td>
<td>1.88</td>
<td>0.18</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>8891 (3946)</td>
<td>9505 (7107)</td>
<td>1.56</td>
<td>1</td>
<td>0.41</td>
<td>0.52</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>9065 (6680)</td>
<td>8388 (4489)</td>
<td>1.56</td>
<td>1</td>
<td>&lt;0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>Thalamus left</td>
<td>7532 (754)</td>
<td>7789 (926)</td>
<td>1.56</td>
<td>1</td>
<td>0.43</td>
<td>0.51</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>7455 (609)</td>
<td>7599 (905)</td>
<td>1.56</td>
<td>1</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>Caudate left</td>
<td>3413 (526)</td>
<td>3515 (498)</td>
<td>1.56</td>
<td>1</td>
<td>0.22</td>
<td>0.64</td>
</tr>
<tr>
<td>Caudate right</td>
<td>3571 (541)</td>
<td>3645 (566)</td>
<td>1.56</td>
<td>1</td>
<td>0.06</td>
<td>0.81</td>
</tr>
<tr>
<td>Hippocampus left</td>
<td>3368 (475)</td>
<td>3560 (454)</td>
<td>1.56</td>
<td>1</td>
<td>1.13</td>
<td>0.29</td>
</tr>
<tr>
<td>Hippocampus right</td>
<td>3421 (207)</td>
<td>3533 (445)</td>
<td>1.56</td>
<td>1</td>
<td>0.04</td>
<td>0.84</td>
</tr>
<tr>
<td>Putamen left</td>
<td>4855 (616)</td>
<td>5051 (571)</td>
<td>1.56</td>
<td>1</td>
<td>2.62</td>
<td>0.11</td>
</tr>
<tr>
<td>Putamen right</td>
<td>4890 (707)</td>
<td>5106 (609)</td>
<td>1.56</td>
<td>1</td>
<td>1.22</td>
<td>0.27</td>
</tr>
<tr>
<td>ICV</td>
<td>1396441 (244835)</td>
<td>1378837 (170161)</td>
<td>1.57</td>
<td>1</td>
<td>0.78a</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**ANCOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, duration of ventilation, and intracranial volume (ICV). Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.**

*not adjusted for ICV

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**White matter – MT voxel-based**

No clusters of significant group differences in white matter MT values exceeded the extent threshold.
White matter – Diffusion voxel-based

The infection/NEC group had lower SMT FA and higher SMT RD and lower intra-neurite volume fraction in the corpus callosum and left superior longitudinal fasciculus, superior corona radiata, corticospinal tract, and anterior thalamic radiation. The tensor was flatter in the body of the corpus callosum and cingulum of the infection/NEC group (Figure 27).
<table>
<thead>
<tr>
<th>Tensor</th>
<th>SMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional Anisotropy (FA)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Radial Diffusivity (RD)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Mode of Anisotropy (MO)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 27: TBSS results of group differences between preterm individuals with and without neonatal infection/NEC

(Figure continues on next page)
Statistical brain maps showing significant differences in diffusion metrics within the white matter skeleton between preterm subjects with and without neonatal infection/NEC ($p<0.05$ TFCE corrected). Red: infection/NEC < infection/NEC, blue: infection/NEC > no infection/NEC. Boxplots of diffusion metric values in voxels with significant group differences, adjusted for sex, age, maternal education, duration of ventilation, GA, and small for GA status. Left: diffusion tensor model metrics, right: spherical mean technique (SMT) model metrics.

Figure 27: TBSS results of group differences between preterm individuals with and without neonatal infection/NEC.

White matter diffusion - cognitive outcome

SMT FA in voxels with significant differences between preterm subjects with and without infection/NEC was positively correlated with the verbal list learning A6 ($\beta=72$, $p=0.02$), RCFT copy ($\beta=104$, $p=0.01$) and delayed recall ($\beta=182$, $p=0.02$), HSCT 2 time ($\beta=1248$, $p=0.009$) and error ($\beta=99$, $p=<0.001$), verbal fluency ($\beta=586$, $p=0.002$), and all IQ scores (PRI: $\beta=604$, $p=<0.001$, VCI: $\beta=514$, $p=0.007$, FSIQ: N/A).
\(\beta=613, p<0.001\), and negatively associated with pegboard both hands (\(\beta=-110, p=0.002\); Figure 28). The relationship with HSCT 2 time was no longer significant when one outlier was removed from the analysis.

*Figure 28: Significant relationships between SMT FA and cognitive test scores adjusted for maternal education, sex, age, GA, birthweight, and duration of ventilation*
The relationships with intra-neurite volume fraction were very similar: a higher intra-neurite volume fraction was associated with higher scores on the HSCT 2 time ($\beta=35$, $p=0.01$) and error ($\beta=27$, $p=<0.001$) measures, verbal fluency ($\beta=161$, $p=0.005$), PRI ($\beta=168$, $p=<0.001$), FSIQ ($\beta=164$, $p=0.002$), and negatively associated with the pegboard both hands score ($\beta=-28$, $p=0.007$). The relationship with HSCT 2 time was no longer significant when one outlier was removed from the analysis.

White matter – Diffusion per tract

There were no significant differences in tract-averaged FA values that survived multiple comparisons corrections (Table 30).
Table 30: Comparisons of neonatal infection/NEC groups in white matter tract FA

<table>
<thead>
<tr>
<th>Tract averaged FA</th>
<th>Infection/NEC (n=11)</th>
<th>No infection/NEC (n=56)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df</td>
</tr>
<tr>
<td>Arcuate fasciculus left</td>
<td>0.550 (0.035)</td>
<td>0.557 (0.042)</td>
<td>1,56</td>
</tr>
<tr>
<td>Arcuate fasciculus right</td>
<td>0.511 (0.034)</td>
<td>0.506 (0.04)</td>
<td>1,56</td>
</tr>
<tr>
<td>Anterior thalamic radiations left</td>
<td>0.423 (0.031)</td>
<td>0.42 (0.04)</td>
<td>1,56</td>
</tr>
<tr>
<td>Anterior thalamic radiations right</td>
<td>0.39 (0.036)</td>
<td>0.394 (0.028)</td>
<td>1,56</td>
</tr>
<tr>
<td>Cingulum left</td>
<td>0.542 (0.057)</td>
<td>0.535 (0.045)</td>
<td>1,56</td>
</tr>
<tr>
<td>Cingulum right</td>
<td>0.492 (0.056)</td>
<td>0.508 (0.028)</td>
<td>1,56</td>
</tr>
<tr>
<td>Corticospinal tract left</td>
<td>0.502 (0.051)</td>
<td>0.48 (0.027)</td>
<td>1,56</td>
</tr>
<tr>
<td>Corticospinal tract right</td>
<td>0.545 (0.037)</td>
<td>0.524 (0.061)</td>
<td>1,56</td>
</tr>
<tr>
<td>Corpus callosum genu</td>
<td>0.551 (0.07)</td>
<td>0.556 (0.038)</td>
<td>1,56</td>
</tr>
<tr>
<td>Corpus callosum splenium</td>
<td>0.553 (0.042)</td>
<td>0.561 (0.038)</td>
<td>1,56</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus left</td>
<td>0.548 (0.038)</td>
<td>0.536 (0.043)</td>
<td>1,56</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus right</td>
<td>0.618 (0.053)</td>
<td>0.6 (0.156)</td>
<td>1,56</td>
</tr>
<tr>
<td>Uncinate fasciculus left</td>
<td>0.469 (0.041)</td>
<td>0.468 (0.029)</td>
<td>1,56</td>
</tr>
<tr>
<td>Uncinate fasciculus right</td>
<td>0.455 (0.029)</td>
<td>0.452 (0.046)</td>
<td>1,56</td>
</tr>
</tbody>
</table>

ANOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, and duration of ventilation.
± relationship differs between males and females

Network

There were no significant differences in network measures between the groups (Table 31).
Table 31: Comparisons of neonatal infection/NEC groups in network graph theoretical measures

<table>
<thead>
<tr>
<th>Network measures</th>
<th>Infection/NEC (n=10) Mean (SD)</th>
<th>No infection/NEC (n=56) Mean (SD)</th>
<th>Group difference df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edge density</td>
<td>37.27 (3.21)</td>
<td>36.19 (2.7)</td>
<td>1,56</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td>Shortest path length</td>
<td>1.24 (0.07)</td>
<td>1.26 (0.06)</td>
<td>1,56</td>
<td>0.20</td>
<td>0.66</td>
</tr>
<tr>
<td>Global efficiency</td>
<td>0.43 (0.02)</td>
<td>0.43 (0.02)</td>
<td>1,56</td>
<td>0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Local efficiency</td>
<td>0.46 (0.01)</td>
<td>0.46 (0.01)</td>
<td>1,56</td>
<td>&lt;0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>Cluster coefficient</td>
<td>0.42 (0.01)</td>
<td>0.42 (0.02)</td>
<td>1,56</td>
<td>0.01</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*ANCOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, and duration of ventilation.*

8.5.5 **Moderating effect of infection on the relationship between human milk and outcomes**

After multiple comparisons, there was no interaction effect between nutrition and infection with regards to cognitive outcomes at childhood or adulthood or with regards to brain volumes, tract averaged FA values or network measures.
8.6 Results summary

- Higher proportion of human milk in the neonatal diet was associated with a lower incidence of infection/NEC.

Cognition

- Results supporting the hypotheses:
  - Neonatal infection/NEC was associated with lower VIQ and FSIQ scores in childhood
  - Neonatal infection/NEC was associated with poorer performance on the HSCT task in females

- Results not supporting the hypotheses:
  - There were no significant group differences on the other cognitive test outcomes

White matter

- Results supporting the hypotheses:
  - Compared to other preterm participants in this study, those with neonatal infection/NEC showed altered white matter microstructure, characterised by decreased FA and intra-cellular volume fraction, and increased RA and MD in the corpus callosum on both sides and left hemispheric association tracts in SMT measures. Additionally, the neonatal infection/NEC group had a more planar tensor in the corpus callosum compared to the rest of the preterm sample.
• SMT FA in voxels with significant differences between preterm subjects with and without infection/NEC was correlated with multiple cognitive test scores.

• Results not supporting the hypotheses:
  o There were no significant differences in MT values
  o There were no significant differences in white matter capacity; white matter volume or edge density

• There was no evidence that human milk moderated the negative effect of infection/NEC on outcomes

8.7 Discussion

8.7.1 Human milk intake – infection/NEC

As hypothesised, human milk intake was associated with lower rates of infection/NEC in the original cohort. This is in accordance with previous reports suggesting a relationship between maternal milk intake and reduced infection and NEC (Lucas and Cole, 1990; Corpeleijn et al., 2012) and supports the hypothesis that the anti-infectious, anti-microbial, and anti-inflammatory agents are important components in human milk that are not present in formula. Alternatively, it is possible that formula increased the incidence of infection/NEC due to contamination with microorganisms (Edmond and Bahl, 2006).
8.7.2 Cognition

Although only the relationship between infection/NEC and HSCT in females is statistically significant after correcting for multiple comparisons, the group with neonatal infection/NEC also had lower average scores on RCFT delayed recall and childhood FSIQ and PIQ (but higher computer switch scores) at the $p=0.05$ level. For example, compared to the other preterm subjects, the infection/NEC group scored on average 7 PIQ points lower during childhood and 6 PIQ points lower during adulthood than the group without infection/NEC.

There are multiple explanations for the fact that these differences do not reach statistical significance after multiple comparisons correction. Firstly, since there were only eleven subjects with infection/NEC in the adult follow-up, the study was underpowered to detect IQ differences. In order to find a statistically significant effect with the 7 points FSIQ differences shown in the childhood follow-up, there would have needed to be at least 73 subjects in each group. Secondly, selective attrition might have caused underestimation of the effects of neonatal infection/NEC. Subjects with neonatal infection/NEC who did not participate in the adult follow-up had on average lower childhood IQ scores than those who did participate as adults.

It is possible that the difference between groups based on neonatal infection/NEC becomes smaller between childhood and adulthood, as other (environmental) factors come into play and participants went through more brain developmental changes.
during adolescence. However, the PIQ difference remained 6 points in adulthood (compared to 7 in childhood) and results from the mixed model do not suggest that the effect of infection on outcome diminishes over time.

