PHYSICAL AND PSYCHOLOGICAL DEVELOPMENT OF HEALTHY INFANT TWINS

THE BIRMINGHAM REGISTRY FOR TWIN AND HERITABILITY STUDIES

by

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ABSTRACT

Twins often show signs of physical and cognitive developmental delays compared with singletons at school age, but tend to catch up by young adulthood. Many previous studies focused on early childhood and beyond, and twins with health issues. In this thesis, I investigated the infancy period of healthy twins in the Birmingham Registry for Twin and Heritability Studies. More specifically, I explored twins' developmental trajectories compared with singleton standards; ante-, peri-, and postnatal factors related to developmental skills; and the association of maternal occupation with twin pregnancy outcomes. Additionally, I studied the heritability of body mass index (BMI) over a lifespan in a meta-analysis of twin studies.

Twins had worse developmental skills and were small for their age compared with singleton standards. Birth weight was not strongly associated with developmental skills as in previous studies; however, larger antenatal head circumference was negatively associated with postnatal development. Higher occupational psychological strain was related to shorter gestations. Finally, heritability of BMI remained high over a lifespan.

Results were discussed in light of current clinical and health and safety guidelines. Suggestions for further research and dissemination of findings to parents of multiples were also discussed in the final chapter of this thesis.

PUBLICATIONS ARISING FROM THIS THESIS

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To those who believed that

little girls can have big dreams

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Cassandra

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LIST OF ABBREVIATIONS

A	additive genetic influence
ART	assisted reproductive technique
ASQ-3	Ages and Stages Questionnaire (3rd edition)
BCH	Birmingham Children's Hospital
BHH	Birmingham Heartlands Hospital
BiRTHS	Birmingham Registry for Twin and Heritability Studies
BMI	body mass index
BWH	Birmingham Women's Hospital
C	common environmental influence
CAATSA	Carolina African American Twin Study of Aging
CI	confidence interval
City	Birmingham City Hospital
cm	centimetre
DZ	dizygotic
E	unique environmental influence
EEG	electroencephalogram
EFPTS	East Flanders Prospective Twin Study
EPDS	Edinburgh Postnatal Depression Scale
FTC	Finnish Twin Cohort
HC	head circumference
IPD	individual participant dataset
IQ	intelligence quotient
IQR	Interquartile range
ISCO-88	International Standard Classification of Occupations
IVF	in vitro fertilisation
(k)g	(kilo)gram
LLTS	Leuven Longitudinal Twin Study
MBR	Medical Birth Registry
MET	metabolic equivalent
MGR	Multi-Generation Register
mm	millimetre
MTR	Murcia Twin Register
MTR & MTSADA	Minnesota Twin Registry & Minnesota Twin Study of Adult Development and Aging
MZ	monozygotic
N	sample size
NHLBI	National Heart, Lung and Blood Institute Twin Study
NTR	Netherlands Twin Registry
SD	standard deviation
SDS	standard deviation score
Tamba	Twin and Multiple Births Association
TEDS	Twins Early Development Study

CHAPTER 1

INTRODUCTION

"All that is valuable in human society depends upon the opportunity for development accorded the individual." – Albert Einstein

Development, whether it is physical or intellectual, is a result of our individual potential and the opportunities that we have been given. For example, our individual potential to obtain a certain height is mostly determined by genes that have been passed down to us from our parents[1, 2], however, nutrition will also influence our physical growth[3]. Many traits, including cognitive development, physical growth and disease are a result of the interplay between genetic predisposition and environmental influences[4] or opportunity[5]. Currently, the trend in research is to find risk factors for negative physical and mental health outcomes at the earliest possible stage. This has resulted in studies that have looked into intrauterine conditions and perinatal outcomes, which will be discussed in §1.5. Results from previous studies are mostly based on children with poor intrauterine growth[6, 7], or other comparable groups at risk for unfavourable physical and mental health outcomes[8, 9]. However, there is a group of children, which is consistently excluded from these studies. This group consists of healthy twins and higher multiples.

Twin and higher multiple pregnancies generally result in offspring with lower mean birth size and are at higher risk for a variety of complications compared with singleton pregnancies[10], which will be discussed in later paragraphs in this chapter. Although their prematurity and small birth size may be appropriate for multiple births, twins may still be at risk for the same complications as singletons with comparable gestational age and birth size.

Very little is known about the causes of ante- and perinatal risk factors for physical growth and developmental skills, or how postnatal physical growth affects psychological development in multiples. This thesis will therefore outline physical growth and developmental skills of healthy multiples, focusing on twin pregnancies, as this is the most common type of multiple pregnancies. I will also investigate genetic and environmental influences on body mass index in relation to the current obesity epidemic. Finally, I will provide clinically relevant conclusions and suggestions for implementation of the findings in clinical practice.

In the following paragraphs, I will describe the incidence of twinning, the different types of twins and complications in twin pregnancies to illustrate the importance of this study. There will be a brief description of what is already known about ante- and postnatal growth and psychological development. Detailed background information on each topic in this thesis will be provided in each individual results chapter (Chapters 4-8). I will explain the principles of the twin design, which is used to estimate genetic and environmental influences (Chapter 8). This introductory chapter will be concluded with an outline of the overarching hypothesis for this thesis and a brief summary of the sub-questions, which will be considered in following chapters.

Chapter 2 then provides a description of the protocol of the Birmingham Registry for Twin and Heritability Studies (BiRTHS), from which data was derived for this thesis. A full description of data management and the characterisation of the sample can be found in Chapter 3.

The first of the results chapters (Chapter 4) will provide a description of how perinatal outcomes influence developmental skills in the first two years of life. Next, results will be presented for the influence of antenatal head circumference on developmental skills in infancy (Chapter 5), followed by a study of the influence of postnatal growth on developmental skills (Chapter 6). I will then provide an outline of maternal occupational influences on perinatal outcomes (Chapter 7). To elaborate more on how these genetic and environmental influences can change over a lifespan, I will conclude the results section of this thesis with a chapter, which describes the heritability of body mass index (BMI) in three age categories (Chapter 8). Each results chapter will have its own discussion at the end of the chapter. This thesis will therefore be concluded with a general summary and discussion of the findings and implications for future studies and clinical practice (Chapter 9).

1.1 Twinning

Twinning occurs when one fertilized egg or zygote (from the Greek ζυγωτός zygōtos=joined) splits and develops into two foetuses, resulting in a monozygotic twin pair (=from one zygote), more commonly known as identical twins[11]. Alternatively, a fraternal or dizygotic twin pair is the result of two zygotes.

Approximately 1 in 100 natural pregnancies is a dizygotic twin pregnancy[12]. Monozygotic twinning happens in about 1 in 300 natural pregnancies[11]. These numbers have increased as assisted fertility treatments have become more and more accessible to parents who are unable to conceive on their own[13]. To increase the chances of having a viable pregnancy, it is common that two fertilized eggs are replaced into the uterus. Sometimes both eggs develop into embryos, which results in a multiple pregnancy. In rare occasions, one viable egg will split and develop into a monozygotic twin pair.

Other factors that increase the chances of twin pregnancies are family history, ethnicity, increasing maternal age and fertility drugs[13]. Family history of multiple pregnancies mostly applies to dizygotic twin pairs, as monozygotic twins are considered a chance happening[11]. In recent years, it has become more common for women to have children at older ages. The chance of having twins significantly increases after the age of 35[13].

1.2 Types of twins

The distinction of monozygotic and dizygotic twin pairs is often used in conversation. However, another distinction is considered more relevant in obstetric care, namely that of chorionicity and amnionicity.

The outer membrane that surrounds the foetus is the chorion, which is connected to the placenta through the umbilical cord and allows for transfer of nutrients from the mother to the foetus[11]. The amnion lies within the chorion and is filled with amnionitic fluid that protects the foetus. Chorionicity and amnionicity can be determined by ultrasound scan, where sonographers can determine whether there are one (mono-) or two (di-) chorions and amnions surrounding the twins. Following this, a distinction can be made between monochorionic-monoamniotic, monochorionic-diamniotic and dichorionic-diamniotic twins. Dizygotic twins are always dichorionic-diamniotic, however, monozygotic twins could be monochorionic-monoamniotic, monochorionic-diamniotic or dichorionic-diamniotic. This determination is dependent on the timing of division of the fertilized egg. The majority of monozygotic twins share one placenta, but have separate amnions (monochorionic-diamniotic), which is a result of the egg splitting between 4 to 7 days after fertilization. When the egg splits within 3 days of fertilization, it results in a dichorionic-diamniotic pregnancy with two separate placentae, which occurs in about 25-30% of twin pregnancies. Only about 1-2% of monozygotic twins are monochorionic-monoamniotic[10], a result of the fertilized egg splitting in the second week. A schematic overview of the different chorion and amnion types can be found in Figure 1.1 below.

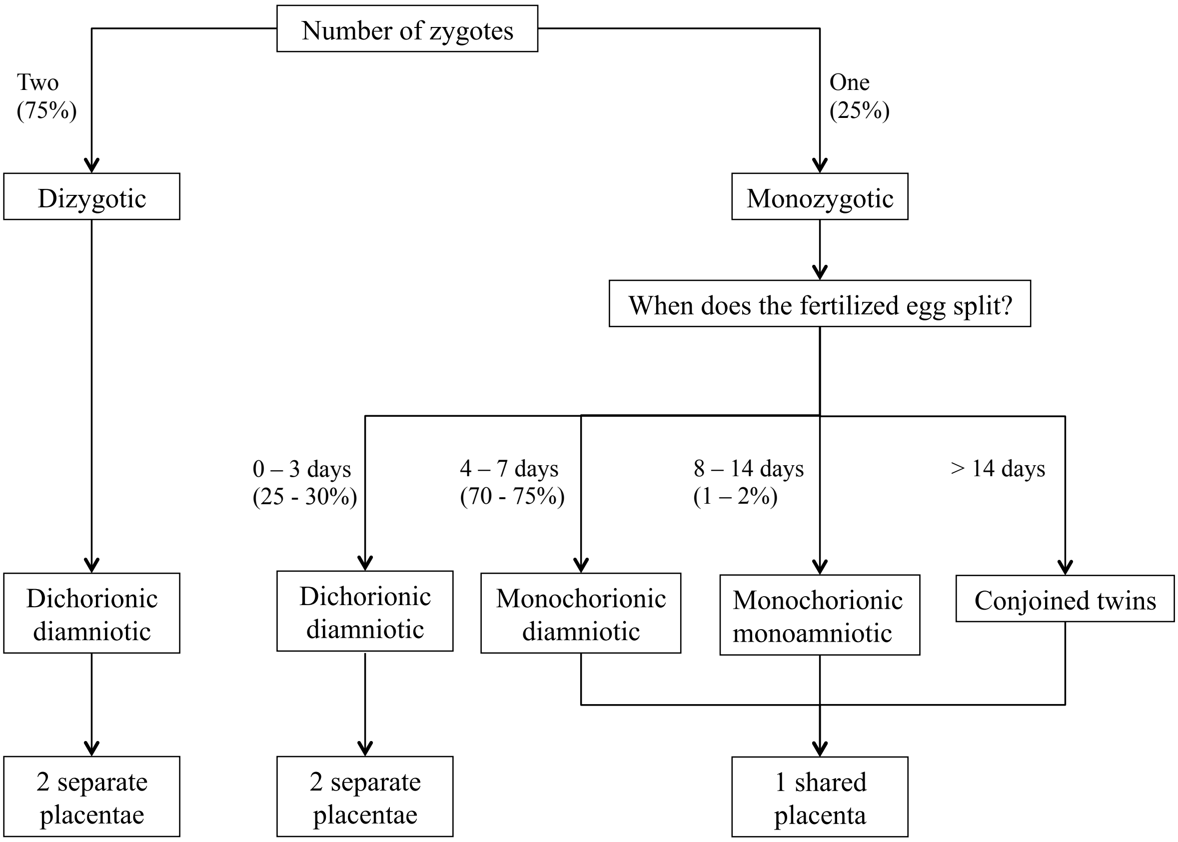


Figure 1.1 Overview of types of twins based on chorionicity and amnionicity with their respective incidence rates.

