

Chapter 1

Introduction, aims and outline of thesis



INTRODUCTION

An optimal intrauterine environment is vital for normal fetal development. Adverse prenatal circumstances such as uteroplacental insufficiency can interfere with normal development. Irrespective of its cause, the effects of fetal malnutrition can extend into adulthood (Barker 2006, Swaab 2010). Striking examples include the association between lower birth weight and long-term cardiovascular and metabolic diseases in offspring of mothers who suffered from the famine during the 2nd world war (Dutch “hunger winter”) (Roseboom *et al.*, 2011; Painter *et al.*, 2005; Roseboom *et al.*, 2000). The most common cause of intrauterine growth restriction (IUGR) in Western countries is uteroplacental insufficiency, which is often caused by pregnancy-induced hypertension (Henriksen and Clausen 2002; Kingdom *et al.*, 2000).

Children with IUGR can be identified prenatally with serial ultrasound measurements (Bertino *et al.*, 2007). When serial ultrasounds have not been performed during pregnancy, a growth-retarded child is identified at birth by a small size (Bertino *et al.*, 2007). When the birth weight and/ or length of a child is too low for its gestational age, the child is considered small-for-gestational-age (SGA). However, having experienced IUGR does not always mean that the child is born SGA.

Being born SGA is associated with increased neonatal mortality and morbidity (Clayton *et al.*, 2007), short stature, cardiovascular disease, insulin resistance, diabetes mellitus type 2, dyslipidaemia and end-stage renal disease. In addition to a negative influence on these physical and metabolic parameters, decreased levels of intelligence and cognition have been described in children born SGA.

Recent advances in non-invasive functional neuroimaging techniques have enabled investigating brain-behaviour associations *in vivo*. During the last decade, techniques such as structural Magnetic resonance Imaging (sMRI), functional MRI (fMRI) and Magneto-Encephalography (MEG) have considerably increased our knowledge on human brain structure and function in both healthy individuals and clinical populations.

In this thesis, neuroimaging techniques will be used to investigate brain structure and function in children born SGA.

Definitions

Based on birth weight and length, children can be defined as SGA, appropriate-for-gestational-age (AGA) or large-for-gestational-age (LGA) when birth size is below, in between or above predefined cut-offs using population-specific growth charts (Clayton *et al.*, 2007). In the Netherlands, cut-off limits of \leq or \geq 2 standard deviations (SD) are used to define SGA and LGA, respectively (Niklasson *et al.*, 1991; Lee *et al.*, 2003). Accurate gestational dating and measurement of birth weight and length are crucial for identifying children who are born SGA. In 2010 in The Netherlands, 184,397 infants were

born alive and approximately 4,000–4,500 of them were born SGA (Central Bureau of Statistics, Voorburg, the Netherlands).

These children can be subdivided into symmetric or asymmetric growth restriction, depending on whether weight, length and head circumference at birth are equally reduced. 85-90% of all children born SGA exhibit postnatal catch-up growth to a height above -2 SD (SGA with postnatal catch-up growth, SGA+). The remainder (i.e. 10-15%) of them remain short (short-statured SGA, SGA-) (Hokken-Koelega *et al.*, 1995; Albertsson-Wikland *et al.*, 1998). Catch-up growth is most pronounced during the first 6 postnatal months and is completed before the age of 2 years in those born at term. In children born prematurely, catch-up growth may take up to 4 years (Hokken-Koelega *et al.*, 1995; De Ridder *et al.*, 2008), or even longer.

Long term outcome of children born SGA

Metabolic consequences and other long term sequelae

Being born SGA is associated with cardiovascular disease and the metabolic syndrome comprising hypertension, dyslipidaemia, and impaired glucose tolerance or diabetes mellitus (Clayton *et al.*, 2007). Interestingly, these metabolic disturbances have mainly been observed in adults who showed spontaneous catch-up growth as children. Impaired reproductive function, reproductive tract anomalies, premature adrenarche, obstructive airway disease, visual impairment, sensorineural hearing loss, liver cirrhosis and abnormal bone development have also been associated with being born SGA.

Hypotheses

Several hypotheses have been formulated to explain long-term cardiovascular and metabolic consequences of being born SGA. For all these hypotheses, studies supply supporting information and at this moment, we do not know which of these hypotheses is the most valuable.

- The Barker hypothesis (fetal origins of adult disease hypothesis) postulates that the risk of developing chronic diseases in adulthood in low birth weight populations is programmed by malnutrition in utero (Barker and Osmond 1986). In response to adverse prenatal circumstances, the fetus adapts by changing its metabolism, altering its production of hormones and the sensitivity of tissues to these, redistributing its blood flow, and slowing its growth rate. Adaptations to malnutrition that occur during development thus permanently alter the structure and function of the body and will result in an increased risk for developing adult diseases.
- In 1999, Hattersley formulated the fetal insulin hypothesis (Hattersley and Tooke 1999). This hypothesis proposes that the association between low birth weight and adult disease is genetically mediated. Parental genes involved in insulin (important

growth factor in utero) resistance pass the placenta and can result in impaired fetal growth, insulin resistance and development of type 2 diabetes mellitus in childhood/adulthood.