8.7.3 Brain structure

The TBSS results further underlined the importance of prevention of neonatal infection/NEC. Those with infection/NEC showed signs of reduced white matter integrity; higher SMT RD, and MD and lower SMT FA and intra-neurite volume fraction in both sides of the corpus callosum and in the left hemispheric association tracts. It was hypothesised that infection/NEC would primarily affect the white matter because the inflammatory response to infection can damage white matter and hinders oligodendrocyte maturation (Dean et al., 2014). Neonatal infection and NEC, have been associated with cerebral white matter injury (Graham et al., 2004), lower FA (Chau et al., 2012), higher MD (Lee et al., 2014) and less FA increase in preterm newborns (Adams et al., 2010), while there was often no association with brain volumes (Rand et al., 2015). The current study shows that even in a small group of people in the infection/NEC category, the impact of neonatal infection/NEC is still clearly present in adulthood.

The tensor-based measures of white matter integrity that are widely used, failed to pick up on the group differences. The results in chapter 6 already showed a reduced sensitivity of the tensor metrics to suboptimal performance with fewer areas showing
group differences than the SMT measures. The group differences in white matter properties might be just too small for tensor metrics to be significantly different or, despite the lack of significant differences in ODE, increased fibre coherence might have attenuated the tensor.

Mean SMT FA and intra-neurite volume fraction in the voxels that showed significant differences between the infection/NEC group and the other preterm subjects was significantly associated with multiple cognitive test scores. Since the voxels covered multiple association tracts in the left hemisphere, it is not surprising that a wide spectrum of cognitive skills was affected; verbal memory (verbal list trial A6), visual-motor processing and memory (RCFT), inhibition (HSCT errors), arithmetic, verbal fluency, and all IQ scores. Interestingly, higher SMT FA was associated with worse performance on the pegboard score when they used both hands.

The observed relationships between white matter microstructure and cognitive performance may suggest that lower white matter integrity, secondary to infection/NEC, impairs cognitive development. This might be a direct effect or a more complex one, where white matter alterations early in life might have impaired the ability and motivation to learn, and thereby limited the input that promotes the development of new white matter connections, which in turn affects cognitive outcome.
Here, all confirmed cases of NEC were included in the infection/NEC category. Two out of the 11 people in this category had surgery to treat their NEC. Surgery is only performed in case of severe NEC, and is itself a taxing procedure which involves anaesthesia and requires significant recovery time. Therefore, infants with surgical NEC would be expected to have worse outcomes than infants with non-surgical NEC, but the sample size does not allow for this sub-group analysis.

Infection/NEC was a predictor of suboptimal performance (chapter 6), and some of the results presented here resemble the differences in white matter alterations between SP and NP groups. However, seven out of eleven subjects with neonatal infection/NEC did not fall in the suboptimal performance category. Furthermore, there were only significant infection/NEC group differences in cognition on the HSCT in females. In other words, infection/NEC is not the only predictor of suboptimal cognitive outcome and vice versa, not all subjects who suffered from neonatal infection/NEC will end up in the suboptimally performing category.

8.7.4 Human milk – infection/NEC - outcome

Since infection/NEC is a risk factor for white matter injury and brain development disturbances, and human milk intake is associated with reduced infection/NEC risk, it was hypothesised that the beneficial effect of human milk on infant brain development could, at least in part, be attributed to its protective effects against
infection (Keunen et al., 2014). This study shows a clear link between human milk intake and infection/NEC and between infection/NEC and white matter microstructure in adulthood, and between white matter microstructure and cognitive outcome (Figure 29). However, human milk intake and neonatal infection/NEC were not consistently associated with the same outcomes in adulthood. Human milk intake was related to RCFT outcomes, left white matter volumes in males and network measures in females, while infection/NEC was associated with lower HSCT scores in females and altered white matter microstructure. The data did show that increased human milk intake and absence of infection/NEC are both associated with higher cognitive scores on most cognitive test measures and larger brain volumes, but the differences were too small to reach statistical significance. Therefore, no path analysis was performed to assess the mediatory role of infection/NEC in the relationship between human milk and outcomes.

Figure 29: Potential relationships between human milk intake, infection/NEC, and outcome
There are explanations to explain the relationship between human milk, infection/NEC, and outcomes other than a mediating role of infection between nutrition and outcome. Infants who suffer illness often have reduced feeding tolerance. This was demonstrated by the association between infection/NEC and reduced dietary intake. The resulting undernutrition could be partly responsible for the adverse relationship between infection/NEC and adult cognitive and brain outcomes. Those with neonatal infection/NEC also had significantly lower GA and birthweight. However, the effects of GA and birthweight were controlled for in the analyses.

It was hypothesised that human milk could also moderate the inflammatory response, reducing the harmful effects of infection/NEC on the brain, but there was no significant interaction effect of human milk on the relationship between infection/NEC and outcomes. This effect was difficult to test in adulthood due to the small number of subjects, but even in the larger dataset at age 7 there was no evidence that the proportion of human milk moderated the effects of infection/NEC on IQ scores.

Preventing infection/NEC and inflammation is only one of many ways in which human milk affects infant brain development. Therefore, the direct relationship between human milk and outcomes might be less clear, particularly since MBM and BBM were combined into one human milk variable. Although BBM was equally protective against infection/NEC as maternal milk, it had a much lower nutritional
value (see table 3, which may have attenuated the results. The next two chapters will further investigate the effect of the plane of nutrition and early growth on the brain.

8.8 Conclusion

Neonatal infection/NEC is associated with compromised white matter microstructure in preterm born adults, which show a significant relationship with cognitive abilities. Therefore, prevention of neonatal infection/NEC should be a priority in neonatal care units. A higher proportion of human milk in the neonatal diet is a modifiable factor that was associated with a lower chance of neonatal infection/NEC.
9 The effect of a high nutrient diet on outcome in adults born preterm

9.1 Background

Human milk is accepted to be important for preterm infants, but it may not provide sufficient nutrients and energy to preterm infants with limited feeding tolerance and high energy requirements (Dusick et al., 2003; Tudehope, 2013). Animal models have demonstrated the potential consequences of malnutrition during a critical period of brain development, including a reduction in brain cells, synapses, and neurotransmitters as well as myelin production. The resulting abnormal maturation leads to reduced complexity of cortical grey matter and lower FA in the white matter (Georgieff and Rao, 2001; Keunen et al., 2014).

A meta-analysis showed that infants were twice as likely to survive without neurodevelopmental impairment if they had received an enteral nutrient enhanced diet, but this was not reflected in developmental quotient scores in infancy (Chan et al., 2016). However, some observational studies with low birthweight infants have pointed towards improved neurodevelopmental scores in infancy with a neonatal diet higher in energy, protein, and lipid content (Stephens et al., 2009; Eleni dit Trolli et al., 2012). There is a paucity of studies examining the long-term effects of nutrient
enhancement diet interventions on brain development. One study suggested that the effect of increased postnatal energy intake on outcome diminished between infancy and young adulthood (Brandt, Sticker and Lentze, 2003), but reports on the cohort studied here indicated that the effect persisted into adolescence (Isaacs, Morley and Lucas, 2009).

9.1.1 **Results of previous follow-up studies**

Past studies on the cohort studied here have shown the effect of the nutrient content of their early diet on cognitive outcome and brain volumes (see in section 3.2.2). In short, compared to BBM, the PTF group had higher developmental quotient scores at nine months post term (Lucas *et al.*, 1989), but the effect was no longer statistically significant at 18 months post term (Lucas *et al.*, 1994). Compared to TF, the PTF group did have higher developmental quotient scores at 18 months (Lucas *et al.*, 1990; Morley and Lucas, 1993). The advantage of PTF compared to TF remained significant at age 7, in particular for VIQ scores (Lucas, Morley and Cole, 1998). Lower VIQ scores were reported in both BBM and TF groups compared to the PTF groups in adolescence, when only children born at 30 weeks GA or less were included. This was statistically significant when combining the BBM and TF in a standard nutrient group and the PTF groups of the two studies in a high nutrient plane group (Isaacs, Morley and Lucas, 2009). Furthermore, the high nutrient group had larger caudate volumes than the standard nutrient group (Isaacs *et al.*, 2008).
The effects of nutrient group were strongest in males who were SGA (Lucas et al., 1989, 1990, 1994; Morley and Lucas, 1993).

In summary, previous studies suggest that in preterm infants, who have a high nutrient requirement and low feeding tolerance, a high dietary nutrient content promotes tissue growth and development of both grey and white matter and there is some evidence that this is associated with beneficial cognitive outcomes. The smallest infants, who have to grow the fastest in their first few weeks, are likely to benefit most from an increased nutrient intake. Previous reports in the cohort studies here have indicated a particular effect of nutrient intake on verbal IQ scores and caudate volumes.

9.2 Aims

This chapter aims to investigate if the advantage of a high nutritional plane early diet on brain outcomes persists into adulthood.

9.3 Hypotheses

Nutrient content hypothesis: A neonatal diet high in nutrients has a beneficial impact on cognitive and brain structure outcomes in people born preterm.
**Cognition**

- Participants in the high nutrient group have better cognitive outcomes compared to the standard nutrient group
  - Cognitive measures that reflect verbal ability; verbal list learning, verbal fluency, HSTC time 1, and VCI

**Grey matter**

- The high nutrient group has increased grey matter volumes compared to the standard group nutrient group
  - Larger caudate volumes and higher intracranial volume (ICV)

**White matter**

- The high nutrient group has increased white matter volumes compared to the standard group nutrient group

- The effects are strongest in people born <30 weeks GA
9.4 Methods

9.4.1 Data collection

*Neonatal*

Subjects were randomly allocated to receive either a high nutrient preterm formula (PTF) or the standard diet, which was either banked donor breast milk (BBM) or standard term formula (TF; see Figure 30). Details on the nutrient content of the neonatal diets can be found in table 3 in section 3.1.3.

*Figure 30: Study design with standard and high nutrient groups*
**Childhood follow-up**

At age 7.5-8, 768 children of the original cohort completed the abbreviated IQ test (Wechsler, 1974).

**Adult follow-up**

The MRI acquisition details, the study population descriptions, computation of brain volumes and network measures, and voxel-based imaging analysis techniques (TBSS, VBM, VBQ) are outlined in chapter 4.

9.4.2 **Statistical analyses**

Group differences in age were examined with a Mann-Whitney test and differences in sex and maternal education were examined with a chi-square test. An ANCOVA was used to calculate group difference in cognitive test measures, brain volumes, tract-averaged FA, and graph theory measures. Age, sex, maternal education, GA, SGA, and duration of ventilation were included as covariates in group comparisons with cognitive tests and voxel-based imaging. Intracranial volume (ICV) was added as a covariate for analyses with brain volumes and VBM. Results are adjusted for multiple comparisons using the Benjamini Hochberg method (Benjamini and Hochberg, 1995).
Previous studies have reported on the differences in IQ scores at age 7 between the PTF group and the TF and BBM groups (Lucas et al., 1992; Lucas, Morley and Cole, 1998; Isaacs, Morley and Lucas, 2009). The TF and BBM groups were not combined into a standard nutrient group in the initial outcome reports (Lucas et al., 1992; Lucas, Morley and Cole, 1998), and only a subset of participants was included in the report that did compare standard and high nutrient groups (Isaacs, Morley and Lucas, 2009). Here, the analyses with IQ data from the childhood follow-up is done in the same way and with the same covariates as the analyses with the adult cognitive data. This facilitates comparison with previous reports on the trial outcome in this study. Using the full childhood dataset retains the high statistical power due to the large sample size. Subjects included in the analysis were all part of the childhood follow-up, but not all subjects had IQ scores at the adult follow-up. A mixed model was used to test a change in the effect of a high nutrient diet on IQ scores over time for those with IQ data at both time points.

9.5 Results

9.5.1 Demographics and neonatal characteristics

There were no significant differences between the nutrient groups in age, sex, and maternal education. The volume of nutrition intake per day is lower in the high nutrient group (Table 32). The standard and high nutrient groups studied here are 7% and 8%, respectively, of the nutrient groups in the original sample. This
difference in follow-up rates between the nutrient groups was not statistically significant and consequently there was no significant difference in the proportion of subjects in each nutrition group between the followed-up and the lost to follow-up groups (see table 6).