1.2.1 Determining zygosity

Zygosity can be determined in several ways. The most reliable is DNA testing. Monozygotic twins have the same genetic make-up, whereas dizygotic pairs only share about 50% of their DNA like regular siblings. Validated zygosity questionnaires are also available as a cost-efficient alternative to DNA testing[14]. Other ways of determining zygosity are by chorionicity and gender, as monochorionic pairs are always monozygotic, and therefore of the same gender. Dichorionic-diamniotic can unquestionably be classed as dizygotic when co-twins are not of the same gender. However, if they are of the same gender, it is impossible to determine zygosity based on chorionicity, and another method will need to be considered. Zygosity determination methods used in this thesis will be outlined in Chapters 2-3.

1.3 Obstetric complications in twin pregnancies

It is commonly known that twin pregnancies differ from singleton pregnancies in that they have higher rates of complications such as intrauterine growth restriction[15], intrauterine demise[10], preeclampsia, anaemia and preterm delivery. Another important difference is that of size for gestation, whereby twins are often smaller. This will be discussed in detail in §1.5.

Besides differences with singleton pregnancies, there are also specific differences between the various types of twins. Monochorionic pregnancies are generally more complicated than dichorionic pregnancies, with shorter gestations and an increased risk of intrauterine mortality not explained by prematurity[16]. Intrauterine growth retardation is equally common in monochorionic as in dichorionic pregnancies, however, the neonatal outcome is significantly worse in monochorionic pregnancies[17]. Intrauterine growth retardation in monochorionic pregnancies has been related to significantly lower gestational age at birth, lower birth weight and higher rates of intrauterine demise. Rates of foetal death are higher in monochorionic pregnancies from 24 weeks onwards[18]. A complication that is specific to monochorionic twins is twin-to-twin transfusion syndrome[10, 19], which occurs in about 15% of monochorionic twin pregnancies. This is a condition in which one twin 'steals' nutrients from the other due to umbilical cord abnormalities, causing an imbalance in blood flow. This can be detected by ultrasound scanning as the donor twin will show signs of insufficient growth and is surrounded by very little amniotic fluid (oligohydramnios), whereas the recipient twin will have an overload of amniotic fluid (polyhydramnios)[20]. This is a life threatening condition for both foetuses, with mortality rates ranging between 60% and 100%. A common method of treatment is to separate the arteries by means of laser surgery.

1.4 Management of twin pregnancies

Twin pregnancies normally fall under consultant care instead of midwifery led care. The following outline is based on the protocol that is currently used at the specialised antenatal clinics at Birmingham Women's Hospital[20], Birmingham Heartlands Hospital and Birmingham City Hospital.

Booking takes place around 10 weeks of gestation, similar to singleton pregnancies. Monochorionic pregnancies are seen fortnightly for scans and clinic consultations from booking onwards, in order to detect any signs of twin-to-twin transfusion syndrome as early as possible. Dichorionic pregnancies require monthly appointments from 24 weeks onwards. The traditional triple test for Down's syndrome is not possible in twin pregnancies, because it would be impossible to tell which result belongs to each baby. Therefore, a nuchal translucency test is offered between 10 and 14 weeks. A detailed mid-term scan is performed at 20 weeks for all pregnancies. These scans include foetal growth measurements as well as detailed measurements of foetal wellbeing and functioning of organs. Delivery plans are discussed around 34 weeks for monochorionic pregnancies, and 36 weeks for dichorionic pregnancies. There is some debate about the optimal gestation at which twins should be delivered[21, 22]. This ranges from caesarean sections at 34 weeks for monochorionic twins to natural delivery at 40 weeks. However, the preference at the involved hospitals is to deliver by 38 weeks. Natural delivery is encouraged where possible, although parents may choose for an elective caesarean section. Obviously, complications that could compromise health of the mother or the babies are an indication for deviations from the planned timing and mode of delivery.

1.5 Development of twins

1.5.1 Antenatal growth

As stated in §1.3, birth weight and antenatal or intrauterine growth in twins differ from measurements in singletons. Antenatal growth of twins generally follows the same pace as singletons, but slows down around 32 weeks of gestation[23]. Some studies have even found deviating growth patterns as early as 28 weeks[24]. This deviation leads to smaller size at birth.

It has been hypothesised that lack of intrauterine space to grow and placental inability to keep pace with the nutritional needs of two growing babies are the underlying contributing factors[19]. These twin specific causes of reduced intrauterine growth cannot be easily solved if at all. However, aside from the obstetric risks described in §1.3, antenatal growth in twin pregnancies is also affected by the same environmental risk factors as in singleton pregnancies, for which recommendations can be made. Maternal smoking, both active[25] and passive[26], has been related to intrauterine growth retardation[7, 27] and altered placental appearance[28]. It has even been associated with higher risk of obesity in adulthood[29]. Decreased exposure to cigarette smoke has been shown to reduce intrauterine growth retardation[26]. Maternal alcohol consumption[30] and substance abuse[31], and exposure to chemicals[32] during pregnancy have been found to have similar effects on foetal growth and development.

Evidently, poor antenatal growth results in low birth weight and could be related to low Apgar scores, complex deliveries and higher rates of admissions to special care wards[8]. These perinatal outcomes are often used in research as a proxy for unfavourable intrauterine developments and are described in the following paragraph.

1.5.2 Perinatal outcomes

Investigations into the consequences of low birth weight, as a measure of insufficient intrauterine growth, have resulted in more knowledge about immediate obstetric outcomes. Low birth weight has been related to higher rates of neonatal mortality and morbidity, including Apgar scores <4, assisted ventilation and neonatal seizures[15]. Furthermore, low birth weight has been associated with obesity[33], hypertension and type II diabetes[34, 35] later in life. The effects of low Apgar scores extend to cognitive problems at school age[36], while delivery by caesarean section has been linked to later respiratory problems[37]. Of the many maternal factors, nutrition has not been found to have a significant effect on placental weight or birth weight[38]. Maternal stress and stressful life events, on the other hand, have been found to have a negative effect on gestational age and birth weight[39, 40]. Evidence has also been found for prolonged effects of maternal stress, which were expressed as delayed mental development[41], behavioural and temperament problems in early life[39].

Maternal occupation is a contemporary environmental factor, which has been studied in relation to perinatal outcomes. Results remain inconclusive and difficult to compile, because the classification of occupation largely varies from physical activity and psychological stress to environmental exposure. However, it can generally be concluded that an excess of physical activity and exposure to hazardous environments have a negative effect on pregnancy outcomes[42]. Psychological stress specifically related to work has not been found to have a significant effect on pregnancy outcome in many studies[6, 43, 44]. The added physical and mental burden of a twin pregnancy[45] lead us to believe that smaller amounts of occupational physical activity and stress will already have an effect on pregnancy outcomes compared with singleton pregnancies. The fact that the modern woman is likely to continue working during her pregnancy provides a good reason to investigate the effect of maternal occupation on twin pregnancy outcomes (Chapter 7).

1.5.3 Postnatal growth

Birth anthropometry and neonatal wellbeing are associated with adult height[46]. Although twins are normally small at birth, their height at the age of 18 is within normal range compared with singleton siblings and the general population[47]. However, children who are small at birth and weigh less than average will need to initially catch up to existing growth standards. Research has suggested that twin-specific growth differs significantly from that of singletons[48]. This difference decreases most between 6 and 12 months[49], indicating catch-up growth.

Catch-up growth has been associated with increased body fat[50]. According to Barker's hypothesis, this is the result of the 'thrifty phenotype', which is due to malnourishment during critical developmental periods in utero and results in lower metabolic rates[51]. It is suggested that catch-up growth and the 'thrifty phenotype' are predictors of later heart disease and obesity[52, 53]. The causes and consequences of excessive weight gain will be discussed in further detail in Chapter 8.

1.5.4 Developmental skills

Studies on the effects of low birth weight on developmental skills are sparse, and mostly performed on singleton populations[54-56]. It appears that premature children with low birth weight show signs of developmental delays within the first two years[57]. Researchers hypothesise that a possible explanation for this could be that a focus on physical growth and muscle gain is prioritized over psychological development[55]. Developmental catch-up has not been found to have similar patterns for all developmental skills. For example, delays in motor development seem to be more pronounced and prolonged in premature children compared with other areas of development[58].

Early developmental delays have been found to have an effect on later cognitive development[59]. Although findings from previous studies mostly agree that preterm and low birth weight children have generally caught-up by age two[60], some studies have found evidence of higher use of school services at school age and later working memory deficiencies[61], which may lead to general learning and behavioural problems.

It is possible that twins generally follow a developmental path, which is comparable to singletons born preterm or with low birth weight. In Chapter 4, I will verify this and determine which perinatal factors are influential at various ages between 3 and 24 months.

Direct links between antenatal growth and postnatal consequences have been studied in a health related context. For example, early rapid foetal growth is related to wheezing disorders at age 3[62], and small for gestational age babies show continued slower growth after birth[63]. Relationships between antenatal head size and cognitive ability later in life have been found in a few studies[64, 65]. However, the relationship between antenatal head *growth* and developmental skills has not yet been investigated. I will provide a detailed report on the effect of antenatal head growth on postnatal developmental performance in Chapter 5, illustrating that there is indeed a relationship.

Similarly, I will outline the relationship between postnatal growth and developmental skills in Chapter 6. There is some evidence to believe that the focus on physical growth may influence the age at which children reach early developmental milestones[55]. Previous studies have found that height[66] and, particularly, head circumference are predictive of cognitive performance in later childhood[67].

1.5.5 Genetic influences

"You can't expect to give birth to an elephant, when you yourself are a mouse", a doctor once said to an expecting mother. Indeed, the growth and size of a child are highly dependent on

body composition of the parents[68-70]. More specifically, birth weight is more influenced by maternal weight, whereas height and weight of both parents influence weight gain equally[2]. It is therefore not surprising that BMI has a strong genetic component[71, 72]. I will describe results from a meta-analysis, which was performed as part of my doctoral research, to illustrate how genetic and environmental influences on body composition vary with age (Chapter 8). The classical twin design is described in §1.6, which is used to disentangle genetic and environmental influences in heritability studies.

1.6 Use of the classical twin design in research

Individual differences can be explained by genetic factors, environmental factors, both or an interaction between the two[73, 74]. The estimated proportion of the observed variance that can be explained by genetic factors is called heritability[75]. It is specific to a particular population at a particular time, meaning that it does not refer to measurements in a completely different population or era[76].

Adoption studies can be used to determine whether a trait is due to genetics or environment and parental rearing style. In this type of study, resemblance between children and biological parents represent genetic effects and resemblance between children and their adoptive parents are a result of environmental effects. Limited access to adoption records poses a complication in this design[76]. Studies with twins raised apart and twins raised together are similar to adoption studies, and are complicated as well as limited by the fact that twins are very rarely raised apart nowadays. Extended family and pedigree studies provide more details on traits that run in the family, and are not as limited in distinguishing genetic from environmental influences as the classical twin study[76, 77].