- Singhal and Lucas formulated the growth acceleration hypothesis (Singhal and Lucas 2004), stating that not low birth weight per se but subsequent adverse effects of early rapid postnatal growth are responsible for the increased risk for diseases later in life.
- Leunissen and co-workers proposed a modified growth acceleration hypothesis, the fat accumulation hypothesis (Leunissen *et al.*, 2008). Rapid weight gain during the first three months of life with a higher body fat percentage is associated with cardiovascular disease in adulthood.

Intelligence and cognition

In addition to a negative influence on these physical and metabolic parameters, there is substantial evidence that this group of children is at risk for an inferior neuropsychological outcome with lower intelligence, lower cognitive abilities and behavioural problems (Lundgren *et al.*, 2001; Strauss 2000). The nature and severity of these intellectual and cognitive vulnerabilities differ widely between study populations and the overall outcome is the result of a complex interaction between intrauterine and extrauterine factors (Noeker 2005). For instance, prematurity itself can be an additional risk factor (van Baar *et al.*, 2009) for inferior outcome on intelligence and cognition in prematurely born SGA children compared to SGA children born at term (Gimenez *et al.*, 2004). Moreover, postnatal catch-up growth of both head circumference and body height is associated with better neuropsychological outcome (Frisk *et al.*, 2002; Geva *et al.*, 2006b; Lundgren *et al.*, 2001). Growth hormone (GH) therapy for children born SGA with persistent short stature, with the intention to increase the bodily catch-up growth, has boosted the research on cognitive function in children born SGA and its relation with growth hormone therapy (Lagrou *et al.*, 2007; Van Pareren *et al.*, 2004).

Growth hormone therapy

Short children born SGA can be treated with GH according to the International Small for Gestational Advisory Consensus Board Development Conference Statement (Lee *et al.*, 2003). Studies have demonstrated a beneficial effect of GH treatment on catch-up growth in short children born SGA. In contrast, conflicting results exist regarding an effect of GH treatment on intelligence and cognition in these children. Interestingly, one study in The Netherlands described a positive effect of GH therapy on intelligence and attention scores in short children born SGA (Van Pareren *et al.*, 2004). After 8 years of GH treatment, estimated IQ scores of SGA children had increased 5–10 IQ-points and remained in the normal range ($90 < IQ < 110$). In this group, the change in head circum-

ference was related to the improvement of estimated IQ scores during GH treatment. These findings are conflicting with the results of a cohort of children born SGA from Belgium (Lagrou *et al.*, 2007). In a randomized controlled trial, no beneficial effect of GH treatment on IQ scores could be observed after 2 years of treatment in spite of a clear effect of GH therapy on head circumference. Future long-term studies must reveal whether GH therapy can influence intellectual functioning in children born SGA. Given the observed association between change in head circumference and increase in intelligence scores, it would be interesting if research on this topic could be expanded with studying the brain of children born SGA before start of and during GH treatment.

Brain structure and function in children born SGA

The observed decreased intellectual and cognitive abilities in SGA children raise the question whether impaired cognitive function is associated with a different brain architecture or neural substrate/ brain function in children born SGA. Furthermore, it would be interesting to determine whether the outcome differs between SGA children with and without postnatal catch-up growth. Until now, our knowledge on brain anatomy and function in human SGA populations is very limited and based on one histopathological post mortem study and a few structural MRI (s-MRI) studies (Chase *et al.*, 1972; Borradori-Tolsa *et al.*, 2004; Martinussen *et al.*, 2005; Martinussen *et al.*, 2009; Skranes *et al.*, 2005). In contrast, many animal studies have been published (both histopathological and imaging research) investigating the effect of low birth weight on brain architecture (Hayakawa *et al.*, 1999; Lister *et al.*, 2005; Lister *et al.*, 2006; Mallard *et al.*, 2000; Mallard *et al.*, 1998; Mallard *et al.*, 1999; Rees *et al.*, 1988; Rehn *et al.*, 2004). These studies consistently demonstrate an underdevelopment of the brain. The medial temporal lobe, including the hippocampus has been of specific interest because of its key role in cognition/memory. The brain has a particular vulnerability to hypoxia, stress hormones and malnutrition resulting in reduced hippocampal volumes in premature IUGR neonates (Lodygensky *et al.*, 2008; Sizonenko *et al.*, 2006). Moreover, lower hippocampal volumes in IUGR children have been related to inferior functional behavioural outcome at term equivalent age (Lodygensky *et al.*, 2008). Unfortunately, no functional MRI studies investigating the neural substrate of associated cognitive impairments exist. Importantly, there is increasing evidence that higher brain functions such as various aspects of cognition depend on the integrated activity of various distributed brain regions instead of discrete regions. Therefore, growth and differentiation of the neural network as well as synchronization of neural activity may play an important role in higher brain functions and has not been investigated yet in SGA populations (Thompson and Varela 2001; David *et al.*, 2002).