Table 32: Comparisons of standard and high nutrient groups in covariates

<table>
<thead>
<tr>
<th></th>
<th>Standard nutrient (n=33)</th>
<th>High nutrient (n=38)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (SD), years</td>
<td>33.5 (1.0)</td>
<td>33.5 (1.0)</td>
<td>-0.18*</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>39</td>
<td>53</td>
<td>0.77*</td>
</tr>
<tr>
<td>Maternal education &lt; A-levels, %</td>
<td>10/21*</td>
<td>15/23*</td>
<td>0.14*</td>
</tr>
<tr>
<td>Gestational age mean (SD), weeks</td>
<td>30.3 (2.3)</td>
<td>30.0 (2.4)</td>
<td>0.53*</td>
</tr>
<tr>
<td>Birthweight mean (SD), g</td>
<td>1275 (995)</td>
<td>1336 (309)</td>
<td>-0.84*</td>
</tr>
<tr>
<td>Birthweight z-score mean (SD)</td>
<td>-0.9 (1.3)</td>
<td>-0.5 (1)</td>
<td>-1.52*</td>
</tr>
<tr>
<td>Small for gestational age, %</td>
<td>39</td>
<td>18</td>
<td>2.87*</td>
</tr>
<tr>
<td>Mechanical ventilation (no/1-5 days/&gt;6 days), %</td>
<td>30</td>
<td>39</td>
<td>0.14*</td>
</tr>
<tr>
<td>Neonatal infection/NEC, %</td>
<td>18</td>
<td>13</td>
<td>0.06*</td>
</tr>
<tr>
<td>Intake diet mean, mL/day</td>
<td>203</td>
<td>165</td>
<td>2.27*</td>
</tr>
</tbody>
</table>

* t-test, b chi-squared test, c One way ANOVA

9.5.2 Cognition

As shown in Table 33, there were no significant differences in cognitive test scores in adulthood between the nutrient groups after correction for multiple comparisons, but the standard nutrient group did have significantly lower VIQ scores in childhood.
Table 33: Comparisons of standard and high nutrient groups in cognitive test scores

<table>
<thead>
<tr>
<th>Cognitive test scores</th>
<th>Standard nutrient (n=33; 7% of original cohort)</th>
<th>High nutrient (n=38; 8% of original cohort)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df^b</td>
</tr>
<tr>
<td>Computer prepare(^a) (s)</td>
<td>-386 (164)</td>
<td>-638 (741)</td>
<td>1.46</td>
</tr>
<tr>
<td>Computer switch(^a) (s)</td>
<td>-76 (88)</td>
<td>-138 (126)</td>
<td>1.48</td>
</tr>
<tr>
<td>Computer inhibit(^a) (error)</td>
<td>-1 (1)</td>
<td>-1 (2)</td>
<td>1.46</td>
</tr>
<tr>
<td>CVLT trial A6 (correct items)</td>
<td>11 (3)</td>
<td>10 (3)</td>
<td>1.61</td>
</tr>
<tr>
<td>CVLT trial B (correct items)</td>
<td>7 (2)</td>
<td>6 (2)</td>
<td>1.60</td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td>33 (3)</td>
<td>33 (3)</td>
<td>1.61</td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td>20 (6)</td>
<td>18 (7)</td>
<td>1.61</td>
</tr>
<tr>
<td>HSCT 1 time(^a) (s)</td>
<td>-9 (9)</td>
<td>-10 (13)</td>
<td>1.61</td>
</tr>
<tr>
<td>HSCT 2 time(^a) (s)</td>
<td>-27 (23)</td>
<td>-39 (44)</td>
<td>1.61</td>
</tr>
<tr>
<td>HSCT 2(^a) error</td>
<td>-2 (2)</td>
<td>-2 (2)</td>
<td>1.60</td>
</tr>
<tr>
<td>Arithmetic (correct items)</td>
<td>16 (7)</td>
<td>15 (6)</td>
<td>1.61</td>
</tr>
<tr>
<td>Pegboard both hands (score)</td>
<td>4 (3)</td>
<td>4 (3)</td>
<td>1.61</td>
</tr>
<tr>
<td>Pegboard assembly (score)</td>
<td>17 (8)</td>
<td>15 (8)</td>
<td>1.61</td>
</tr>
<tr>
<td>Zoo map error(^a)</td>
<td>-1 (2)</td>
<td>-2 (3)</td>
<td>1.61</td>
</tr>
<tr>
<td>Zoo map 1 time(^a) (s)</td>
<td>-218 (103)</td>
<td>-184 (95)</td>
<td>1.59</td>
</tr>
<tr>
<td>Verbal fluency (correct items)</td>
<td>64 (17)</td>
<td>62 (16)</td>
<td>1.61</td>
</tr>
<tr>
<td>VCI</td>
<td>104 (15)</td>
<td>108 (17)</td>
<td>1.62</td>
</tr>
<tr>
<td>PRI</td>
<td>107 (13)</td>
<td>107 (16)</td>
<td>1.61</td>
</tr>
<tr>
<td>FSIQ</td>
<td>106 (14)</td>
<td>108 (16)</td>
<td>1.61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Standard nutrient (n=382; 82% of original cohort)</th>
<th>High nutrient (n=386; 84% of original cohort)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>VIQ age 7</td>
<td>98 (19)</td>
<td>101 (19)</td>
</tr>
<tr>
<td>PIQ age 7</td>
<td>101 (16)</td>
<td>101 (17)</td>
</tr>
<tr>
<td>FSIQ age 7</td>
<td>100 (15)</td>
<td>101 (16)</td>
</tr>
</tbody>
</table>

CVLT=California Verbal Learning Test; RCFT=Rey Complex Figure Test; HSCT=Hayling Sentence Completion Test; VCI=Verbal Comprehension Index, PRI=Perceptual Reasoning Index; FSIQ=Full Scale Intelligence Quotient.

ANCOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, and duration of ventilation. Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

\(^a\) Values multiplied by -1, higher scores indicate better performance. \(^b\) degrees of freedom may vary due to missing data points.

\(^c\) Age not included as covariate, adjusted for age at scoring. \(^d\) Across entire childhood sample (n=768)

± relationship differs between males and females.
**Longitudinal IQ**

IQ scores in both the standard and high nutrient groups did not differ significantly between the childhood and adult follow-up studies, nor was there a significant interaction term in the mixed model.

9.5.3 **Brain structure**

*Grey matter – volume voxel-based*

There were no significant differences in local grey matter volumes between the standard and high nutrient groups.

*Volume – per structure*

After multiple comparison corrections, there were no group differences in brain volumes (Table 34).
Table 34: Comparisons of the standard and high nutrient groups in brain volumes

<table>
<thead>
<tr>
<th>Brain volumes (mm³)</th>
<th>Standard nutrient (n=31)</th>
<th>High nutrient (n=37)</th>
<th>Group difference</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical white matter left</td>
<td>223599 (30726)</td>
<td>229851 (37345)</td>
<td>1.56</td>
<td>0.51</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Cortical white matter right</td>
<td>226658 (29142)</td>
<td>230949 (36499)</td>
<td>1.56</td>
<td>0.05</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>7822 (3396)</td>
<td>10687 (8278)</td>
<td>1.56</td>
<td>3.15</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>7045 (2785)</td>
<td>9678 (5819)</td>
<td>1.56</td>
<td>5.79</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Thalamus left</td>
<td>7781 (901)</td>
<td>7719 (911)</td>
<td>1.56</td>
<td>0.85</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Thalamus right</td>
<td>7676 (827)</td>
<td>7495 (890)</td>
<td>1.56</td>
<td>2.85</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Caudate left</td>
<td>3455 (470)</td>
<td>3533 (527)</td>
<td>1.56</td>
<td>0.57</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Caudate right</td>
<td>3592 (516)</td>
<td>3666 (596)</td>
<td>1.56</td>
<td>0.14</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Hippocampus left</td>
<td>3564 (441)</td>
<td>3499 (479)</td>
<td>1.56</td>
<td>0.88</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Hippocampus right</td>
<td>3599 (376)</td>
<td>3445 (439)</td>
<td>1.56</td>
<td>3.52</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Putamen left</td>
<td>4959 (539)</td>
<td>5067 (611)</td>
<td>1.56</td>
<td>0.13</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Putamen right</td>
<td>5053 (652)</td>
<td>5085 (612)</td>
<td>1.56</td>
<td>0.21</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>ICV</td>
<td>1360153 (139169)</td>
<td>1399221 (211335)</td>
<td>1.57</td>
<td>0.09*</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

*ANCOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, duration of ventilation, and intracranial volume (ICV).

*adjusted for age, sex, and maternal education

**White matter – MT voxel-based**

There were no significant differences in voxel-wise MT values between the standard and high nutrient groups.

**White matter – Diffusion voxel-based**

There were no significant differences between the standard and high nutrient groups in the white matter skeleton in a voxel-wise analysis.
There were no significant relationships between nutrient group and averaged FA in white matter tracts (Table 35).

Table 35: Comparisons of standard and high nutrient groups in white matter tract FA

<table>
<thead>
<tr>
<th>Tract averaged FA</th>
<th>Standard nutrient (n=31)</th>
<th>High nutrient (n=36)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>Arcuate fasciculus left</td>
<td>0.549 (0.035)</td>
<td>0.553 (0.038)</td>
<td>1.56</td>
</tr>
<tr>
<td>Arcuate fasciculus right</td>
<td>0.5 (0.033)</td>
<td>0.519 (0.035)</td>
<td>1.56</td>
</tr>
<tr>
<td>Anterior thalamic radiations left</td>
<td>0.426 (0.033)</td>
<td>0.419 (0.032)</td>
<td>1.56</td>
</tr>
<tr>
<td>Anterior thalamic radiations right</td>
<td>0.388 (0.038)</td>
<td>0.392 (0.032)</td>
<td>1.56</td>
</tr>
<tr>
<td>Cingulum left</td>
<td>0.535 (0.047)</td>
<td>0.546 (0.061)</td>
<td>1.56</td>
</tr>
<tr>
<td>Cingulum right</td>
<td>0.487 (0.05)</td>
<td>0.501 (0.054)</td>
<td>1.56</td>
</tr>
<tr>
<td>Corticospinal tract left</td>
<td>0.492 (0.04)</td>
<td>0.504 (0.054)</td>
<td>1.56</td>
</tr>
<tr>
<td>Corticospinal tract right</td>
<td>0.537 (0.039)</td>
<td>0.546 (0.044)</td>
<td>1.56</td>
</tr>
<tr>
<td>Corpus callosum genu</td>
<td>0.555 (0.083)</td>
<td>0.548 (0.047)</td>
<td>1.56</td>
</tr>
<tr>
<td>Corpus callosum splenium</td>
<td>0.624 (0.057)</td>
<td>0.607 (0.093)</td>
<td>1.56</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus left</td>
<td>0.569 (0.036)</td>
<td>0.543 (0.041)</td>
<td>1.56</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus right</td>
<td>0.55 (0.038)</td>
<td>0.544 (0.04)</td>
<td>1.56</td>
</tr>
<tr>
<td>Uncinate fasciculus left</td>
<td>0.463 (0.047)</td>
<td>0.474 (0.031)</td>
<td>1.56</td>
</tr>
<tr>
<td>Uncinate fasciculus right</td>
<td>0.455 (0.032)</td>
<td>0.454 (0.033)</td>
<td>1.56</td>
</tr>
</tbody>
</table>

ANOVA test of group differences, adjusted for age, sex, maternal education, GA, SGA, and duration of ventilation. ± relationship differs between males and females.

Network

There were no significant differences in any of the graph theory measures examined between the two groups (Table 36).
9.5.4 Effects of nutrition group on infants <30 weeks GA

In subjects who were born <30 weeks GA (n=31), a high nutrient diet was associated with increased childhood VIQ (F(1,209)=4.13, p=0.04), PIQ (F(1,204)=6.01, p=0.02), and FSIQ (F(1,202)=7.61, p<0.001) compared to the standard nutrient group. Mean adult IQ scores were higher in the high nutrient group compared to the standard nutrient group (VCI: 109 vs 105, PRI: 112 vs 105, FSIQ: 112 vs 106, respectively), but the differences were not statistically significant. There were no significant group differences in other cognitive scores or brain volumes.
Results summary

Cognition

• Results supporting the hypotheses:
  o The high nutrient group had significantly higher VIQ scores in childhood compared to the high nutrient group.
  o In a subgroup of subjects born <30 weeks GA, the high nutrient group had significantly higher childhood VIQ, PIQ, and FSIQ scores.

• Results not supporting the hypotheses:
  o The high nutrient group did not perform significantly better on any of the cognitive scores compared to the standard nutrient group in adulthood.
  o In a subset of subjects born <30 weeks GA, the high nutrient group did not have significantly higher IQ scores in adulthood.