The twin design is one amongst few that does not have these limitations. It relies on the differences and similarities between monozygotic and dizygotic twin pairs. Aside from the different degrees of genetic alikeness between monozygotic and dizygotic twins, both types of twins share some of their environment, such as the intrauterine environment, family, home meals and schools. On the other hand, there is a part of their environment that is unique to each twin, like sports and hobbies. Using correlations within monozygotic and dizygotic twin pairs, the proportion of variation on a trait that is explained by additive genetic (A), shared environmental (C) and unique environmental factors (E) can be estimated.

1.6.1 Assumptions in the twin design

The twin design is based on several assumptions[77]. One of these is that any difference within a monozygotic twin pair is likely to be due to unique environmental influences, as their genetic and shared environmental factors are the same. Any differences within dizygotic pairs could either be due to genetic or unique environmental variations (Figure 1.2). Another assumption is that there is a genetic factor if monozygotic twins are more alike than dizygotic twins. In other words, if the within pair correlation for monozygotic twins is higher than for dizygotic twins, a genetic factor can explain all or part of the observed variance. Other important assumptions within this design are that the variance and means in monozygotic and dizygotic twins are not significantly different and that of random mating. The latter assumes that people are as likely to choose a partner that is similar to themselves on a trait, as they are to choose someone who is completely different.

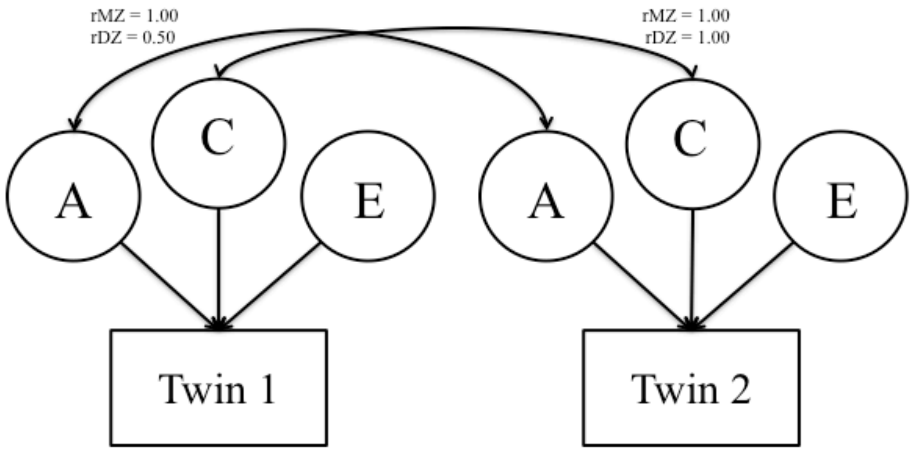


Figure 1.2 The correlation (*r*) of genetic (A), common environmental (C) and unique environmental (E) factors within monozygotic (MZ) and dizygotic (DZ) twin pairs.

1.7 Theoretical model and objectives of this thesis

Developmental delays in childhood have been previously associated with preterm birth[55], low birth weight[54] and low Apgar scores[36, 37], which in turn are associated with maternal and environmental factors (e.g. maternal occupation[42-44], which is further investigated in this thesis). Based on previously described literature, I created a simplified diagram that describes what is known about the relationship between antenatal/maternal factors, perinatal outcomes and developmental delays and delayed growth (displayed as solid lines in Figure 1.3). However, this is a mainly superficial explanation, involving unfavourable antenatal conditions (e.g. insufficient nutrition[19]) as the source of delays in cognitive development and physical growth, but does not explain the biological mechanisms.

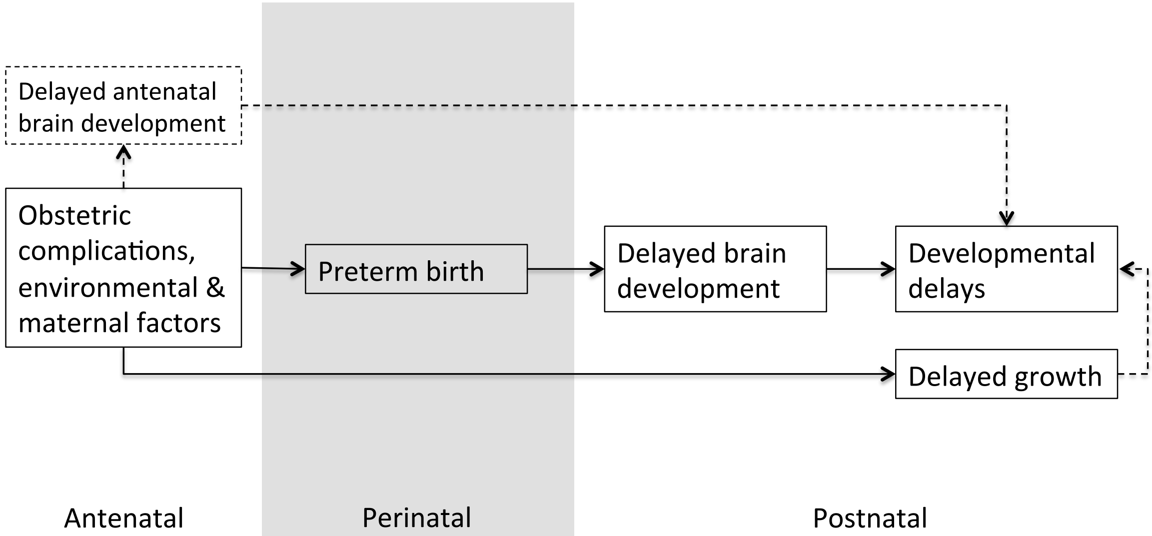


Figure 1.3 Diagram of the theoretical neurodevelopmental context of physical and developmental delays.

Solid lines indicate established relationships. Dashed lines (---) indicate possible relationships, which will be investigated in this thesis.

Considering the literature on child development and the obstetric characteristics of twin pregnancies, there is reason to believe that the effects of known ante- and perinatal risk factors on postnatal development of singletons may be amplified in twins. As these effects have not been previously studied in detail in a sample of healthy twins, I aim to shed more light on the subject with this thesis. Aside from describing twins' developmental and growth trajectories (Chapters 4 and 7), I was interested to investigate the literature-based diagram I created in Figure 1.3 with the following research questions:

- what is the influence of perinatal outcomes on development in the first two years of life? (Chapter 4);
- what is the association of maternal occupational physical activity and psychological strain on twin pregnancy outcomes? (Chapter 7).

I was further interested in studying the alternative paths to developmental delays I suspected (dashed lines in Figure 1.3) with the following research questions:

- what is the relationship between antenatal head circumference and developmental skills in infancy? (Chapter 5);
- what is the association of postnatal growth with concurrent and later developmental skills in infancy? (Chapter 6).

In my last results chapter (Chapter 8), I investigated the change in relative genetic and environmental influence on BMI over a lifespan in a meta-analysis using the classical twin design. This thesis is concluded with a discussion of my findings and suggestions for future studies and clinical practice in Chapter 9. However, I will begin by presenting my work as project manager for BiRTHS (Chapter 2) and the data that was used in this thesis (Chapter 3).

CHAPTER 2

THE BIRMINGHAM REGISTRY FOR TWIN AND HERITABILITY STUDIES (BIRTHS)

The Birmingham Registry for Twin and Heritability Studies (BiRTHS)[78] is a prospective multiple birth registry, which was established in 2008. As far as we know, there is currently no other multiples registry in which parents are recruited and asked to complete questionnaires regarding social support, maternal physical activity and maternal depression antenatally. The registry serves as a database of families with twins or higher multiples in Birmingham (and surroundings), and aims to investigate development of healthy multiples in an ethnically diverse population. It is a platform for relevant studies, for which families from the registry can be invited to participate.

The first of these studies is the current one, which has investigated physical and psychological development in infant twins, following the aims and research questions described in Chapter 1. In order to achieve these goals, I followed the included families from recruitment until the twins were 2 years of age.

This chapter describes the BiRTHS study protocol, after the latest protocol update in May 2010, and outcomes of the recruitment process. Descriptions of tasks that were not performed by me or decisions that were made before I started my doctoral study are written in *italics*.

2.1 Ethical approval

Ethical approval for this study was obtained from the Solihull Local Research Ethics Committee on May 9th 2007. After this, site-specific approval for all proposed sites, namely Birmingham Women's Hospital, Birmingham City Hospital and Heartlands Hospital was granted. Approval from the Research and Development departments at these sites was granted following site-specific approval, and project activities were undertaken from then onwards.

2.2 Eligible families

All families were eligible if they were expecting twins or higher multiples, and were seen at Birmingham Women's Hospital, Birmingham Heartlands Hospital or Birmingham City Hospital. Mothers were between the 12th and 28th week of their pregnancy in order to be eligible for an introduction to the study. This was decided to ensure that the chance of any intrauterine demise or undetected severe growth issue would be minimal, and that we would allow enough time for parents to complete and return the initial questionnaires before delivery to reduce the chance of losing data. To illustrate, the recruitment process will be explained in detail in later paragraphs.

2.2.1 Exclusion criteria

Pregnancies in which severe growth problems were found or which resulted in foetal demise were excluded from the study. Families who did not understand English or did not have access to an interpreter were not invited to participate.

2.3 The BiRTHS research team

Figure 2.1 gives a full overview of the BiRTHS research team on 15th July 2011, with the exception of Mr. H. Gee, who was lead consultant at Birmingham Women's Hospital until December 2009. The Chief Investigator oversaw the entire project, while day-to-day management was the responsibility of the project manager. The Principal Investigators are represented as the first consultant listed for each hospital in Figure 2.1, and acted as line managers for me while I was at the hospitals. The research assistants were graduate psychology students, who worked on the project during overlapping periods of 6 months between August 2010 and August 2011. A research midwife was assigned to the project from

August 2009 to October 2010 at Birmingham Women's Hospital, and between March 2010 and July 2011 at Birmingham Heartlands Hospital. Consultants, research assistants and midwives were trained to identify eligible patients, introduce the study and take consent. Both honorary research associates were involved in the set-up of the study between May 2006 and August 2008 as full team members, and remained involved in organising events and newsletters in honorary posts.