Neuroimaging

To investigate human brain development several research modalities are available. Neuroimaging techniques such as MRI and MEG are valuable tools for investigation of brain structure and function.

MRI

With MRI it is possible to investigate brain anatomy and volume with exquisite anatomic detail. Functional MRI (f-MRI) enables to investigate brain-behaviour associations. Task related f-MRI measures changes in blood oxygenation levels following activity of neurons. This is called the blood oxygenation level dependent (BOLD) effect (Logothetis *et al.*, 2001; Raichle 2001). Due to its non-invasive nature f-MRI has permitted new investigations across typical and atypical development in adult as well as pediatric populations. Advanced techniques such as resting state functional connectivity (rs-fc) MRI have further expanded our insight in brain function. Rs-fc MRI investigates functional connectivity between various distributed regions in the brain by studying time courses of low frequency fluctuations of the BOLD signal across the brain in the absence of salient stimuli or a cognitive task (Biswal *et al.*, 1995; Fox and Raichle 2007).

Electroencephalography (EEG) and Magneto encephalography (MEG)

EEG and MEG are neuroimaging techniques with very good temporal resolution. With these techniques it is possible to investigate oscillatory brain activity that is assumed to originate from large neuronal networks that synchronize their activity in the brain areas underlying the sensors. An advantage of MEG over EEG is that it is more child-friendly, since it contains a helmet with inbuilt sensors, obviating the need to of glued electrodes which can be a source of distress for children. Moreover, the MEG scanner has a 151-sensor array resulting in higher spatial resolution and it is insensitive to the effects of skull-thickness and skin conductance. MEG is particularly useful for studies of the functional connectivity in the brain. Simultaneous recording of MEG and fMRI scans, and thereby combining both a high spatial and temporal resolution, has been done although interpretation of data is still hampered by technical difficulties associated with combining these two modalities.

Neuropsychologic evaluation of intelligence, cognition and behaviour

Intelligence comprises a set of abilities to understand, learn and apply knowledge and can be expressed in terms of an intelligence quotient (IQ). Intelligence scores can be estimated using standardized intelligence tests such as The Wechsler's scales and Raven's Matrices. Cognition refers to mental processes and can be discerned in the following cognitive domains: speech and language, visuospatial and visuoconstructive skills, learning and memory, attention, and executive functions such as planning,

problem-solving and self-monitoring. Cognitive functioning can be investigated applying neuropsychological tests (NPT) that each pertain to a (combination of) specific cognitive functions. Behaviour refers to action in conjunction to your environment and is regulated by means of social norms and control. Children's behaviour, and the acceptability of it, is evaluated by parents and teachers questionnaires.

Challenges when scanning young children

Several challenges arise when performing research in pediatric populations and specifically in MRI research. The design and dimensions of most MRI systems can be intimidating (huge machine, loud noise, and narrow bore), especially for young children (Bunge and Wright 2007; Davidson *et al.*, 2006; Westra *et al.*, 2011). Together with a lack of comprehension, this may induce anxiety and distress, resulting in poor or non-adherence and excessive movement. MRI scans, and especially fMRI scans, are highly sensitive to movement artefacts. It is difficult to obtain scans of good quality in young children, and sedation or general anaesthesia is used in most patients under 7 years of age undergoing MRI investigation in a clinical setting (Cote *et al.*, 2000; Lawson 2000; Smart 1997). Ethics committees in the Netherlands do not allow the use of sedation procedures to perform MRI in a non-therapeutic research setting in pediatric populations. Besides ethical concerns, task-related fMRI requires an awake and cooperative participant which precludes sedation. Therefore, optimal preparation and guidance of children by qualified personnel is a prerequisite to successfully perform research in young pediatric population to guarantee well-being of each participant.

AIMS AND OUTLINE OF THESIS

This thesis is part of a longitudinal study on effects of GH on brain development and cognition in children born SGA using structural and functional MRI, MEG and neuropsychological testing. (Dutch Trial Register: NTR 865). In this thesis, baseline MRI and neuropsychology data will be presented. Longitudinal data are not yet available and therefore beyond the scope of the current thesis.

The following research questions were formulated:

1. What is the current knowledge on brain anatomy and cognitive functioning in children born SGA? (Review of the literature, Chapter 3).
2. Can a mock scanner training protocol be used for preparing young children for magnetic resonance imaging in a research setting? (Chapter 4).
3. Does brain structure differ in (4-7 year old) SGA children in comparison with children born AGA? (s-MRI, Chapter 5).

4. Does brain function differ in (4-7 year old) SGA children in comparison with children born AGA? (rs-fc MRI, task related f-MRI, Chapter 6, 7).
5. Do intelligence, cognitive functioning and behaviour differ in (4-7 year old) SGA children compared to AGA children? (neuropsychological testing, Chapter 8).
6. Do SGA+ and SGA- children differ with respect to brain structure, function and intelligence & cognition? (s-MRI, task-related fMRI, NPT, Chapter 5, 7, 8).