Grey matter

• Results not supporting the hypotheses:
  o There were no significant differences in grey matter volumes between the standard and high nutrient groups.

White matter

• Results not supporting the hypotheses:
There were no significant differences in white matter volumes between the standard and high nutrient groups.

### 9.6 Discussion

#### 9.6.1 Cognition

Adults in the high nutrient PTF group did not have significantly higher cognitive scores compared to the standard nutrient group. This seems to be in contrast with reports of an advantage of the high nutrient group on VIQ scores in childhood (Lucas, Morley and Cole, 1998), and adolescence (Isaacs, Morley and Lucas, 2009). However, VCI scores remained on average 4 points lower compared to the standard nutrient group, which is in fact a larger mean difference than the 3-point group difference in childhood VIQ scores. A quick power calculation indicates that in order to achieve 80% power at a level of significance of p<0.05 (two-sided) with a 3-point VCI difference in means (the difference in mean VIQ at age 7) and a SD of 15 (the SD in VCI of the complete adult sample), the study would require at least 393 subjects in each group. There were 33 and 38 subjects in the standard and high nutrient groups in adulthood, respectively. With a larger sample size, the difference in VIQ scores in adulthood may have reached statistical significance.

The other cognitive test measures in this adult study do not follow the same trend. Only three cognitive outcome measures are higher in the high nutrient group.
compared to the standard nutrient group (one of which is FSIQ and the effect is driven by the increased VIQ scores), while eleven scores are lower in the high nutrient group compared to the standard nutrient group.

9.6.2 Brain structure

There were no significant group differences in brain volumes. As was mentioned before, caudate volumes in the adolescent follow-up were larger in the high nutrient compared to the standard nutrient group (Isaacs et al., 2008). In the adult sample, caudate volumes in the high nutrient group were 74-78 mm$^3$ larger compared to the standard nutrient group, but this is a much smaller difference than was reported in adolescence and did not reach statistical significance. The other brain volumes were not consistently larger in the high nutrient group compared to the standard nutrient group.

9.6.3 Explanations for non-significant findings

Although data from the childhood, adolescent, and adult follow-ups seem to be consistent with regards to the effect of a high nutrient diet on verbal IQ scores and caudate volumes, the relationships do not reach statistical significance in this adult follow-up. Section 9.6.1 already mentioned that this may be due to insufficient statistical power, but there are alternative explanations, some of which can be tested with the available data.
First, it is possible that for some confounding reason or by chance, those with the lowest adolescent VIQ scores and caudate volumes were not recruited for the adult study. However, adolescent VIQ scores ($t (32) = 1.02$, $p=0.31$) and caudate volumes (left: $t (31)= -0.25$, $p=0.80$, right: $t (30) = -0.54$, $p=0.60$) of those who were included in the adult study did not differ from those who were not included.

Second, the effect of a high nutrient diet may have reduced over time. However, VIQ scores ($t (21) = 0.50$, $p=0.96$) and caudate volumes ($t (13)= -0.50$, $p = 0.63$) did not change more between the two time points in the high nutrition group compared to the standard nutrition group. As presented in section 9.5.2, the longitudinal analysis showed no significant change in the effect of nutritional plane on IQ scores over time.

Third, since the adolescent study only included participants born <30 weeks GA, the effect of a high nutrient diet might only apply to low GA infants. After all, the sample size of the adolescent publication was $n=76$ (Isaacs et al., 2008), which should also be too small to detect a difference in IQ scores according to the power calculation. The mean group differences in adult IQ scores in the subset of participants of <30 weeks GA was the same (4 points) for VCI, but larger (6 points) for PRI and FSIQ compared to the complete sample. With only 13 and 18 subjects in the standard and high nutrient groups of this analysis, respectively, this did not reach statistical significance. In childhood too, there were significant IQ differences between the nutrient groups in this subset of participants, with the strongest differences in the
FSIQ and PIQ scores. Thus, although the advantage of a high nutrient diet on IQ scores seems to be stronger in low GA infants (<30 weeks GA), this is not specific to VIQ scores.

Fourth, as can be seen in Table 32, the average nutrient intake per day was lower in the high nutrient group compared to the standard nutrient group. This was the case in the original sample as well ($t=3.1$, $p=0.001$), but not in the sample studied in adolescence ($t = 0.78$, $p = 0.44$). This means that the difference in absolute nutrient intake was smaller than expected based on nutrient content of the diets alone in the original and adult samples, but not in the adolescent study. Chapter 10 will examine if the high nutrient diet group had higher weight gain compared to the standard nutrient group, indicating that the high nutrient diet was effective, and further investigate the relationship between weight gain and outcomes.

### 9.7 Conclusion

Higher VIQ and caudate volumes in the high compared to the standard nutrient group remained observable in adulthood. However, this did not reach statistical significance and there was no clear indication that a high nutrient diet resulted in better cognitive outcomes or higher brain volumes in the adult sample. There may be a benefit of a high nutrient diet in infants with low GA. A larger sample size is required to test this with sufficient statistical power.
10 The effect of neonatal weight gain on outcome in adults born preterm

10.1 Background

Previous studies have shown a positive relationship between early growth rates and neurodevelopment (Ehrenkranz et al., 2006; Franz et al., 2009; Sammallahti et al., 2014; Jaekel et al., 2018). Growth is thought to be related to tissue growth and development of both grey and white matter and thereby with improved cognitive outcomes. The beneficial effect of growth on neurodevelopment has been reported from infancy through adolescence (Latal-Hajnal et al., 2003; Pongcharoen et al., 2012; Ramel et al., 2012). This has lead researchers to recommend a more aggressive feeding strategy (Dusick et al., 2003).

However, the effect of extra-uterine growth rate on outcome might be small (Ehrenkranz, 2010) and increased ‘catch-up growth’ is associated with metabolic and cardiovascular risk factors such as higher body fat percentage, waist circumference, cholesterol levels (Kerkhof et al., 2012), and early stages of arterial dysfunction (Singhal et al., 2004). Since cardiovascular disease often manifests itself in adulthood while the majority of studies on people born preterm have focused on the
developmental period, it is important to know if the short-term cognitive benefits that have been associated with increased neonatal weight gain persist into adulthood.

Previous studies in this cohort have reported a relationship between nutritional plane and early growth. Length and head circumference growth during hospital stay was greater in the PTF group compared to the BBM group, and weight gain and head circumference growth was greater in the PTF group compared to the TF group (Morley and Lucas, 1993). At the time of the infant follow-ups, however, TF and BBM were not combined into a low nutrient plane (standard diet) group. Previous publications on this cohort have not yet examined the relationship between neonatal growth and later cognitive outcomes.

10.2 Aims

This chapter aims to examine if a high nutrient diet is associated with increased neonatal weight gain, and if there is a relationship between neonatal growth and brain outcomes in adulthood.

10.3 Hypotheses

- A high nutrient diet is associated with increased weight gain, length gain, head circumference gain, and days to regain birthweight.
Cognition

- Increased neonatal weight gain is associated with higher cognitive test scores

Grey matter

- Increased neonatal weight gain is associated with increased grey matter volumes

White matter

- Increased neonatal weight gain is related to increased white matter capacity
  - Increased white matter volume
  - Increased number of connections (edge density) and increased local connectivity

- The effect is most pronounced in infants born <30 weeks GA
10.4 Methods

10.4.1 Data collection

During trial

Early growth rates were recorded prospectively by research nurses during the hospital stay of the infants. Larger infants gain more weight per day than smaller infants, so the natural logarithm of the weight was taken to remove the confounding effect of body weight. Weight gain in g/day was calculated by taking the regression slope from the first of 3 consecutive days above birthweight and the final weight measurement. Only cases where there are three or more data points were included. Neonatal weight gain was taken as the measure reflecting neonatal growth, since weight was measured at regular intervals in the largest number of infants and it is routinely acquired in neonatal care units.

Subjects were randomly allocated to receive either a high nutrient preterm formula (PTF) or the standard diet, which was either banked donor breast milk (BBM) or standard term formula (TF). Details on the nutrient content of the neonatal diets can be found in table 3 in section 3.1.3.
Childhood follow-up

At age 7.5-8 years, 768 children of the original cohort completed the abbreviated IQ test (Wechsler, 1974).

Adult follow-up

The MRI acquisition details as well as the study population descriptions, computation of brain volumes, and voxel-based imaging analysis techniques TBSS, VBM, VBQ, and connectivity are outlined in chapter 4.

10.4.2 Statistical analysis

Correlations between neonatal weight gain and age at the time of the study visit were examined with a Pearson’s correlation test and differences in human milk intake between males and females as well as low and high maternal education groups were examined with a t-test.

A multiple linear regression was used to calculate the relationship between weight gain and cognitive test measures, brain volumes, tract-averaged FA, and graph theory measures. Age, sex, maternal education, GA, SGA, and duration of ventilation were included as covariates in with cognitive tests and voxel-based imaging. Intracranial volume (ICV) was added as an additional covariate for analyses with brain volumes as outcomes.
The IQ data from the childhood follow-up was used to examine the relationship between neonatal weight gain and cognition with more statistical power due to the larger sample size. Subjects included in the analysis were all part of the childhood follow-up, but not all subjects had IQ scores at the adult follow-up. A mixed model was used to test a change in the effect of neonatal weight gain on IQ scores over time for those with IQ data at both time points.

Results were adjusted for multiple comparisons using the Benjamini Hochberg method (Benjamini and Hochberg, 1995).

10.5 Results

10.5.1 Nutritional plane - growth

Infants in the high nutrient group exhibited significantly higher weight, length, and head circumference gain as well as fewer days to regain birthweight compared to infants in the standard nutrient group in the original sample (Table 37).
Table 37: Comparisons of standard and high nutrient groups in neonatal growth measures

<table>
<thead>
<tr>
<th>Growth</th>
<th>Standard nutrient (n=464)</th>
<th>High nutrient (n=462)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g/day)</td>
<td>13.3 (3.5)</td>
<td>15.7 (4.0)</td>
<td>1,65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p &lt;0.001</td>
</tr>
<tr>
<td>Length gain (cm/day)</td>
<td>1.3 (0.6)</td>
<td>1.4 (0.7)</td>
<td>1,65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p 0.031</td>
</tr>
<tr>
<td>Head circumference gain</td>
<td>1.3 (0.5)</td>
<td>1.4 (0.5)</td>
<td>1,65</td>
</tr>
<tr>
<td>(cm/day)</td>
<td></td>
<td></td>
<td>F 0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p 0.001</td>
</tr>
<tr>
<td>Days to regain birthweight</td>
<td>15.9 (7.1)</td>
<td>13.5 (6.7)</td>
<td>1,65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p &lt;0.001</td>
</tr>
</tbody>
</table>

Results that are significant after correcting for multiple comparisons (FDR method, p<0.05) are printed in bold.