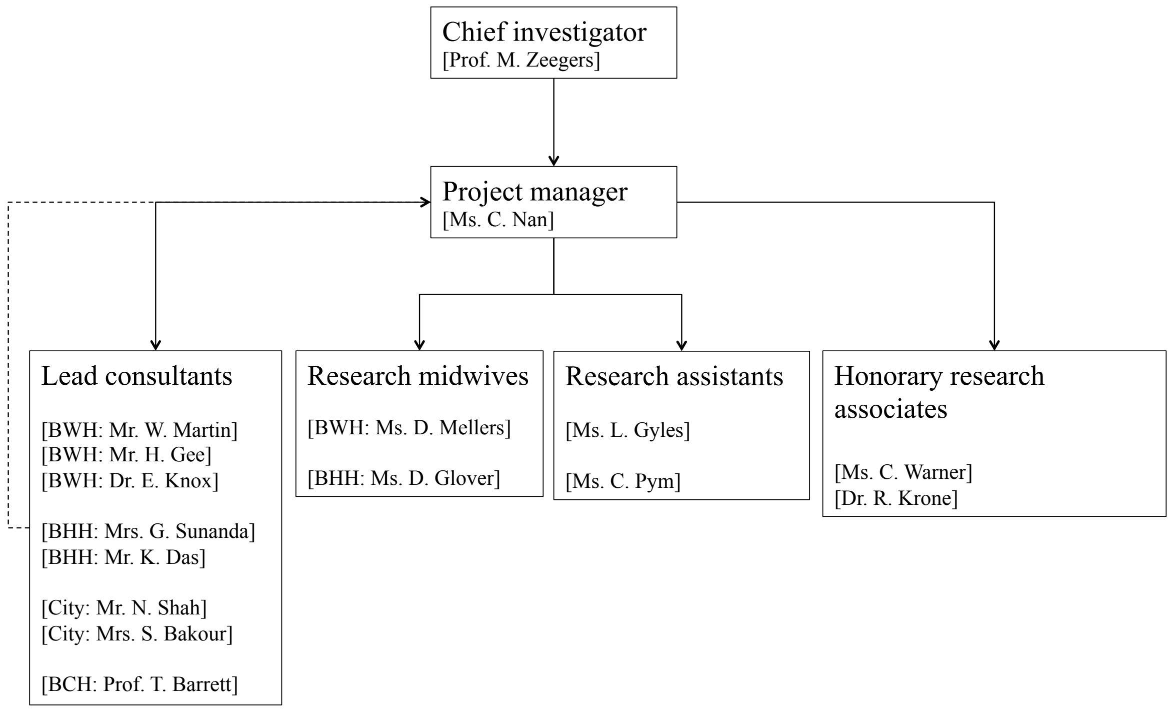


Figure 2.1 Organisational chart of the BiRTHS research team.
[the dashed line (---) represents line management by lead consultants from each hospital. (BWH = Birmingham Women's Hospital, BHH = Birmingham Heartlands Hospital, City = Birmingham City Hospital, BCH = Birmingham Children's Hospital)].

2.4 Recruitment

Families were recruited antenatally from specialized multiple clinics at Birmingham Women's Hospital, Birmingham Heartlands Hospital and Birmingham City Hospital between 4th August 2008 and 15th July 2011. Consultant obstetricians led these clinics with their team of specialist midwives and registrars. The following is a description of the antenatal process from introduction to consent and data collection (Figure 2.2). Together with the staff in antenatal clinic, I identified eligible families by reviewing patient medical notes when they attended their appointments. Families were mainly approached by myself, and in the final 1.5 years, by a trained research midwife and research assistant as well. Eligible families were informed about the study and given a study information sheet (Appendix A-I.i) to take home and read. To aid in the identification of these families at their next appointment, mothers' next appointment dates were kept in the BiRTHS recruitment diary. The second stage of recruitment was the consent procedure (Appendix A-I.ii). Previously approached families were asked for consent at their next antenatal appointment. They were given the opportunity to ask questions regarding the study, after which they had the choice of consenting to the registry only, the 2-year follow-up study or declining to consent altogether. Consenting to the follow-up study automatically meant that families were a part of the registry. Those who declined were not approached again regarding the study. Contact details for those who did consent were completed on the contact details sheet (Appendix A-I.iii). Antenatal questionnaires (§2.5.1) were given out at consent and parents were instructed on how to complete them and to return them to a BiRTHS team member at their next appointment, which was marked in the BiRTHS recruitment diary.

A BiRTHS team member approached parents who had not yet returned their questionnaires at each consecutive antenatal appointment, until completed questionnaires were returned. Families had completed the antenatal pathway for the project when I was in possession of the completed questionnaires and would not be approached again until after birth of their babies.

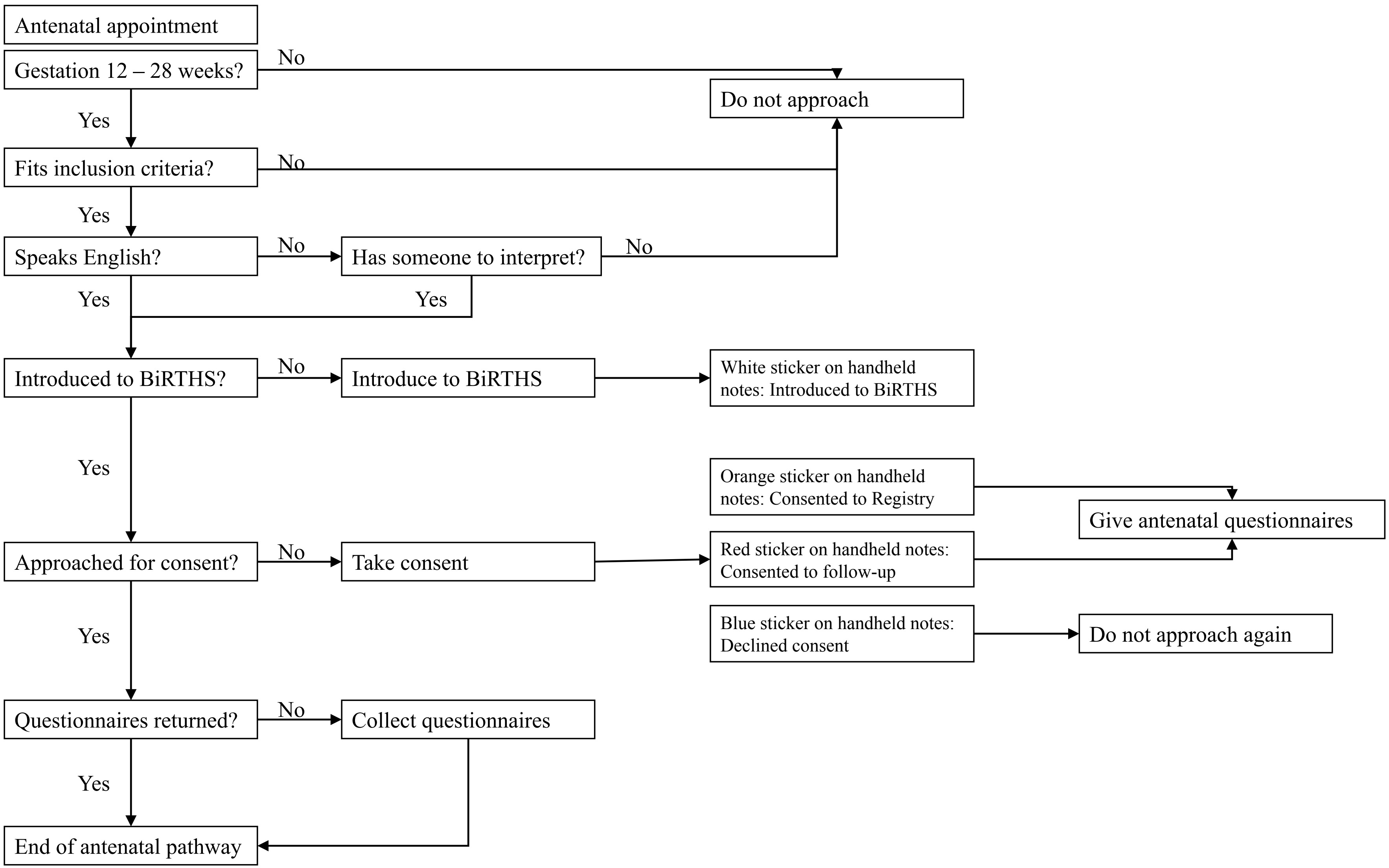


Figure 2.2 Complete pathway for the antenatal recruitment into the Birmingham Registry for Twin and Heritability Studies (BiRTHS).

2.4.1 Exclusion criteria after consent

Although families had the right to discontinue their participation in the project at any time and without stating a reason, there were several criteria at birth that needed to be met in order to be definitely included.

- First of all, deliveries had to meet the International Classification of Disease for a viable live birth (10^{th} ed.)[79]. This meant that:
    - gestational age at birth should be at least 22 weeks;
    - newborns should weigh more than 500 grams.
- Secondly, there should be no severe malformations or developmental impairments that became apparent at birth.

A diagram of the entire follow-up process is displayed in Figure 2.3.

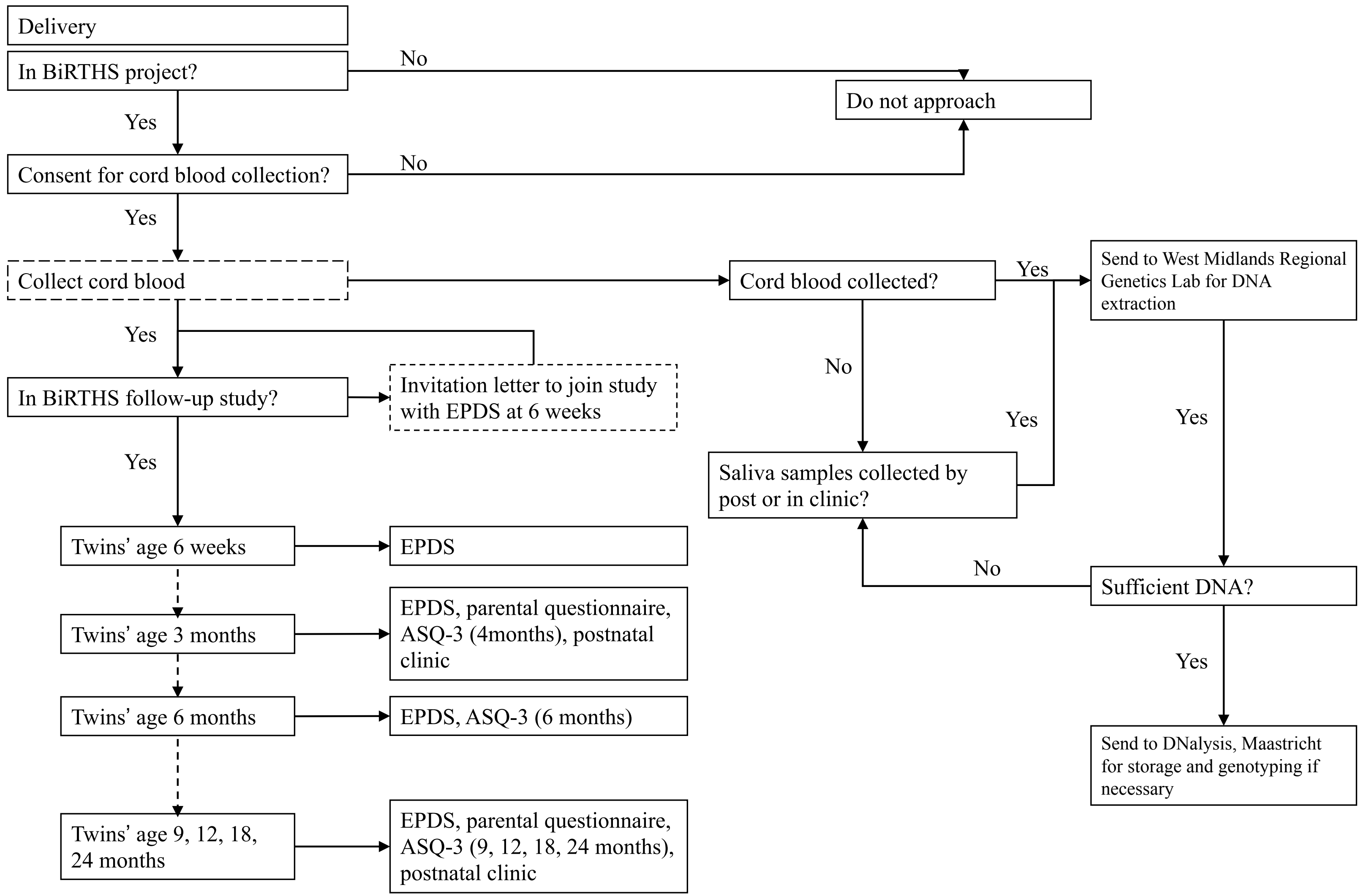


Figure 2.3 Complete perinatal and postnatal pathway for the Birmingham Registry for Twin and Heritability Studies (BiRTHS).
EPDS=Edinburgh Postnatal Depression Scale, ASQ-3=Ages and Stages Questionnaires (3rd edition), DNA=Deoxyribonucleic acid. Dashed (---) borders represent optional items.