10.5.2 Demographics and neonatal characteristics

There was also a significant difference between the standard and high nutrient groups in neonatal weight gain in the adult sample. There were no significant differences in age at testing, sex, maternal education, GA, birthweight, SGA status, and mechanical ventilation (Table 38).
Table 38: Associations between neonatal weight gain and demographic and neonatal characteristics

<table>
<thead>
<tr>
<th>Neonatal weight gain</th>
<th>df</th>
<th>r^2/F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (SD), years</td>
<td>-</td>
<td>60</td>
<td>0.24^a</td>
</tr>
<tr>
<td>Male/female, mean (SD)</td>
<td>15(4)/14(3)</td>
<td>45</td>
<td>0.26^b</td>
</tr>
<tr>
<td>Maternal education &lt; A-levels, %</td>
<td>14(5)/15(3)</td>
<td>-</td>
<td>323^d</td>
</tr>
<tr>
<td>Gestational age</td>
<td>-</td>
<td>60</td>
<td>-0.19^a</td>
</tr>
<tr>
<td>Birthweight</td>
<td>-</td>
<td>60</td>
<td>-0.13^a</td>
</tr>
<tr>
<td>Birthweight z-score</td>
<td>-</td>
<td>60</td>
<td>0.09^a</td>
</tr>
<tr>
<td>Small for gestational age, mean (SD)</td>
<td>15(3)/15(4)</td>
<td>44</td>
<td>0.05^b</td>
</tr>
<tr>
<td>Mechanical ventilation mean (SD)</td>
<td>14(4)/15(4)/15(4)</td>
<td>1.60</td>
<td>0.51^c</td>
</tr>
<tr>
<td>(no/1-5 days/&gt;6 days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection/NEC, mean (SD) (no, yes)</td>
<td>15(4)/13(4)</td>
<td>14</td>
<td>1.23^b</td>
</tr>
<tr>
<td>Standard/high nutrient group</td>
<td>14(3)/15(4)</td>
<td>-</td>
<td>322^d</td>
</tr>
</tbody>
</table>

*a Pearson’s correlation test  
b t-test  
c One way ANOVA, 
d Wilcoxon test

10.5.3 Cognition

Higher neonatal weight gain was associated with higher childhood PIQ, VIQ and FSIQ scores (Table 39).
Table 39: Associations between neonatal weight gain and cognitive test measures

<table>
<thead>
<tr>
<th>Cognitive test scores</th>
<th>Neonatal weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
</tr>
<tr>
<td>Computer prepare* (s)</td>
<td>0.38</td>
</tr>
<tr>
<td>Computer switch* (s)</td>
<td>0.19</td>
</tr>
<tr>
<td>Computer inhibit* (error)</td>
<td>0.10</td>
</tr>
<tr>
<td>CVLT trial A6 (correct items)</td>
<td>0.20</td>
</tr>
<tr>
<td>CVLT trial B (correct items)</td>
<td>0.10</td>
</tr>
<tr>
<td>RCFT copy (score)</td>
<td>0.11</td>
</tr>
<tr>
<td>RCFT delay (score)</td>
<td>0.12</td>
</tr>
<tr>
<td>HSCT 1 time* (s)</td>
<td>0.16</td>
</tr>
<tr>
<td>HSCT 2 time* (s)</td>
<td>0.14</td>
</tr>
<tr>
<td>HSCT 2* error</td>
<td>0.14</td>
</tr>
<tr>
<td>Arithmetic (correct items)</td>
<td>0.11</td>
</tr>
<tr>
<td>Pegboard both hands (score)</td>
<td>0.06</td>
</tr>
<tr>
<td>Pegboard assembly (score)</td>
<td>0.24</td>
</tr>
<tr>
<td>Zoo map error*</td>
<td>0.10</td>
</tr>
<tr>
<td>Zoo map 1 time* (s)</td>
<td>0.14</td>
</tr>
<tr>
<td>Verbal fluency (correct items)</td>
<td>0.14</td>
</tr>
<tr>
<td>VCI*</td>
<td>0.28</td>
</tr>
<tr>
<td>PRI*</td>
<td>0.20</td>
</tr>
<tr>
<td>FSIQ*</td>
<td>0.28</td>
</tr>
<tr>
<td>VIQ age 7ª,d</td>
<td>0.17</td>
</tr>
<tr>
<td>PIQ age 7ª,d</td>
<td>0.13</td>
</tr>
<tr>
<td>FSIQ age 7ª,d</td>
<td>0.19</td>
</tr>
</tbody>
</table>

CVLT=California Verbal Learning Test; RCFT=Rey Complex Figure Test; HSCT=Hayling Sentence Completion Test, VCI=Verbal Comprehension Index, PRI=Perceptual Reasoning Index, FSIQ=Full Scale Intelligence Quotient.

Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation. Results that are significant after correcting for multiple comparisons (FDR method, $p<0.05$) are printed in bold. ± Results that are significant after correcting for multiple comparisons (FDR method, $p<0.05$) are printed in bold. ± Results that are significant after correcting for multiple comparisons (FDR method, $p<0.05$) are printed in bold. * Values multiplied by -1, higher scores indicate better performance. * Degrees of freedom may vary due to missing data points. ± Age not included as covariate, adjusted for age at scoring. ± Across entire childhood sample ($n=768$)
Longitudinal IQ

There was no significant effect of time on the effect of neonatal growth on IQ in a mixed model.

10.5.4 Brain structure

Grey matter – volume voxel-based

There were no significant grey matter changes associated with growth.

Volume – per structure

Neonatal weight gain was not significantly associated with increased brain volume (Table 40).
Table 40: Associations between neonatal weight gain and brain volumes

<table>
<thead>
<tr>
<th>Brain volumes (mm³)</th>
<th>Neonatal weight gain</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Cortical white matter left</td>
<td>0.74</td>
<td>708.3</td>
<td>0.28</td>
</tr>
<tr>
<td>Cortical white matter right</td>
<td>0.69</td>
<td>826.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>0.22</td>
<td>112.8</td>
<td>0.65</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.36</td>
<td>-29.8</td>
<td>0.85</td>
</tr>
<tr>
<td>Thalamus left</td>
<td>0.38</td>
<td>-5.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>0.39</td>
<td>-11.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Caudate left</td>
<td>0.36</td>
<td>-11.4</td>
<td>0.45</td>
</tr>
<tr>
<td>Caudate right</td>
<td>0.41</td>
<td>-14</td>
<td>0.38</td>
</tr>
<tr>
<td>Hippocampus left</td>
<td>0.14</td>
<td>20.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Hippocampus right</td>
<td>0.14</td>
<td>8.0</td>
<td>0.61</td>
</tr>
<tr>
<td>Putamen left</td>
<td>0.45</td>
<td>3.7</td>
<td>0.83</td>
</tr>
<tr>
<td>Putamen right</td>
<td>0.55</td>
<td>0.9</td>
<td>0.96</td>
</tr>
<tr>
<td>ICV</td>
<td>0.38</td>
<td>-5757.6ᵃ</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, duration of ventilation, and intracranial volume (ICV).

β coefficients indicate a change in score per g/day weight gain

ᵃ not adjusted for ICV

White matter – Diffusion: MT voxel-based

There were no significant clusters of MT value correlations with neonatal weight gain.
White matter – Diffusion voxel-based

There were no significant relationships between diffusion metric values and neonatal weight gain.

White matter – Diffusion per tract

There were no significant relationships between neonatal weight gain and tract averaged FA (Table 41).
Table 41: Associations between neonatal weight gain and white matter tract FA

<table>
<thead>
<tr>
<th>Tract averaged FA</th>
<th>Neonatal weight gain</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$\beta$</td>
<td>$p$</td>
</tr>
<tr>
<td>Arcuate fasciculus left</td>
<td>0.74</td>
<td>708.3</td>
<td>0.28</td>
</tr>
<tr>
<td>Arcuate fasciculus right</td>
<td>0.69</td>
<td>826.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Anterior thalamic radiations left</td>
<td>0.22</td>
<td>112.8</td>
<td>0.65</td>
</tr>
<tr>
<td>Anterior thalamic radiations right</td>
<td>0.36</td>
<td>-29.8</td>
<td>0.85</td>
</tr>
<tr>
<td>Cingulum left</td>
<td>0.38</td>
<td>1.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Cingulum right</td>
<td>0.39</td>
<td>-11.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Corticospinal tract left</td>
<td>0.36</td>
<td>-11.4</td>
<td>0.45</td>
</tr>
<tr>
<td>Corticospinal tract right</td>
<td>0.41</td>
<td>-14.0</td>
<td>0.38</td>
</tr>
<tr>
<td>Corpus Callosum genu</td>
<td>0.14</td>
<td>20.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus left</td>
<td>0.14</td>
<td>8.0</td>
<td>0.61</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus right</td>
<td>0.45</td>
<td>3.7</td>
<td>0.83</td>
</tr>
<tr>
<td>Corpus callosum splenium</td>
<td>0.55</td>
<td>0.9</td>
<td>0.96</td>
</tr>
<tr>
<td>Uncinate fasciculus left</td>
<td>0.38</td>
<td>-5757.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Uncinate fasciculus right</td>
<td>0.74</td>
<td>708.3</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation
*Note: $\beta$ coefficients indicate a change in score per g/day weight gain

Network

There were no significant relationships between neonatal weight gain and graph theory network measures (Table 42).
Table 42: Associations between neonatal weight gain and network measures

<table>
<thead>
<tr>
<th>Network measures</th>
<th>Neonatal weight gain</th>
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<td>β</td>
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<td>Cluster coefficient</td>
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Multiple linear regression adjusted for sex, age, maternal education, GA, SGA, and duration of ventilation. β coefficients indicate a change in score per g/day weight gain.

10.5.5 Effects of nutrition group on infants <30 weeks GA

There was no significant relationship between neonatal weight gain and outcomes in people born <30 weeks GA.
Results summary

- There was a significant relationship between nutritional plane and measures of neonatal growth; neonatal weight gain, length gain, head circumference gain, and the number of days to regain birthweight.

Cognition

- Results supporting the hypotheses:
  - There was a positive association between neonatal weight gain and childhood IQ scores.

- Results not supporting the hypotheses:
  - There were no significant relationships between neonatal growth and cognitive performance in adulthood.

Grey matter

- Results not supporting the hypotheses:
  - There were no significant relationships between neonatal growth and grey matter volumes.

White matter

- Results not supporting the hypotheses:
There were no significant relationships between neonatal weight gain and white matter capacity.

- There were no significant results when only subjects of <30 weeks GA were included in the analysis

10.6 Discussion

The high nutrient group showed significantly higher weight, length, and head circumference gain compared to the standard nutrient group. This confirms that the high nutrient group indeed received a higher nutrient diet despite a lower volume intake, and that this translated into increased neonatal growth.

There was a clear relationship between neonatal weight gain and childhood IQ scores. Each g/day extra weight gain was associated with 0.6 IQ point increase in childhood. There was no significant effect on adult cognitive scores or brain structure. With the exception of the computer test scores, zoo map error and PRI scores, the beta coefficients indicated a positive direction of the relationship between early weight gain and cognitive scores, but not nearly strong enough to reach statistical significance. As there is no significant difference in weight gain between the original sample and the adult follow-up sample, the most likely explanation for the lack of findings in this adult study is that the effects are relatively small and the study is underpowered.
The relationships with brain structure, on the other hand, do not indicate a homogeneously positive relationship with early growth. There are no significant associations between neonatal weight gain and brain structure measures. The test estimates also do not uniformly show positive correlations.

10.7 Conclusion

Although neonatal weight gain was positively associated with childhood cognitive outcome, the effect was not sufficiently strong to be detected in this study in adulthood. There was no indication of a positive effect of neonatal weight gain on brain structure in adulthood. Research in larger samples are needed to further investigate whether a benefit of neonatal weight gain on cognitive outcome persists into adulthood.
11 General discussion

This thesis investigates if and how nutrition can affect the brain structure alterations and the resulting cognitive problems that are caused by preterm birth using cognitive tests as well as multiple MRI modalities. In this section, the main findings, strengths and limitations will be discussed, along with suggestions for future research.

11.1 Main findings

The main findings will be discussed with respect to the three hypotheses, focusing particularly on consistencies and discrepancies with previously published trial results.

11.1.1 The effects of premature birth

*Prematurity hypothesis: The effects of premature birth on the brain and cognition persist into adulthood*

Supporting the prematurity hypothesis, adults born preterm had lower cognitive test scores, lower grey matter volumes, and widespread decreases in fibre coherence compared to term born peers. In contrast to previous studies, there was little
evidence for impaired white matter microstructural integrity across the preterm sample as a whole. It is possible that in this sample there were fewer white matter alterations than in other cohorts or the white matter alterations disappeared over time, potentially as a result of beneficial environmental influences.

However, upon closer examination of the distribution of performance across all cognitive tests in chapter 6, it became clear that there was a tail of preterm subjects scoring significantly below the mean on a number of tests. No control subjects fell into this category, supporting the notion that the cognitive scores of the suboptimally performing group (SP) group were not within the normal range. Furthermore, the SP group exhibited striking differences in diffusion metrics in the white matter compared to both the rest of the preterm subjects (NP) and control subjects (C). Decreased FA, increased RD and decreased intra-neurite volume fraction are indicators of compromised white matter integrity and axonal packing. The results are in line with previous reports of preterm subjects in adulthood (Nosarti et al., 2014; Meng et al., 2015). Diffusion metrics in these areas were associated with cognitive scores in the preterm subjects, indicating that impaired white matter microstructure is associated with cognitive problems in adults born preterm.

This suggests that the white matter differences reported in the literature were present in some, but not all preterm individuals in this study. As was discussed in section 1.7 and chapter 6, a complex interaction between biological, environmental, and behavioural factors could affect if a preterm infant develops suboptimally. This
has implications for future studies, as it shows that information can be missed when preterm populations are treated as one homogenous group. Compared to modern standards, the care of all infants in this study would be considered sub-optimal in many ways. Although the criteria for the SP category were stringent and only included severely underperforming subjects, and it is impossible to know how the preterm subjects would have performed had they been born full term, the fact that the majority of the preterm participants perform within the normal range is encouraging and demonstrates the resilience of preterm infants.