2.5 Collected data

Data were collected through questionnaires, BiRTHS postnatal clinics and clinical record forms. Any questionnaires regarding the twins or measurements from postnatal clinics were not corrected for prematurity. Additionally, I set up a DNA depository for DNA samples of the twins. The following paragraph provides a description of the materials and procedures that were used for data collection and DNA sampling. A detailed description of data handling and data management can be found in the next chapter.

2.5.1 Antenatal questionnaires

Antenatal questionnaires (Appendix A-II.i-A-II.ii) were given to parents when they consented to participate in the registry or in the follow up study. Parents were asked to complete questions about their ethnic background, employment, body composition, alcohol and smoking habits, family history of twinning and familial relationships between the parents. Furthermore, parents were asked to complete the 8-item Duke UNC Functional Social Support Questionnaire about their perceived social support[80]. Additionally, mothers were asked to complete a short exercise questionnaire[81] in which they estimated the number of hours spent on exercise each week before and during pregnancy. Finally, maternal depression was assessed with the Edinburgh Postnatal Depression Scale (EPDS; Appendix A-II.iii)[82]. Data from the parental questionnaires (age and body composition) are used as potential confounders in analyses described in subsequent results chapters. Maternal occupation is used in the main analysis in Chapter 7.

2.5.2 Postnatal questionnaires

Postnatal questionnaires were posted to parents who agreed to participate in the follow-up study. If parents had initially consented to the registry, they were given the option to participate in the follow-up study from 6 weeks onwards. If they agreed, they would follow the same process as parents, who had consented to the follow-up study during the antenatal consent procedure. Mothers received the EPDS at 1.5, 3, 6, 9, 12, 18 and 24 months. The mother's general practitioner was made aware of any total scores above 12. This happened for four women in the follow-up study, and was done via email or a letter and confirmed via telephone. Mothers received a copy of the letter to their general practitioner. All but one general practitioner were aware of a history of or ongoing depression for the concerning mothers. The four women, who had a high EPDS score, were also in the group that showed cause for concern in their antenatal EPDS. In conjunction with the EPDS, a parental questionnaire (Appendix A-II.iv) was completed at 3, 9, 12, 18 and 24 months. This questionnaire was similar to its antenatal counterpart, with the exception of questions about handedness and a zygosity questionnaire about the twins' alikeness.

2.5.3 The zygosity questionnaire

The zygosity questionnaire consisted of four questions, and was based on a longer validated questionnaire[14]. Each answer corresponds to a score, with which a total score can be calculated at the end of the questionnaire. A zygosity quotient is calculated by dividing the actual total score with a theoretical total score, which is the sum of the maximum possible scores for each question. The zygosity quotient ranges from 0 to 1, with 0 representing twins that are likely monozygotic and 1 representing twin pairs that are likely to be dizygotic. Similar to the original zygosity questionnaire, twins were classed as dizygotic when their zygosity quotient was above the median. The original validated questionnaire has a sensitivity of 98.8%, specificity of 88.9% and 90% overall agreement between questionnaire and genotype. Information regarding the validity of the questionnaire used in BiRTHS can be found in Chapter 3.

2.5.4 Ages and Stages Questionnaires (ASQ-3)

Developmental skills of the twins were assessed by a parent-completed questionnaire, the third edition of the Ages and Stages Questionnaires (ASQ-3). This was done at all follow-ups, except for 1.5 months. The ASQ-3 System is a series of validated and standardized questionnaires that measure developmental milestones of children at regular intervals between 2 and 60 months, and is meant as a simple method for initial screening for any developmental delays. Norm scores are based on healthy singleton children born at 40 weeks. Based on an American sample of 15,138 children, sensitivity and specificity for all questionnaires were estimated between 70% and 100%, while validity was between 82% and 88%. Furthermore, there was a 84% overall agreement between the ASQ-3 and the Bayley Scales of Infant Development[83].

I used ASQ-3 questionnaire sets for 6, 9, 12, 18 and 24 months for the corresponding follow-ups. The 2 and 4 months questionnaires have been validated for the ages between 2 months 0 days and 2 months 30 days, and between 3 months 0 days and 4 months 30 days, respectively. The 4 months questionnaire was used for the 3 months follow-up by default. However, depending on the timing of posting the questionnaires, it was more appropriate to use the 2 months questionnaire in some cases. This occurred four times in the course of the study, because parents notified me of planned holidays, which would overlap with their intended follow-up. For these cases, I decided to send the questionnaires earlier to avoid missing data completely. The 48-month ASQ-3, which is available as a free sample questionnaire online, can be found in Appendix A-II.v.

The questionnaires consist of simply phrased questions based on developmental milestones at each follow-up age. The questionnaires measure five domains of development, namely communication, gross motor, fine motor, problem solving and personal social skills. Parents complete questions about their children's ability to perform certain age specific movements (e.g. picking up toys) or whether they show signs of communication and understanding (e.g. laughing). The questions are designed in a way so that parents can easily complete them regardless of educational background. They answer each question with 'always', 'sometimes' or 'not yet'. In accordance with the ASQ-3 protocol, a score of 10 is assigned for 'always', 5 for 'sometimes' and 0 for 'not yet' answers, resulting in a total score ranging from 0 to 60 on each of the five subscales. These scores correspond to three areas in which a child can score: 'normal development' (above -1 standard deviation (SD)), 'cause for concern and further monitoring needed' (between 1 and 2 standard deviations below the mean) and 'referral for further developmental assessment needed' (more than 2 standard deviations below the mean). Because there are no norms for premature twins and the ASQ-3 was not used as a clinical screening tool, I did not make any referrals. Children who would have needed a referral according to the outcome of the questionnaire were already under assessment at the Birmingham Children's Hospital or local institutions specialising in infant development. I will describe and analyse the ASQ-3 data in more detail in Chapters 4-6.

2.5.5 Postnatal clinics

Families were invited to BiRTHS postnatal clinics when the twins' chronological age was 3, 9, 12, 18 and 24 months. The baby clinic at Birmingham Women's Hospital was the main location. However, if the available dates or the location was not convenient for the mother, they were offered an appointment at the Wellcome Trust Clinical Research Facility at the Birmingham Children's Hospital.

A detailed overview is provided in Figure 2.4. A monthly list was produced with follow-ups that were due within the next 6 months. Each week this list was checked for follow-ups within the next month. Mothers were then contacted by telephone and scheduled for a date as close to the follow-up age as possible, but not more than 8 weeks later. In the event that the mother was not available to arrange an appointment, I called at a later time or left a message and an email was sent with possible dates and appointment slots. I also checked whether cord blood was taken at birth and whether the DNA yield from this sample was sufficient or whether an additional saliva sample was needed. If a saliva sample was necessary, I explained the procedure, which is described in §2.5.8, to the parents beforehand so that they could decide whether they would allow us to take the samples in clinic. Two families declined this.

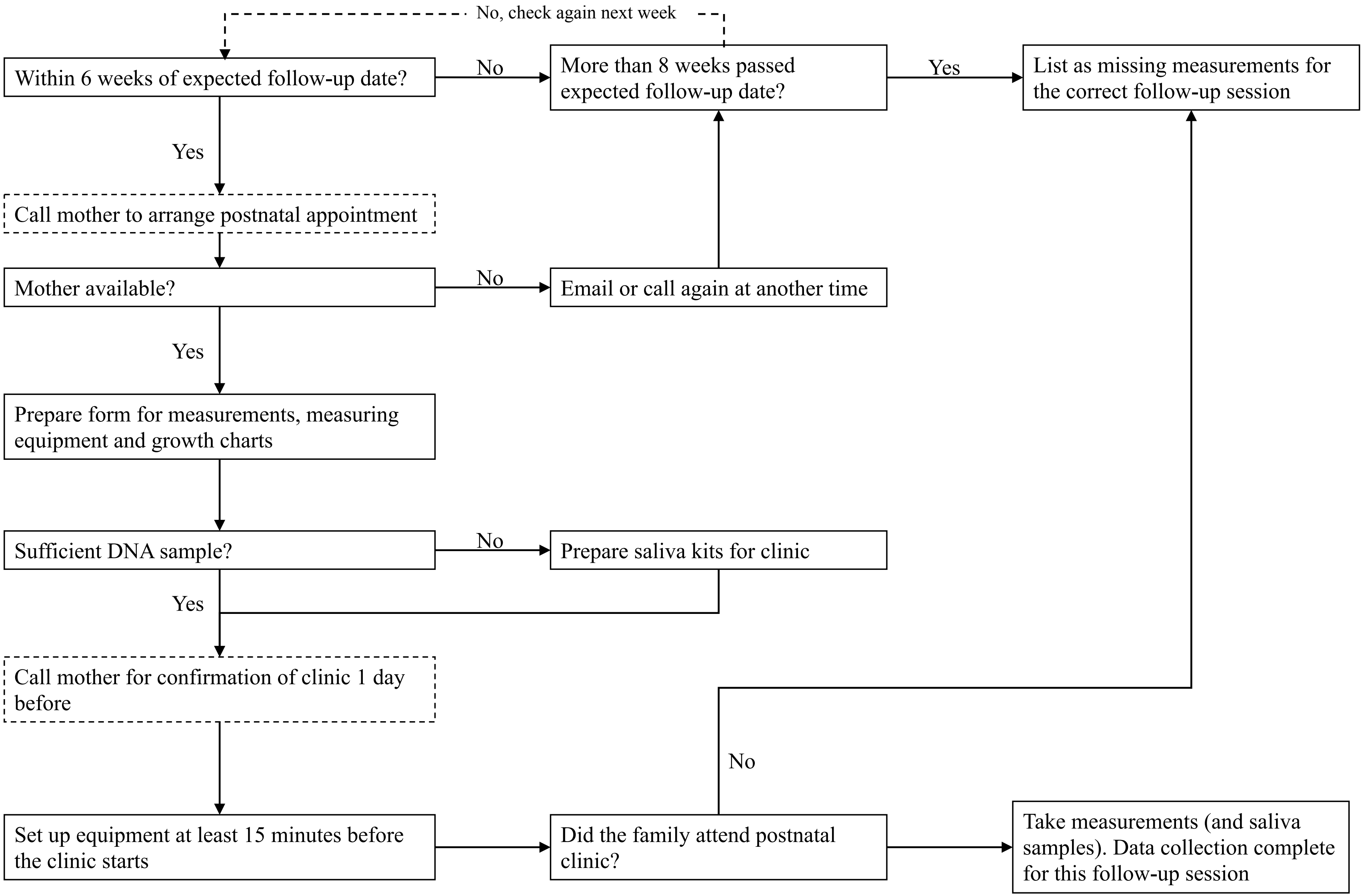


Figure 2.4 Complete pathway for postnatal follow-up appointments in the Birmingham Registry for Twin and Heritability Studies (BiRTHS).

A trained BiRTHS researcher measured weight, length, head circumference, mid-upper arm circumference and skinfold thickness (triceps and subscapular) at each postnatal appointment. Each measurement, except for weight measured with digital scales, was taken three times to minimize variability. If one of the three measurements differed more than 1 centimetre (cm) compared with the others, it was assumed that the measurement was incorrect and all three measurements were redone. Over the course of 2.5 years, I conducted about 75% of the measurements, while the trained research assistants and midwives took account of the remaining measurements. Due to the amount of time it took to measure one set of twins, it was not feasible to measure the same children twice for a large enough number of twin pairs in order to check the inter-observer variability. Therefore, the research assistants and midwives took the measurements in their training phase and compared them to the measurements that I took immediately afterwards. I then checked whether the discrepancy between both sets of measurements was more than 1cm, similar to intra-observer variability. Because measurements were extensively practiced on myself and other volunteers (research assistants, students and friends) beforehand, measurements that were taken in postnatal clinics did not vary more than 1cm in most cases. Exceptions were occasions where the children were fidgety, in which case more time was taken to calm the children down and take accurate measurements. Finally, feedback was given to parents in the form of World Health Organisation growth charts, on which I did correct for prematurity, as this is common practice for most health visitors and to not worry the parents unnecessarily. A full description of how the measurements were taken and their reliability is provided in Appendix III. Postnatal growth measurements are used in the analyses in Chapter 6.

2.5.6 Maternal medical records – clinical record forms

All mothers who are under antenatal care have a medical record (Appendix A-IV.i), which is a combination of information on all the mother's hospital visits, a summary of the current pregnancy and, finally, delivery details (Appendix A-IV.ii). Mothers complete questions regarding their ethnic background, date of birth, employment status, previous pregnancies, medical history and family history of disease for themselves and their partners. Midwives record mothers' height and weight at their booking appointment, which normally happens between 10-12 weeks of gestation. The following delivery details were extracted: estimated date of delivery, gestational age, gender, mode of delivery, presentation, birth weight, length, head circumference, Apgar scores at 1 and 5 minutes, resuscitation details and the location after birth. Children were transferred to special care, high dependency, intensive care, transitional care or regular postnatal wards. Indications for admission to wards other than transitional care or postnatal wards are birth weight below 1500 grams, gestational age younger than 25 weeks or major health complications that require constant monitoring.

I extracted the above information from maternal medical notes after the twins were born, and recorded the data on clinical record forms. Parity, gender and gestational age at birth were used as potential confounders in subsequent results chapters. Birth weight, delivery mode, postnatal hospital admission and Apgar scores are investigated as outcomes of maternal occupation in Chapter 7.

2.5.7 Ultrasound measurements – clinical record forms

I extracted biparietal diameter, femur length, head circumference, abdominal circumference and estimated foetal weight from ultrasound scan reports (A-IV.iii). All growth measurements are expressed in millimetres (mm), while estimated foetal weight is measured in grams (g). Gestational age was extracted for each scan report. These scans reports are generated after each antenatal appointment. Scans were made using three ultrasound scan machines: Siemens Acuson S2000, GE Voluson 730 Expert and GE Voluson E8.

Antenatal growth data will be described in detail in Chapter 5.

2.5.8 DNA depository

Cord blood samples

Parents were asked for permission to take a cord blood sample at birth from each baby (Appendix A-I.ii, item 4). This cord blood was taken by midwives and collected into 10ml EDTA bottles, which were labelled with the BiRTHS logo and a patient identifier. Midwives were instructed to fill in date of birth, time of birth and birth order on the patient identifier label. A single clip on the umbilical cord of the first-born and two clips on the second-born twin's cord of the second-born determined birth order. This is common practice at the three hospitals from which patients were recruited for this study.

The samples were sent to the West Midlands Regional Genetics Laboratory based in the Birmingham Women's Hospital for DNA extraction with Qiagen Puregene DNA extraction kits. This was done according to the lab protocol in which non-nucleated red blood cells were separated from nucleated white blood cells first. DNA was then preserved while any contaminating substances, such as cytoplasmic and nuclear proteins, were removed with salt precipitation. The genomic DNA was isolated by precipitation with alcohol, after which the DNA was re-hydrated in a buffered solution. Reports, in which the quantities of the DNA yield were stated, were sent to the BiRTHS office and samples were stored at -80 degrees Celsius at the laboratory. A total of 30 cord blood samples were taken, of which 20 yielded enough DNA for further analysis.

Saliva samples

In the event that cord blood was not taken, or the DNA yield had insufficient volume for further analyses, saliva samples (N=97) were taken using Oragene·DNA self-collection kits (OG-250) in disc format with the collection accessory for infants and young children from DNA Genotek[84]. I did this at a postnatal appointment, or posted the kit to the parents, according to Royal Mail guidelines for human specimens. Kits mailed to parents included simple instructions describing how to collect saliva samples, identifier labels, DNA Genotek liquid-tight biospecimen bags with absorbent material to soak up 4mL of liquid and bubble return envelopes with First Class postage and labelled 'Exempt Human Specimen'.

The DNA extraction and storage procedure was the same as from cord blood samples.

Genotyping

Batches of extracted DNA samples were sent to DNalysis in Maastricht, the Netherlands, where they were permanently stored at -20 degrees Celsius. Genotyping was done for those where zygosity could not be conclusively determined by gender and chorionicity. The AmpFℓSTR® NGM™ PCR Amplification Kit was used for the genotyping of most samples. The kit amplifies the gender determination locus Amelogenin, 1 trinucleotide repeat locus (D22S1045) and 14 tetranucleotide repeat loci (D10S1248, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D22S1045, D19S433, TH01, FGA, D2S441, D3S1358, and the highly polymorphic D1S1656 and D12S391 loci). DNA samples of two twin pairs were analysed with the AmpFℓSTR® SEfiler Plus™ PCR Amplication Kit due to problems with the amplification of several loci. This kit amplifies Amelogenin, and 11 tetranucleotide repeat loci (vWA, D16S539, D2S1338, D8S1179, D21S11, D19S433, TH01, FGA, D18S51, D3S1358 and the highly polymorphic SE33 locus).

Twins were classified as monozygotic if DNA profiles were identical.

CHAPTER 3

DATA MANAGEMENT AND CHARACTERISATION OF THE SAMPLE

3.1 Data management

All included individuals were assigned a unique identifier, which consisted of a family and person identifier. A password-protected database was created in Microsoft Access to hold the collected data and saved on the University of Birmingham network. A general summary form was used as starting point for each entry. This form provided contact details, children's names and a summary of sent, received and entered questionnaires for each family. It also delivered a list of family specific follow-up dates, based on their twins' date of birth. Separate database forms were created for each questionnaire and clinical record form. Each database form was connected to an underlying database table, where the entered data was saved automatically. This resulted in two specific antenatal forms (mother's antenatal questionnaire and father's antenatal questionnaire), four specific postnatal forms (mother's postnatal questionnaire, father's postnatal questionnaire, twins' ASQ-3 and twins' anthropometric measurements), one combined antenatal and postnatal form (EPDS questionnaire) and three clinical record forms (mother's medical details, twins' perinatal details and ultrasound scan details).

The data tables were then imported into STATA 11 (Statacorp, 2009)[85] using Open Database Connectivity, where they were merged into one large anonymised dataset. Parts of this dataset were used for this thesis, and will be described in subsequent chapters.

3.2 Data cleaning

Harmonisation of the data was mostly done during the data entry stage. For example, parents could give their height and weight in either metric or imperial measurements. Because the database was built to only allow the metric system, imperial measurements were converted before they were entered into the database. Missing data that could be easily calculated, such as parental age, was completed in the data entry stage. Discrepancies in the physical activity questionnaires were checked. For example, if mothers filled in that they walked 10 hours per week in the year previous to their pregnancy, but left the corresponding age category for walking blank, the data would be added in the appropriate age category.

By allowing only certain types of numbers (e.g. bytes and integers), based on possible answers specific for each question, the number of discrepancies within the answers of each question was minimised. The general summary sheet of the database allowed for automated entry of family member identifier and follow-up age on each subsequent form and eliminated the chance of mistakes in assigning incorrect identifiers or follow-up age. All of these built-in procedures resulted in a merged dataset that was uniform in presentation.

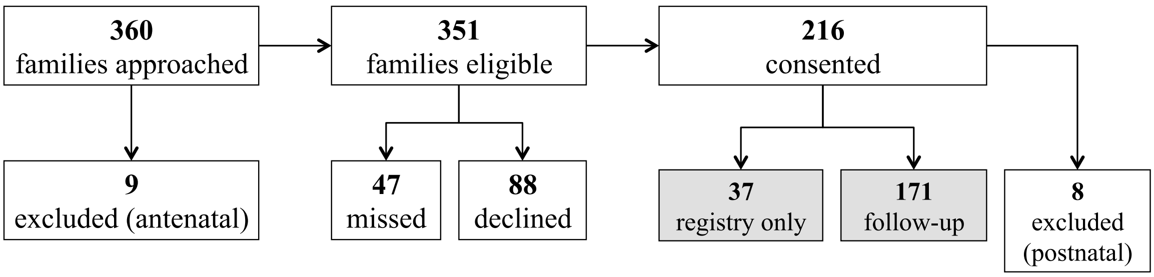
Logical checks for impossibilities within the merged dataset were then performed in STATA 11[85]. Anthropometric measurements and ultrasound scans were checked for outliers that were physically impossible. A length of 8.97cm for example would be impossible and would very likely be mistakenly entered instead of 89.7cm. Gestational age for ultrasound scans was checked against gestational age at birth, in order to confirm that all scans were performed before birth. All discrepancies were checked against hard copies of the data and corrected. Four errors were found and corrected in the entry of ultrasound scan measurements. No other errors were identified in the data that is described in this thesis. The following paragraphs describe the collected data after cleaning.

3.3 The recruitment process in numbers

Figure 3.1 shows the BiRTHS recruitment process in numbers. Of the 360 families we approached in the antenatal clinics, nine (3%) were not eligible because they had miscarried or received news about congenital malformations. Of the remaining 351 eligible families, 47 families (13%) were missed for consent, because they had delivered their babies before we saw them again or because they had been transferred to another hospital. A total of 88 families (24%) declined consent. Reasons were the husband not agreeing in families of Asian ethnic backgrounds, the study being too time consuming, the registry being too invasive of privacy and families not being interested at all in research. One family declined, because they were planning to move abroad and would not be able to take part.

A total of 216 families (61.5%) consented to take part in the project. Eight families (4%) were excluded after consent (postnatal) due to miscarriages or congenital malformations in the twins. This resulted in a total of 208 families in the BiRTHS cohort, of which 171 also participated in the 2-year follow-up study. One family pulled out of the study after consent due to the time commitment, but remained part of the registry.

Figure 3.1 Recruitment in numbers.



3.4 Baseline data

A lot of data was collected during the course of the BiRTHS project. However, I will only describe the data that are relevant to the analyses in this thesis. Response rates for questionnaires and postnatal anthropometric follow-ups can be found in Table 3.1. The number of eligible families was dependent on the age of the twins at the time that the study closed; over half the twins had not yet reached their first birthday when the study closed.

Table 3.1 Response rates for all parent completed questionnaires and clinic appointments.