Identifying those who performed suboptimally in adulthood lead not only to replication of previous findings of white matter structural abnormalities, but also to identification of neonatal risk factors and MRI biomarkers associated with suboptimal functioning in adulthood. Interestingly, classic risk factors such as GA, birthweight, and ventriculomegaly did not predict SP classification. Neonatal infection/NEC on the other hand was associated with SP classification. This is encouraging, as it may be easier to reduce the incidence of neonatal infection/NEC via improvements in clinical practice than to alter GA, birthweight, and ventriculomegaly.

In summary, the preterm group as a whole was characterised by reduced grey matter volumes and fibre coherence. Although some grey matter volumes were significantly smaller in SP individuals, white matter volumes and microstructure best differentiated the SP group from the NP and C groups. Neonatal infection/NEC significantly increased the odds of performing suboptimally.
11.1.2 The direct relationships between neonatal nutrition and cognitive and brain outcomes during the different life phases

Based on previous trial reports (see section 3.2.2), two aspects of infant nutrition were hypothesised to improve adult outcomes; i) a high proportion of human milk and ii) a high nutrient content. These two are likely to interact, but due to the unique design of the trial, both could be studied.

*Human milk hypothesis: Neonatal human milk has a beneficial impact on cognitive and brain structure outcomes in people born preterm*

Previously published trial results suggested a positive relationship between human milk intake and cognitive outcome. In infancy, human milk in the neonatal diet was associated with improved developmental scores (Morley and Lucas, 1993; Lucas et al., 1994). During childhood, MBM was associated with higher IQ scores (Lucas et al., 1992), but BBM was only analysed together with TF in a standard nutrient group, which showed reductions in IQ scores compared to PTF (Isaacs, Morley and Lucas, 2009). Membership of the BBM and MBM groups combined was associated with improved childhood PIQ at trend level (p=0.05). The positive associations with IQ were stronger when subjects who received BBM were excluded from the analysis. In adolescence, MBM was positively related to VIQ scores and white matter volumes, whilst BBM and TF in the standard nutrient group were associated with reduced VIQ scores compared to PTF.
The results in adulthood also demonstrate a positive relationship between human milk and (left) white matter volume as well as with improved RCFT delayed recall in males. Although these were the only relationships with cognitive test scores and brain volumes that reached statistical significance, the majority of beta coefficients for the associations with the rest of the cognitive scores and brain volumes were positive. The association with higher PRI scores was significant prior to correction for multiple comparisons. In females, there was a very small reduction in network efficiency, but this was not related to cognitive outcome. There was no significant change in the effect of nutrition on IQ scores between childhood and adulthood.

In summary, this study confirms and extends previous reports of positive associations between the proportion of human milk intake, and in particular maternal milk intake, and childhood IQ scores. The results suggest that beneficial effects, although small, persist into adulthood. Taken together, the findings underscore the importance of incorporating human milk in the diet of preterm infants where possible.

*Nutrient content hypothesis: A neonatal diet high in nutrients has a beneficial impact on cognitive and brain structure outcomes in people born preterm.*

Infants who received the nutrient enriched PTF had higher developmental scores at 18 months compared to those who received TF. The effects were largest in SGA and male infants (Lucas *et al.*, 1990; Morley and Lucas, 1993). Infants who received
BBM also exhibited lower developmental scores at 18 months, but this was only significant in SGA infants (Morley and Lucas, 1993). This study showed that at age 7, compared to children in the PTF group, those in the standard nutrient (BBM/TF) group had lower VIQ scores. One report demonstrated that in a subset of children <30 weeks GA, the group difference was also significant for PIQ and FSIQ scores. The VIQ difference in this very preterm subgroup persisted at adolescence (Isaacs, Morley and Lucas, 2009). There is no indication that the effect of a high nutrient diet changes over time. In adulthood VIQ scores were on average 4 points higher in the high nutrient group compared to the standard nutrient group, but this difference did not reach significance.

Caudate volumes were also increased in the high nutrient group in the very preterm sample at adolescence. This difference was detected but did not reach significance in the adult sample. Interestingly, however, unlike the relationships between human milk and outcomes, where the beta coefficients indicated a fairly consistent yet small positive effect on adult outcomes, the cognitive scores and brain volumes in the high nutrient groups were not consistently higher than those of the standard nutrient group.

Childhood and adolescent data suggest a beneficial effect of a high nutrient diet on IQ outcome, but this effect might be specific to infants born at a lower GA and/or SGA. The effect appears to remain stable over time, but in adults the relationship with a broader range of cognitive and brain outcomes could not be established.
11.1.3 Short-term risk factors: infection/NEC and weight gain

After establishing that, as hypothesised and supported by previous reports, there was a relationship between human milk intake and neonatal infection/NEC as well as between nutrient plane and neonatal weight gain, the effect of these short-term neonatal factors on long-term adult brain outcomes was investigated.

The subject group with neonatal infection/NEC showed altered white matter microstructure, which was related to reduced cognitive performance. It is not surprising that the effect of infection/NEC on the brain is much stronger than that of human milk, despite the relationship between those two. As was described in section 1.1.2, infection and subsequent inflammation can lead to severe disruption of developmental processes. Human milk might facilitate development and protect against injury in preterm infants, but there may be a certain “ceiling effect” pertaining to normal brain development.

This also applies to the relationship between neonatal weight gain and brain outcomes. There were significant positive associations with IQ scores in a much larger sample at age 7, but there were no significant relationships with cognitive or brain outcomes in adulthood. Taken together, the results from previous reports and this study suggest that the beneficial effect of a high nutrient diet, or perhaps the detrimental effect of a standard nutrient diet, might apply specifically to the small and/or very premature infants. This is important as there is a focus on promoting
growth in modern neonatal care units, primarily because of supposed beneficial effects on cognitive and brain outcomes. Given the adverse metabolic and cardiovascular effects that have been associated with this ‘catch-up’ growth (Singhal et al., 2004; Kerkhof et al., 2012), larger studies are needed to determine whether the beneficial effect of weight gain in infancy only applies to a subset of preterm infants. Cardiovascular risk becomes much more prominent later in life. People born preterm are already at increased risk of cardiovascular sequelae as they exhibit altered cardiac shape and increased blood pressure (Bertagnolli et al., 2016; Aye et al., 2017). The risk-benefit balance might change over the life-course and the extent to which it changes could depend on the gestational age and birthweight of the infant.
11.1.4 MRI

Volumes

Volume analyses differentiated preterm from control groups. Left white matter volume differentiated the suboptimally performing subjects from those who performed in the normal range.

Diffusion

This adult follow-up used advanced multi-shell diffusion sequences and is the first study to use spherical mean technique (SMT) to identify differences in white matter microstructure in preterm born individuals. The traditional tensor model was also used as this has been widely reported in neuroimaging studies. In theory, SMT measures should provide a more accurate representation of white matter microstructure, since unlike the tensor model metrics, they are not affected by fibre orientation dispersion and fibre crossings (Kaden, Kruggel and Alexander, 2016). The results presented in this thesis support this.

First, the preterm group exhibited increases in tensor FA and AD in crossing fibre areas compared to term born controls. These were associated with increased fibre coherence (ODE) and a more linear tensor shape (MO), suggesting that the increases in tensor FA and AD were the result of ‘unmasking’. More specifically, “unmasking” refers to the notion that, in regions of crossing fibres, fibre loss could result in a more coherent mean orientation of the remaining fibres. The fibre coherence (ODE) thus increases and the tensor shape (MO) becomes more linear.
As a result, FA increases. People born preterm are unlikely to have degenerating fibres, but some fibre populations might be underdeveloped relative to others. This could also apply to previous findings of local FA increases in areas of crossing fibres in preterm populations (Allin et al., 2011; Eikenes et al., 2011). Whilst Eikenes and colleagues did not provide an explanation for this phenomenon, Allin and colleagues hypothesised that this could either reflect compensatory changes or unmasking. In the absence of ODE or MO measures, these two possibilities could not be further investigated (Allin et al., 2011).

Second, SMT diffusion measures appear to be more sensitive to the performance group differences and neonatal infection/NEC effects than tensor-based metrics. For example, the SMT FA differences between performance groups include bilateral association tracts and the external capsule, while tensor FA only shows these effects on the left side. Similarly, the differences in SMT transverse diffusivity are significant in a larger number of voxels than the traditional RD measures. This could again be due to the fact that the tensor model is more influenced by fibre orientation dispersion. Both models show significant results in the corpus callosum, where fibres are relatively aligned, whereas only the SMT parameters show group differences in the more lateral parts of the brain where fibre orientations are more dispersed. Furthermore, intra-cellular volume fraction differentiated between suboptimally and normally performing preterm subjects, and ODE between preterm and control participants, underlining their utility as potential biomarkers.
Differences in FA and RD could be caused by differences in myelination, but there were no significant group differences or correlations with MT. This could indicate that there are no differences in myelin content and that changes in diffusion are due to other factors such as less dense fibre packing. The latter was supported by findings of reduced intra-neurite volume fraction in areas of decreased FA and increased RD. Alternatively, the differences in white matter myelination between relatively healthy adults in this study might not have been large enough to be detected by MT transfer. This technique has primarily been useful to study the processes of myelination during early development (Xydis et al., 2006) and demyelination due to disease (Brochet, Petry and Dousset, 2003; Tofts, Steens and van Buchem, 2003) and aging (Ge et al., 2002).

The changes in diffusion metrics, indicating widespread differences in white matter quantity, coherence, and microstructure did also not translate into differences in graph theory measures. Graph theory first reduces connections to a weighted matrix, and then extract network characteristics. This inherently summarises information over many connections, which might have averaged out some of the changes observed in the diffusion maps. Another difference between TBSS and network measures is that TBSS analysis only analyses voxels in the white matter skeleton,
the core of the fibre bundles, whereas the network connectome encompasses connections in the whole brain.

11.1.5 Sex differences

This study was not powered to perform subgroup analyses in the voxel-based comparisons, but there was no significant interaction effect of sex and preterm/term group membership on cognitive outcomes, brain volumes, tract-averaged diffusion measures or network measures. Sex was also not a predictor of suboptimal performance in the preterm group. This seems to be in contrast with previous studies reporting lower cognitive scores and brain volumes in preterm born males (see sections 1.3.1 and 1.5). As most of these studies report on younger samples, it is possible that sex differences are more pronounced in early life. The longitudinal analysis showed no such interaction effects in this cohort, but more and larger studies should be performed to investigate this further, especially since a study by Allin and colleagues showed VIQ score decline in males only (Allin et al., 2008), suggesting an increase in sex differences between adolescence and adulthood.

Some early life factors did show differences in effect on males and females. There was a significantly stronger relationship between neonatal human milk intake and higher RCFT delayed recall and left white matter volumes in males than in females. This is in line with infant studies in this cohort, reporting a stronger effect of early nutrition on outcomes in males compared to females (Lucas, Morley and Cole, 1998;
Isaacs et al., 2010). On the other hand, the relationship between human milk intake and network efficiency was stronger in females, but the effect sizes were so small that this is unlikely to have practical implications. Interestingly, the relationship between neonatal infection/NEC and HSCT task performance was stronger in females.

11.1.6 Theoretical context

In line with the neuroconstructivist theory, there are differences in some brain structure and cognitive measures between the preterm and control groups, indicating that preterm birth indeed changes the constraints during development. There is a subset of preterm born individuals that show significant cognitive impairments, but these individuals have a unique profile of neonatal factors, which, most likely in combination with differences in childhood environments, have resulted in problems on different cognitive tests.

Neonatal infection/NEC clearly poses extra biological constraints on development, with long-lasting effects on white matter microstructure. A higher proportion of human milk and higher nutrient content in the neonatal diet appears to affect the developmental trajectory after preterm birth, with persistent effects. Neuroconstructivism proposes that changes in for example neonatal nutrition might also change further interactions with the environment, thereby altering developmental trajectories in unique ways for each individual. Future studies using
MRI tools to study preterm infants from birth to adulthood might be better equipped
to disentangle the more subtle interaction effects of preterm birth itself as well as
neonatal nutrition and infection/NEC.