	Parental questionnaire			Ages and Stages Questionnaires			Twins' anthropometry		
	Eligible (N)	Received (N)	Response rate (%)	Eligible (N)	Received (N)	Response rate (%)	Eligible (N)	Received (N)	Response rate (%)
Antenatal									
Mother	208	207	73	.	.	.	.	.	.
Father	208	213	70	.	.	.	.	.	.
1.5 months	.	.	.	.	.	.	.	.	.
3 months	128	67	52	256	85	33	256	134	48
6 months	.	.	.	254	126	50	.	.	.
9 months	112	60	54	224	110	49	224	110	49
12 months	92	57	62	184	116	63	184	64	35
18 months	64	37	58	128	74	58	128	50	39
24 months	84	25	30	168	50	30	168	38	23

N=sample size. Numbers for parental questionnaires are 'per family', while numbers for the Ages and Stages Questionnaires and anthropometry are for twins as individuals.

3.4.1 Parental demographics

On average, mothers were 31.9 years (SD=5.2) at the time of questionnaire completion at an average of 27 weeks gestation, while fathers were about 34.2 years (SD=5.8). Mothers' average weight at booking during the first trimester was 70.6 kilograms (kg; SD=79.7), and had increased with 9 kilos at the time of questionnaire completion. 63% of the parents were expecting their first children (primiparous). The majority of the parents were British (69%), while the second largest ethnic group was Asian (15%). Over three quarters (83%) of the parents had paid employment, of which a little over half (55%) held a job that required completion of a tertiary degree.

3.5 Pregnancy details

3.5.1 General pregnancy details

Nearly 40% of mothers were admitted to hospital during their pregnancy. The most common reasons were contractions and foetal distress, which required monitoring or delivery of the babies. More than half of the twins (67%) were naturally conceived as opposed to via fertility treatments. Of the twins that were conceived through fertility treatments, conception by in vitro fertilisation was the most common (65%). A quarter of our sample consisted of monochorionic twins, which concurs with the previously explained theory in Figure 1.1.

Table 3.2 Sample size (N) and perinatal details by birth order.

	Twin 1			Twin 2		
	N	Proportion		N	Proportion	
Male	88	49.7		100	56.5	
Mode of delivery						
Emergency caesarean	80	44.9		80	45.2	
Other	98	55.1		97	54.8	
Resuscitated at birth	24	13.5		44	25.0	
Resuscitation method						
Suction	1	4.2		0	0.0	
Oxygen	19	79.2		41	93.2	
Intubation	4	16.7		3	6.8	
Hospital admission after birth						
Special care ward	47	79.7		43	76.8	
High dependency ward	2	3.4		1	1.8	
Intensive care ward	10	17.0		12	21.4	

	Twin 1			Twin 2		
	N	Mean	SD	N	Mean	SD
Birth weight (grams)	176	2382.7	552.2	174	2340.7	549.3
Length (cm)	140	45.6	13.0	137	46.4	4.0
Head circumference (cm)	170	32.4	2.1	167	32.3	2.2
		Median	IQR		Median	IQR
Apgar at 1 minute	174	9.0	8-10	168	9.0	8-9
Apgar at 5 minutes	174	9.0	9-10	168	9.0	9-10

cm=centimetre, SD=standard deviation, IQR=interquartile range.

Table 3.2 describes perinatal details of the twins. On average, there was no difference between birth weight (t=0.69, p=0.49), length (t=-0.71, p=0.48) or head circumference (t=0.66, p=0.51) of first- and second born twins. Notably, the standard deviation for length at birth for first-born twins was three times larger than that of second-born twins. This could reflect the inaccuracy of the measurements due to time pressure in the delivery room. Although there was no large variation in head circumference measurements in the current sample, several delivery suite midwives have expressed their concerns about the precision of these measurements for the same reason as length. Because the accuracy of length and head circumference at birth has not yet been verified, these measurements will not be used in further analyses in this thesis. Median Apgar scores at 1 and 5 minutes were above 7 for both twins – which is considered good – and less than half of the deliveries were by emergency caesarean section (45%). When looking at immediate neonatal problems, a higher proportion of second-born twins needed resuscitation (25%). Of the twins that were admitted to specialist hospital wards after birth, over 75% were admitted to the lowest level of special care wards, compared with 17-21% that was admitted to the intensive care unit (highest level of care).

3.5.2 Growth scans

Data from antenatal growth scans of 180 twin pairs can be found in Table 3.3. The earliest available scan was at 13 weeks, which is in line with the antenatal appointment schedule of booking around 12 weeks and regular appointments from 16 weeks onwards: every fortnight for monochorionic pregnancies and every month for dichorionic pregnancies. The majority of mothers had about 6 scans, while two mothers had 12 scans throughout the entire pregnancy up to an average of 35.9 weeks.

Overall, growth was observed over time for all measurements, with foetal weights at 36 weeks estimated to be a little over 2600 grams. Both twins were about the same size on most measurements. However, twin 1 had a significantly larger biparietal diameter at 20 ($t=2.03$, $p=0.02$), 21 ($t=1.80$, $p=0.04$), 28 ($t=2.39$, $p=0.01$) and 29 ($t=1.84$, $p=0.04$) weeks of gestation.

Foetal position made it impossible to take all measurements at some appointments, which have led to inconsistent sample sizes for measurements at each individual scan.

Table 3.3 Sample size (N) and average antenatal growth measurements for each twin at gestations between 13 and 37 weeks.

	Biparietal diameter (mm)		Head circumference (mm)		Femur length (mm)		Abdominal circumference (mm)		Estimated fetal weight (grams)	
Gestational age	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2
13 weeks										
N	11	12	8	8	6	5	2	2	0	0
Mean (SD)	24.0 (1.3)	23.7 (2)	87.9 (3.2)	87.7 (5.4)	10.6 (0.9)	10.2 (0.4)	68.5 (6.4)	68.8 (10.1)	. (.)	. (.)
14 weeks										
N	8	8	9	9	7	7	3	3	1	1
Mean (SD)	26.6 (1.9)	26.9 (1.8)	100.8 (5.6)	102 (4.3)	15.1 (1.9)	15.1 (2.3)	89.3 (6.4)	86.7 (8.7)	121.0 (.)	124.0 (.)
15 weeks										
N	20	19	20	19	19	19	11	10	4	4
Mean (SD)	30.3 (1.6)	30.8 (1.9)	112.4 (6.1)	114.7 (6.0)	16.7 (1.9)	16.9 (1.4)	93.7 (6.9)	97.4 (7.2)	126.5 (11)	130.5 (8.5)
16 weeks										
N	23	23	33	33	31	32	26	26	11	11
Mean (SD)	35.5 (2.6)	34.9 (2.4)	128.4 (5.9)	126.9 (8.4)	20.3 (1.4)	20.6 (1.6)	106.5 (7.2)	106.1 (7.7)	153.9 (13.9)	152.2 (21.5)
17 weeks										
N	20	20	22	21	21	21	12	12	2	2
Mean (SD)	38.6 (2.2)	37.6 (2.2)	139.9 (6.7)	137.7 (6.4)	23.6 (1.5)	23.3 (1.5)	116.9 (4.3)	113.0 (7.3)	192.0 (28.3)	194.5 (13.4)
18 weeks										
N	18	18	27	27	27	28	19	18	11	11
Mean (SD)	41.4 (1.9)	41.3 (1.5)	152.6 (6.4)	151.5 (5.0)	26.8 (1.9)	26.6 (1.7)	123.4 (24.6)	129.6 (7.6)	234.5 (23.4)	235.7 (25.1)
19 weeks										
N	33	33	34	34	34	34	25	26	2	2
Mean (SD)	45.9 (2.2)	45.1 (2.2)	168.7 (7.4)	167.2 (7.6)	30.1 (1.8)	29.7 (1.8)	142.5 (8.7)	140.1 (8.6)	289.0 (14.1)	260.5 (2.1)
20 weeks										
N	118	120	120	122	122	120	70	70	37	35
Mean (SD)	48.4 (2.4)	47.8 (2.3)	177.9 (8.1)	176.7 (6.6)	32.5 (2.0)	32.7 (1.5)	152.7 (9.4)	153.0 (8.2)	341.1 (44.4)	341.0 (31.6)

N=Sample size, SD=standard deviation, mm=millimetres.

Table 3.3 (*continued*). Sample size (N) and average antenatal growth measurements for each twin at gestations between 13 and 37 weeks.

	Biparietal diameter (mm)		Head circumference (mm)		Femur length (mm)		Abdominal circumference (mm)		Estimated fetal weight (grams)	
Gestational age	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2
21 weeks										
N	30	29	34	32	32	33	25	25	17	17
Mean (SD)	51.4 (2.7)	50.2 (2.5)	184.0 (30.3)	188.3 (7.4)	35.4 (1.9)	36.1 (5.3)	162.6 (9.6)	163.4 (11.2)	409.1 (41.6)	401.0 (40.5)
22 weeks										
N	18	17	29	30	31	31	31	31	28	29
Mean (SD)	53.7 (2.7)	52.4 (2.4)	198.9 (10.1)	197.5 (7.0)	37.7 (1.7)	38.5 (2.1)	173.1 (10.5)	174.2 (8.6)	478.7 (55.6)	489.7 (51.7)
23 weeks										
N	11	10	40	39	41	39	41	39	41	39
Mean (SD)	58.4 (3.4)	57.4 (2.1)	216.4 (11.2)	266.9 (33.8)	41.6 (2.6)	41.0 (2.4)	189.3 (11.5)	188.3 (11.5)	618.6 (95.9)	600.4 (84.0)
24 weeks										
N	21	21	93	94	94	94	94	94	94	94
Mean (SD)	60.3 (2.3)	59.0 (2.3)	220.0 (34.3)	222.4 (7.5)	43.6 (2.1)	43.8 (2.2)	198.8 (10.4)	199.3 (9.9)	697.5 (78.6)	702.1 (74.6)
25 weeks										
N	8	8	27	30	30	30	30	30	30	30
Mean (SD)	63.1 (3.1)	61.5 (3.4)	229.4 (18.0)	232.2 (8.0)	45.9 (1.9)	45.8 (2.6)	209.2 (8.0)	208.5 (11.5)	806 (74.3)	799.1 (98.5)
26 weeks										
N	9	8	31	37	37	37	37	37	37	37
Mean (SD)	64.5 (3.0)	64.6 (3.2)	237 (40.4)	241.6 (11.2)	48.0 (2.5)	46.6 (8.0)	218 (10.7)	216.8 (13.5)	919 (104.1)	904.2 (131.2)
27 weeks										
N	7	7	37	39	42	42	42	42	42	42
Mean (SD)	68.8 (2.8)	67.0 (3.4)	256.8 (8.0)	254.4 (11.0)	51.0 (2.3)	50.5 (2.8)	230.5 (10.1)	229.2 (11.5)	1086.3 (110.7)	1057.1 (123.0)
28 weeks										
N	17	17	95	106	108	108	108	108	108	108
Mean (SD)	71.7 (2.3)	69.4 (3)	262.4 (22.7)	260.9 (22.5)	58.8 (48.5)	54.7 (20.3)	240.0 (12.2)	239.6 (24.7)	1200.9 (169.8)	1218.6 (141.9)

N=Sample size, SD=standard deviation, mm=millimetres.

Table 3.3 (*continued*). Sample size (N) and average antenatal growth measurements for each twin at gestations between 13 and 37 weeks.