In summary, many different factors determine the extent and nature of early damage
and alterations to developmental trajectories that occur after preterm birth and
thereby its consequences with regards to brain structure and cognitive outcomes. In
this thesis, the primary focus was on the long-term effects of neonatal nutrition, and
the short-term risk factors influenced by nutrition; infection/NEC and neonatal weight
gain. Some of the relationships between these and adult brain structure and
cognitive function could still be discerned in adulthood, but it is important to note that
throughout the course of the lives of the participants, interactions with other
biological, as well as social and behavioural influences (see section 1.7) might will
have also played a role in adult outcomes.

11.2 Strengths

The original nutritional intervention trial had a number of strengths that were
exploited in this adult follow-up. It had a large sample size for a neonatal nutrition
intervention trial, including 926 infants. It was a representative cohort since infants
from all backgrounds were included and all parents who were asked to participate
granted consent, eliminating participation bias. The randomised design of the trial
minimised the effect of confounding factors. In addition, most of the infants in the trial
received human milk via a tube rather than directly from the breast, reducing the effects of maternal bonding via suckling.

Detailed neonatal records, collected prospectively by dedicated research nurses, were available, as well as cognitive data from a large childhood follow-up. The childhood follow-up at age 7 was comprehensive, with 92% of subjects who had survived taking part.

The adult follow-up included a control group of term born individuals, providing an essential comparison population. In addition to the IQ tests that were administered for continuity with previous reports, a carefully selected cognitive test battery tailored to the preterm sequelae was added to the protocol. The outcome measures were carefully selected based on a comprehensive literature review, as well as recommendations from an expert in cognitive neuroscience with a specialisation in executive functioning (Prof. Paul Burgess). An unbiased, data-driven principal component analysis was conducted, which balanced the need to keep the number of outcome measures to a minimum with the desire to incorporate all cognitive domains of interest.

A unique, novel approach was used to identify suboptimally performing preterm subjects, incorporating scores from the entire test battery for increased robustness and ecological validity. This enabled identification of a small subset of preterm individuals who demonstrated white matter alterations that were not observed in the
preterm group as a whole. Responses to the quality of life questionnaire and to additional questions about occupation, education, and income, support the notion that this classification was ecologically valid.

Having access to data on the present preterm cohort from neonatal centres as well as studies at infancy, childhood, and adolescence, enabled unique investigation of the relationship between nutrition, neonatal risk factors, and cognition at different stages of life.

In addition, multimodal MR images were acquired with a powerful 3T scanner using advanced imaging sequences. The contemporary data processing pipelines allowed for detailed analysis of brain structure that has not been possible before. Multiple measures of grey matter volumes, white matter volume, white matter structure and structural connectivity painted a more complete picture of alterations in brain structure than any of these measures could have done on their own.

This study was the first to employ The Spherical Mean Technique (SMT) model in adults born pre-term. Using both the SMT and tensor metrics to test the hypotheses enabled a unique and novel insight into white matter microstructure and organisation in people born preterm. Consideration of the relationship between metrics aided interpretation of results. This study demonstrated that SMT is more sensitive to white matter microstructure alterations and less prone to confounding by fibre orientation than the traditional tensor model. The findings underscore the utility of SMT metrics.
as biomarkers of cognitive dysfunction and altered brain development in adults born preterm.

It becomes clear from this thesis that cognitive and MRI biomarkers have complementary value. Cognitive outcomes are a good indicator of academic achievement which largely determines employment, income, and socioeconomic status later in life (Slominski et al., 2011). Chapters 5 and 6 both showed that preterm birth is associated with lower cognitive outcome in adulthood and this should be taken into account when advising new parents of preterm infants. Knowing which cognitive problems a child born preterm is more likely to face may also help them to access the extra support they need in those areas, and this could make a difference in their academic achievement and professional development. Using a population tailored set of tests, as was piloted here in chapter 6, could identify those children born preterm who require extra attention and resources. Cognitive tests do have their limitations; performance is affected by situational factors such as tiredness, time of day, anxiety, and mood. Furthermore, the tests and testing situations are not identical to real-world scenarios, limiting their ecological validity.

By revealing the brain structure, MRI biomarkers can shed light on the biological processes after preterm birth that eventually result in problems with cognition. Since brain structure alterations may be a direct consequence of injury or altered developmental processes, specific MRI measures can sometimes pick up on differences that might not be evident from the outside. For example, diffusion metrics
showed widespread structural alterations in the people with neonatal infection/NEC, and these metrics were related to cognitive outcome even though the relationships between infection/NEC and cognitive outcome were not immediately apparent through direct group comparisons. This demonstrates the potential detrimental effect of infection/NEC on the brain and the diffusion metrics could serve as an outcome measure in trials on inflammation management. MRI biomarkers have the advantage that they can be used in infancy, before cognitive tests can be administered. Furthermore, identifying sensitive, informative, and valid MRI biomarkers in preterm populations in adulthood can be used in new studies on modern cohorts to examine if and how these change over time. Nevertheless, brain structural changes do not always result in poorer outcomes and often times patient populations demonstrate changes in both directions (e.g. increased grey matter in some regions and decreased grey matter in others).

The combination of both types of markers is particularly powerful as cognitive tests help to identify those brain MRI biomarkers that are related to changes in cognitive ability. Furthermore, from a theoretical point of view, using these in tandem could contribute to understanding brain behaviour relationships.

In summary, the present work was strengthened by the use of novel cognitive and imaging analyses, the inclusion of a control group, and the context of a large, comprehensive, and well-designed prospective neo-natal trial, which together
provided a unique opportunity to study the long-term effects of prematurity and nutrition on the preterm brain.

### 11.3 Limitations

#### 11.3.1 Original trial

There are a number of limitations that always play a role in nutrition interventions in preterm infants. First, at the time, there was no gold standard for nutrition in preterm infants to compare the intervention against. As was noted in chapter 1, breast milk is considered gold standard for term infants, but might not meet the requirements of preterm ex-utero development. In this trial, BBM and TF were used as the control diets, but they differ widely in composition and active ingredients. Second, it is unethical to deny mothers the right to provide breast milk for their child. Therefore, infants of mothers who chose to breastfeed received a mixture of breastmilk and trial diet, and the proportion depended on the amount of breast milk the mother could provide. More breastmilk inherently equated to less of the trial diet. As a result, it is impossible to tell whether a significant positive effect of breast milk reflects a reduction in harmful effects of the trial diet.

Third, a mother's choice to provide breastmilk could have introduced bias and confounded results, because women who choose to breastfeed tend to have a higher socioeconomic status than those who choose to use formula (Der, Batty and
Deary, 2006). In the current study, mothers who chose to provide breast milk were more highly educated than mothers who did not provide breast milk \((F(1,764)=82.67, p<0.001)\). Higher socioeconomic status is not only a predictor of later academic achievement, but also associated with a number of factors that affect child development such as obesity and smoking habits (Donath and Amir, 2000; Sebire et al., 2001; Leung, Lam and Ho, 2002; Chung, Kim and Nam, 2008).

11.3.2 Adult follow-up

Attrition

Attrition is a well described problem in long-term randomised trials and prospective studies (Fewtrell et al., 2008). This study was originally designed as a trial rather than longitudinal cohort study, which made it even more difficult to trace participants and invite them back. From the 926 infants that were included in the trial, 72 were recruited for this adult follow-up. This was the maximum number that could be recruited due to time and budget restrictions, along with the high costs of MRI scans. The majority of MRI studies with preterm subjects have even smaller sample sizes (Domenico, 2010; Dutt et al., 2011; Li et al., 2015). Furthermore, the results in this study demonstrate that the sample size was large enough to capture at least some of the variability in cognition and brain structure in the preterm group. Nevertheless, there are 854 participants from the original trial who were not included in this follow-up and therefore the results cannot be interpreted as randomised outcomes. The
childhood IQ scores of the participants who took part in this follow-up were higher than those who were lost to follow-up, suggesting that attrition may not have been random. This may also imply that the percentage of preterm adults performing sub-optimally is higher than the 10% identified in this sample.

Confounding factors

Results in chapter 5 and 6 that were attributed to preterm birth might in fact have been a result of perinatal factors such as pre-eclampsia or medical complications associated with preterm birth. Furthermore, infant nutrition after release from hospital was not considered, nor were environmental factors such as parental IQ, genetic make-up, life trauma, stress, and lifestyle.

Maternal education below or above A-levels was used as a covariate in the analyses, but future studies could include a measure of parental IQ for more precise adjustment.

Statistical power

This study was underpowered to detect most changes in IQ measures, which formed the main cognitive end points in previous follow-up studies. However, test measures such as the RCFT delayed recall demonstrated greater sensitivity to changes in the preterm group. Furthermore, the TBSS results in chapter 8 demonstrated significant brain structure differences even though the IQ scores are not significantly different.
between the groups. IQ measures may be less sensitive due to the inherent averaging of scores across several domains.

Multiple comparisons corrections were consistently applied to avoid type 1 errors, but this could have introduced type 2 errors. For example, the relationship between human milk intake and PRI scores was significant at p=0.03, which in previous studies with only three IQ outcomes would have been considered significant.

This study was also underpowered for subgroup analyses. Previous trial reports have shown an increased sensitivity to diet in infants that were SGA and males (Lucas et al., 1990). Whilst in the original trial there were 141 males that were SGA, only seven took part in this follow-up.

According to our hypotheses regarding human milk and nutritional plane, the optimal nutrition for preterm infants in this trial consisted of a combination of human milk and preterm formula. The human milk would have benefited the infant, partly by preventing infection and moderating systemic inflammation, and the preterm formula would have provided the nutrients that were required to promote growth and development. The results are consistent with this notion. Nowadays, this could be achieved using human milk with fortifiers, but these were not available at the time of the start of the trial.
Taken together, the results of the trial follow-ups also suggest that the optimal diet may depend on GA, birthweight, sex, and neonatal complications to name a few factors. The timing of neonatal complications may also affect the nutritional requirements. A much larger sample size would have enabled investigation of the optimal diet considering different neonatal risk factors, but even if this were achieved, the intricate biological underpinnings are unlikely to have been elucidated.

*Nutrition variables*

Although all infants in this trial were randomly allocated to a high or standard nutrient diet, the trial consisted of two separate studies. Infants in Sheffield and Norwich were allocated to TF or PTF, whereas those in Ipswich, Cambridge, and King’s Lynn to BBM or PTF. These studies were run in parallel, but cannot be regarded as a single randomised controlled trial.

Although a previous report showed that BBM and MBM were equally protective against NEC, indicating that at least some of the anti-infectious properties remained (Lucas and Cole, 1990), pasteurisation and freezing processes could have affected some of the bioactive components in the donor milk. Whilst it may be reasonable to group maternal and banked donor milk for some analyses, for others it may not. As it is difficult to tell exactly which bioactive factors in the BBM remained functional after the pasteurisation and freezing processes, their relative contributions to clinical endpoints cannot not be investigated.
As BBM was used as the standard diet in one arm of the study, infants allocated to the BBM diet always received 100% human milk. This resulted in a slight skew of the data towards the higher end of the human milk intake variable.

*Multiple hit hypothesis*

The multiple hit hypothesis suggests that the risk of abnormalities in people born preterm increases with cumulative exposure to multiple perinatal risk factors. Relating nutrition and infection separately to outcome whilst adjusting for other risk factors (GA, small for gestational age status, the number of days of ventilation) might underestimate their potential effect in combination with other risk factors. For example, the effect of GA on outcomes might be much smaller in those with appropriate birthweight than in those who were small for gestational age. Furthermore, those with low GA who were small for gestational age and had a high number of days of ventilation might have poorer outcomes than could be predicted from any of these factors alone. Recent research indeed suggests that lower GA at birth, fetal growth restriction, increased number of days requiring ventilation and parenteral nutrition, necrotizing enterocolitis and male sex each carry a separate risk which is additive to other risk factors (Barnett et al., 2018).
A term born control group was recruited for this adult follow-up to provide a standard to which preterm outcomes could be compared. Ideally, the control sample should be recruited at the same time as the preterm sample. Inclusion criteria were set to target term born individuals with similar characteristics to the preterm group. This study only included infants born between 1982 and 1985, so the age range at follow-up was inevitably very narrow. The control group was on average 2 years and 7 months younger than the preterm group, with a wider age range. However, effects of age are less prominent during this phase of life. Although brain structure is always changing, a degree of relative stability is observed during adulthood (Tamnes and Østby, 2018). The inclusion criteria of the control group were set at 25-40 years, because after 40 years of age, brain weight starts to decrease at a rate of approximately 5% per decade (Svennerholm, Boström and Jungbjer, 1997). To further reduce any confounding, age was included as a covariate in all analyses.

Only approximately a third of the control group was recruited via the preterm participants. Twenty percent of the control subjects was recruited via personal connections of the research team. Given their demographic characteristics, this could have introduced a bias towards younger, more highly educated people who live in and around London. However, the control and preterm groups did not show any significant differences in maternal education.
Since the term born control subjects were not recruited at birth, there was no data available about their neonatal history. Maternal education and birth history was reported by the participant, which is less reliable than the data that was prospectively collected during the trial for the preterm participants.

Suboptimally performing group

The method for identification of suboptimally performing individuals was novel, and has yet to be validated. As was noted in the discussion of chapter 6, subjects with very specific deficits and those who performed just within the normal range will not have been classified as suboptimally performing (SP), but may still experience difficulties in their daily lives. The fact that none of the term born subjects in this cohort falls into the SP category strengthens the notion that this is not simply the tail end of the normal distribution. The significant differences in white matter structure between the SP and normally performing (NP) preterm provide some indication that the classification is meaningful. The average IQ score of 115 does suggest that the control group was relatively high performing compared to the general population. The preterm group might therefore appear to perform worse compared to the norm than they would do in a more representative sample of society. Nevertheless, two standard deviations below the control mean remains a stringent selection criterium. Additionally, the SP group also perform significantly worse on cognitive tests, and show altered white matter microstructure compared to the NP group. This further confirms that this is a clearly distinguishable group.
Generalisability

The use of surfactants and improved ventilators, training of clinical staff, and improved standard of nutritional care are only a few of the advances that have occurred since the early 1980s that cumulatively have had a significant impact on the standard of care in neonatal units (Stoll et al., 2015). Nevertheless, the findings of this study can still be used to inform decisions about clinical practice. Even in more recent cohorts, preterm birth remains a risk factor for adverse developmental outcomes. Neonatal weight gain is still one of the most consistently recorded growth measures and is often used as a proxy for infant welfare. Neonatal infection and NEC are unfortunately still common in preterm infants. Although current preterm feeding recommendations advocate fortification, these are still based on formula, banked donor breast milk, and maternal milk.

11.4 Future studies

11.4.1 Nutrition

Theoretically, fortifying BBM could improve outcome in preterm infants by retaining the protective effect against infection and providing a high plane of nutrition. There is still no consensus about how and to what extent milk should be fortified, and the long-term effects on brain structure and cognition are not yet known.
There have been a number of trials investigating long-chain polyunsaturated fatty acids (LCPUFAs). These fatty acids are present in breast milk and are important for neural development. As preterm infants are unable to produce these types of fatty acids, they have to receive them via their diet (Schulzke, Patole and Simmer, 2011). LCPUFAs were not added to the formulas in this study since supplementation only started in the late 1980s, but they would have been present in human milk diets. LCPUFA is therefore one candidate component of human milk that could contribute to its beneficial effects on outcomes. However, results of studies on LCPUFA supplementation on cognitive and brain structural outcomes have been mixed and do not provide convincing evidence for longer term benefit (Schulzke, Patole and Simmer, 2011). As of yet there are no adult follow-up studies from the LCPUFA supplementation trials.

11.4.2 Infection

It is of course not possible to do a true randomised controlled trial with infection or NEC as the differential variable, but well-designed observational studies could be informative. These should be large in size, set up prospectively, have rigorous and consistent data collection, and use adequate diagnostic measures and definitions of infection and NEC. With a sufficiently large sample size, NEC and infection could be looked at separately. It would also be interesting to investigate whether the timing of infection has an effect on brain structure.
11.4.3 Multi-system

Future studies on preterm nutrition should include cardiovascular and metabolic outcomes in conjunction with brain outcomes. This would create a more holistic picture of the effects of preterm birth and enable a systems-level understanding of development. That, in turn, facilitates further investigation of risk factors and potential interventions.

11.4.4 MRI

This study has used state of the art imaging sequences, but MRI physics is a rapidly developing field. New acquisition sequences are continuously being developed, with increased contrast and decreased acquisition times. A promising new avenue for diffusion analysis that is likely to be sensitive to the changes observed in this study is fixel-based DWI analysis. This approach has already been shown to reveal microstructural and macrostructural abnormalities in infants born preterm, with increased sensitivity than traditional tensor metrics (Pannek et al., 2018).

11.4.5 Future studies with the collected data from this study

MRI scans

Although beyond the scope of this thesis, the MRI protocol in the current study included arterial spin-labelling (ASL) and functional resting state (rsfMRI) scans. ASL
can be used to study cerebral haemodynamics, and rsfMRI to study intrinsic functional networks. In addition, after processing the MPM scans, R1, R2*, and A maps were generated but not analysed.

*Multi-modal*

This study has shown that effects of premature birth and infection can be observed using images from multiple MRI modalities. Furthermore, each MRI modality can provide multiple contrasts, each representing different aspects of brain structure. A multivariate analysis technique could be used to understand combined variance of the different structural brain maps on a voxel-wise basis. This could show the relationships between the maps within one modality, for example the different diffusion maps, as well as between modalities. Using T1 and DWI scans could further elucidate the relationship between white matter damage and grey matter development. Combining ASL and DWI scans could show whether the white matter structural differences are associated with altered cerebrovascular function. If an association was found, the heart-brain axis in preterm born adults could be examined by looking at the cardiac MRI outcomes at age 25 in this cohort (Lewandowski et al., 2016).

The FLICA toolbox (Groves et al., 2011) or Multifactorial Causal Model of Brain (dis)Organization model (Iturria-Medina et al., 2017) could be used for this purpose. In preterm infants, a recent analysis using the FLICA toolbox showed independent
patterns of neuroanatomical variation that related to clinical factors (Ball et al., 2017). Results also confirmed the relationship between imaging markers of structural brain abnormality and poor cognitive outcome at 2 years. Interpretation of these data-driven approaches can be difficult, but the methods would enable further examination of the hypotheses and multimodal imaging data collected in this study.

Investigation of the relationship between structural and functional connectivity could shed light on possible compensatory mechanisms. White matter damage occurs early in life and the results of this study suggest that structural abnormalities persist into adulthood. People born preterm may develop different functional networks to compensate for structural damage (Papini et al., 2016). It would be interesting to investigate whether structural-functional coupling could be a biomarker of cognitive function. Comparison between suboptimally and normally performing preterm subjects would be informative, particularly between those who exhibit altered white matter microstructure but are in the normally performing group and vice versa.

*Longitudinal*

Chapters 7-10, briefly touched upon longitudinal analyses. However, as the main purpose of this thesis was to assess brain structure and function in adulthood, the longitudinal data were not considered in quantitative analyses. Instead, results were interpreted in the context of those reported previously. If IQ scores from all the previous visits were incorporated, it would be possible to investigate IQ trajectories.
Trajectories could then be used to identify neonatal factors that predict the rate and level of development. Furthermore, there are longitudinal MRI data that could be used to examine changes in brain volume over time. However, only 21 subjects in the present study took part in all the previous follow-ups and only a subset had MRI scans at these timepoints. Further, the scans were acquired at different scanners with different field strengths, which complicates longitudinal modelling.

11.5 Summary

Adults born preterm achieved lower scores on cognitive tests of IQ, executive functioning, visuospatial and verbal memory, mental arithmetic, and language compared to term born controls. They also showed widespread reductions in grey matter volumes, but limited differences in white matter properties.

Within this preterm sample, there was wide variation in outcome. Preterm subjects who performed suboptimally showed widespread reductions in white and grey matter volumes as well as in markers of white matter integrity. Affected grey matter structures included the cerebellum, left thalamus, hippocampus, and putamen. Interestingly, there were no differences between the normally performing preterm subjects and controls in ventricular or white matter volumes or in white matter integrity. This suggests that whilst white matter is not affected in all preterm subjects, its quality is pertinent to normal functioning in adult life.
Thanks to detailed hospital and trial records from the neonatal period, various candidate risk factors for cognitive and brain outcomes could be investigated. Neonatal infection/NEC significantly increased the odds of being in the suboptimal performance category. Individuals with neonatal infection/NEC showed evidence of reduced white matter quality and cognitive performance in adulthood. Although there was little evidence for a direct relationship between neonatal human milk intake or nutritional plane and adult outcomes, this may have been due to insufficient statistical power. Data from the neonatal phase of this trial showed that the odds of neonatal infection/NEC could be significantly reduced by increasing neonatal human milk intake.

Nutritional plane was associated with higher growth rates and weight gain, but a direct relationship between these and cognitive or brain outcomes in adulthood could not be established. Novel MRI analyses techniques could be employed to further elucidate the subtler effects of neonatal factors and nutrition on brain development.
12 Conclusion

The results from this thesis demonstrate that the effects of preterm birth persist into adulthood. This is the first study to show widespread reductions in fibre coherence in adults born preterm compared to term born controls, and the first to report lower grey matter volumes. Despite these brain structure alterations, the majority of participants born preterm perform in the normal range. Preterm participants who perform suboptimally exhibit altered white matter structure and volume. The results underscore the utility of the SMT diffusion model as a valuable tool for characterisation of white matter microstructure. SMT outperforms the traditional tensor model in its ability to differentiate between preterm and control groups as well as normally performing and suboptimally performing individuals.

In addition to characterising the cognitive abilities and brain structure of people born preterm in adulthood, this thesis also considers the long-term effect of neonatal factors. Particular attention is paid to neonatal nutrition, as this may be an important target for intervention to improve outcomes in preterm infants.

The results do not indicate persisting beneficial effects of a high nutrient diet across the preterm sample as a whole. A high nutrient diet may however be effective in a sub-sample of infants of very low GA and/or very low birthweight. By contrast, there does appear to be a long-term benefit of increased human milk intake at the sample level. The direct effects on cognition and brain structure in adulthood are small, but a consistent positive effect is observable across outcome measures. Increased intake of human milk is associated with a reduced rate of infection/NEC. Altered white matter microstructure associated with infection/NEC is detectable in adulthood and related to cognitive dysfunction.
Taken together, the results of this thesis further underscore the importance of an uncomplicated neonatal period for the development of preterm infants. Developmental outcomes may be improved by providing optimal nutrition. Further work should focus on understanding and exploiting the protective effects of human milk.
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13 Supplementary Material

Questionnaire questions

What education had your mother completed when you were born? *

- No educational qualifications
- less than 5 CSE’s
- 5 or more CSE’s plus one or more 0-levels
- A-levels and post-school training (including nurses)
- A-levels and professional training or university degree

What were your parents' jobs when you were born?

How many years of education have you completed? *

What is the highest level of formal education or school you have completed? *.

- No school
- Primary school
- Some secondary school
- Finished secondary school
- Trade training
- University (undergraduate)
- University (postgraduate)

What is your current or last main job? Please provide a detailed description of the kind of organisation you work(ed) for, your everyday responsibilities, and whether it is/was part-time or full-time. *
What is your household gross income (before tax) per year?

- Less than £13999
- £14000-£18999
- £19000-£25999
- £26000-£37999
- £38000-£49999
- More than £50000

How many people are there in your household? (number of people that live from your household gross income)

Please write down any health conditions, illnesses or disabilities you have or had in the past and how long you (have) had them for and what medication you have taken or treatment you have received.

Do you smoke? *

- I currently smoke
- I smoked in the past
- I have never smoked

If you smoke or have smoked, how many cigarettes a day on average?

Were you born preterm (at less than 37 weeks)? *

- Yes
- No

At how many weeks of gestation were you born? Normally pregnancy lasts 40 weeks
Birth weight

What type of milk did you receive during the first four weeks of life?

- Breast milk at least 90% of the time
- Formula at least 90% of the time
- A combination of breast milk and formula
- I don't know

Taking everything into consideration, during the past week how satisfied have you been with your ......

(1=very poor, 5=very good)

physical health? *
mood?
work?
household activities?
social relationships?
family relationships?
leisure time activities?
ability to function in daily life?
sexual drive, interest and/or performance?
economic status?
living/housing situation?
ability to get around physically without feeling dizzy or unsteady or falling?
your vision in terms of ability to do work or hobbies?
overall sense of well being?
medication? (leave blank if you’re not taking any)

How would you rate your overall life satisfaction and contentment during the past week?