	Biparietal diameter (mm)		**Head circumference (mm)**		**Femur length (mm)**		**Abdominal circumference (mm)**		**Estimated fetal weight (grams)**	
Gestational age	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2
29 weeks										
N	8	9	34	39	39	38	39	39	39	38
Mean (SD)	73.6 (1.5)	72.1 (1.8)	274.9 (6.9)	275.1 (9.4)	53.8 (7.9)	55.1 (2.6)	252.0 (15.6)	252.1 (12.0)	1398.3 (190.3)	1394.2 (161.7)
30 weeks										
N	10	11	28	34	36	36	36	36	35	36
Mean (SD)	76.6 (2.0)	74.5 (3)	281.1 (9.9)	278.7 (12.0)	56.3 (3.2)	57.2 (2.9)	254.9 (14.4)	256.7 (14.7)	1476.6 (196)	1504.2 (203.0)
31 weeks										
N	7	8	48	53	55	54	55	55	55	54
Mean (SD)	78.5 (4.8)	77.7 (5.9)	285.5 (39.2)	284.7 (37.5)	59.2 (3.0)	58.4 (2.7)	268.7 (16.9)	270.8 (15.0)	1692.3 (331.2)	1711.4 (229.2)
32 weeks										
N	17	18	72	88	92	95	93	95	93	95
Mean (SD)	80.8 (2.5)	79.4 (2.8)	296.9 (12.4)	297.3 (9.0)	60.7 (2.8)	61.3 (2.6)	278.0 (14.2)	281.3 (14.8)	1878.4 (226.9)	1929.5 (221.2)
33 weeks										
N	4	4	24	32	35	35	35	35	35	35
Mean (SD)	82.5 (6.0)	80.0 (5.7)	294.3 (56.7)	302.8 (11.3)	63.9 (3.0)	63.1 (2.4)	287.6 (16.6)	290.4 (16.7)	2127.2 (271.1)	2113.3 (265.8)
34 weeks										
N	9	9	39	45	49	49	49	48	49	48
Mean (SD)	86.7 (3.7)	82.7 (6.6)	310.4 (10.5)	310.7 (11.9)	64.9 (2.6)	64.5 (2.7)	293.6 (16.1)	293.4 (14.8)	2242.6 (259.6)	2217.8 (250.9)
35 weeks										
N	10	8	37	44	51	51	51	51	51	51
Mean (SD)	87.5 (4.0)	86.8 (3.7)	319.5 (8.2)	318.0 (8.4)	67.7 (5.0)	66.9 (2.6)	305.6 (23.6)	310.5 (16.4)	2495.6 (445.7)	2561.7 (271.9)
36 weeks										
N	4	4	37	48	53	52	53	53	53	52
Mean (SD)	88.6 (3.2)	88.4 (1.5)	324.9 (9.8)	322.1 (11.5)	67.3 (2.7)	67.7 (2.9)	314.6 (15.5)	313.8 (15.6)	2673.4 (303.6)	2649.5 (282.9)
37 weeks										
N	1	2	8	11	12	12	12	12	12	12
Mean (SD)	87.0 (.)	88.8 (1.1)	324.1 (8.3)	324.6 (9.3)	69.7 (3.1)	69.5 (2.6)	323.4 (16.7)	343.1 (89.1)	2882.0 (310.3)	2789.1 (266.5)

N=Sample size, SD=standard deviation, mm=millimetres.

3.6 Follow-up data

Data collection ceased on 17th July 2011. Postnatal response rates at this time can be found in Table 3.1. The ASQ-3 was introduced in March 2009. Therefore, the first 10 families were ineligible for the ASQ-3 at 3 and/or 6 months. Five families changed their contact details without notifying us, leaving them eligible but unable to contribute to further data collection.

Generally, about 50% of the eligible families returned their questionnaires before 17th July 2011. 40% of the eligible families attended the 3-month antenatal clinic appointment before this date, with a decline in attendance towards the age of 24 months (15%).

3.6.1 Parental occupation

Parental occupation was not always completed in the questionnaires. However, in general, mothers in paid employment antenatally, resumed work after 12 months. Fathers were generally in paid employment throughout the entire follow-up period.

3.6.2 Twins

As mentioned in Chapter 2, zygosity was determined based on chorionicity and gender, DNA genotyping and a zygosity questionnaire. Only 8% of the twin pairs could not be identified by any other means than the zygosity questionnaire. There was a good agreement between the questionnaire and zygosity determination by chorionity, gender and DNA genotyping (kappa=0.74). The questionnaire had a positive predictive value of 0.79, sensitivity of 0.90 and specificity of 0.87.

Table 3.4 provides details of follow-up data collected for the twins. The ASQ-3 was completed at each follow-up from 3 months onwards, whereas postnatal measurements and data from the parental questionnaires were collected at every follow-up except for 1.5 and 6 months. Data is presented for first and second born twins. A description of ASQ-3 scores of monozygotic and dizygotic twins is provided in Chapters 4-6 in which the relationships between ante-, peri- and postnatal factors and developmental skills are described.

Table 3.4 Sample size (N), means and standard deviations (SD) for first and second born twins' (displayed as 'N twin 1 / N twin 2' etc.) physical growth and developmental skills over a 2-year period.

	3 months			6 months			9 months		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Ages and Stages Questionnaires									
Communication	41 / 42	39.3 / 41.4	13.9 / 14.5	62 / 62	44.2 / 46.9	11.3 / 11.2	55 / 55	36.1 / 36	14.5 / 15.6
Gross motor	41 / 42	42.6 / 38.5	10.6 / 11.4	61 / 62	28.9 / 30.5	14.3 / 15.5	55 / 55	28.3 / 29.6	17.2 / 17.3
Fine motor	40 / 42	27.2 / 29.3	12.1 / 14.3	62 / 62	37.7 / 38.1	17.4 / 17.7	55 / 55	46.4 / 47.6	15.3 / 14.4
Problem solving	40 / 42	31.2 / 29.2	12.7 / 15.1	62 / 62	41.5 / 41.5	15.9 / 15.7	55 / 55	39.9 / 41	17.8 / 15.3
Personal social	40 / 42	36.5 / 34	13.5 / 15.1	62 / 62	34.1 / 36.5	15.7 / 16.6	55 / 55	38.7 / 39.5	13 / 13.6
Anthropometric measurements									
Weight (kg)	64 / 64	5.7 / 5.8	1.1 / 1.4	.	.	.	55 / 55	8.3 / 8.4	1 / 1.2
Length (cm)	65 / 65	59.9 / 60.9	8 / 4	.	.	.	55 / 55	71.9 / 72.4	3 / 3.3
Head circumference (cm)	66 / 66	40.4 / 40.4	1.9 / 1.8	.	.	.	55 / 55	44.4 / 44.9	4.3 / 1.6
Mid-upper arm circumference (cm)	66 / 65	12.7 / 12.7	1.2 / 1.1	.	.	.	55 / 55	14.2 / 14.3	1.1 / 1.3
Skinfold thickness - Triceps (mm)	64 / 63	9.6 / 9.8	1.8 / 1.6	.	.	.	55 / 55	9.9 / 10.5	1.8 / 2.3
Skinfold thickness - Subscapula (mm)	66 / 66	7.8 / 7.7	1.6 / 1.5	.	.	.	55 / 54	7 / 7.3	2.1 / 2.3
		Median	IQR		Median	IQR		Median	IQR
Gestational age at follow-up (weeks)	66	12	10-14	.	.	.	55	37	35-39

kg=kilograms, cm=centimetres, mm=millimetres, IQR=interquartile range.

Table 3.4 (*continued*). Sample size (N), means and standard deviations (SD) for first and second born twins' (displayed as 'N twin 1 / N twin 2' etc.) physical growth and developmental skills over a 2-year period.

	12 months			**18 months**			**24 months**		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Ages and Stages Questionnaires									
Communication	58 / 58	42.5 / 44.1	13.7 / 14.1	37 / 37	39.3 / 38.2	14 / 17.4	25 / 25	49.2 / 48.8	15.2 / 17.1
Gross motor	58 / 58	34.8 / 37.9	21.1 / 22.1	37 / 37	52.8 / 54.2	13.7 / 12.6	25 / 25	54.8 / 52.8	11 / 13.4
Fine motor	58 / 58	50.3 / 49.1	11 / 14.6	37 / 37	52.6 / 51.4	10.6 / 12.3	25 / 25	51.8 / 51.6	7.3 / 10.6
Problem solving	58 / 58	43.6 / 43.4	15.4 / 15.6	37 / 37	43.2 / 42.6	12.8 / 14.5	25 / 25	46.8 / 45.8	11 / 15.5
Personal social	58 / 58	42.4 / 41	13.9 / 15.4	37 / 37	45.3 / 43.6	10.8 / 14.3	25 / 25	45.6 / 46	11.6 / 12.4
Anthropometric measurements									
Weight (kg)	32 / 32	9.3 / 9.4	1 / 1.1	25 / 25	10.7 / 10.8	1.3 / 1.2	19 / 18	11.8 / 11.7	1.4 / 1.5
Length (cm)	32 / 32	76.1 / 76.2	2.9 / 2.8	25 / 25	82.5 / 83.4	4 / 3.5	18 / 18	88.2 / 87.7	3.8 / 3.4
Head circumference (cm)	32 / 32	46.2 / 46.4	1 / 1.4	25 / 25	47.7 / 46.2	1.5 / 6.8	19 / 18	48.4 / 48	2.1 / 2.1
Mid-upper arm circumference (cm)	32 / 32	15.6 / 14.7	5.6 / 1.3	25 / 25	15.4 / 15.1	1.2 / 1.3	19 / 18	15.1 / 15.3	0.9 / 1
Skinfold thickness - Triceps (mm)	32 / 32	10 / 10.3	2.1 / 2.1	25 / 25	9.2 / 10.7	1.5 / 8.3	19 / 18	9.8 / 9.7	1.7 / 1.5
Skinfold thickness - Subscapula (mm)	32 / 32	7 / 7.4	1.5 / 2.1	25 / 25	7.2 / 7.4	2.2 / 2.5	19 / 18	9.8 / 6.3	15.6 / 1
		Median	IQR		Median	IQR		Median	IQR
Gestational age at follow-up (weeks)	32	51	49-57	25	78	75-80	19	103	100-106

kg=kilograms, cm=centimetres, mm=millimetres, IQR=Interquartile range.

CHAPTER 4

DEVELOPMENTAL SKILLS OF TWINS IN THE FIRST TWO YEARS OF LIFE

"My older son smiled a lot earlier than the twins. They don't seem to communicate with you at all, unless they want something, and then they'll cry." – BiRTHS parent

4.1 Abstract

Background: Low birth weight and low 5-minute Apgar scores have been associated with developmental delay, while older maternal age is a protective factor. Little is known about trajectories and predictors of developmental skills in infant twins, who are generally born with lower birth weights, lower Apgar scores and to older mothers.

Methods: Developmental skills were assessed at 3, 6, 9, 12, 18 and 24 months using the Ages and Stages Questionnaires in 152 individual twins from the Birmingham Registry for Twin and Heritability Studies. Multilevel spline and linear regression models (adjusted for gestational age, gender, maternal age) were used to estimate developmental trajectories and the associations between birth weight, maternal age and Apgar scores on developmental skills.

Results: Twins performed worse than singletons on communication, gross motor, fine motor, problem solving and personal-social skills ($p<0.001$). Twins caught up around 6 months, except on gross motor skills, which did not catch up until after the age of 12 months. A one-year increase in maternal age was significantly associated with decreases in gross motor and personal-social z-scores of up to -0.09, whereas one unit increases in Apgar score increased z-scores up to 0.90 ($p<0.01$).

Conclusions: Healthy twins should be considered at a higher risk for developmental delay. Whether these results are comparable to preterm singletons, or whether there are twin-specific issues involved, should be further investigated in a study that uses a matched singleton control group and has more (longitudinal) data.

4.2 Introduction

Comparisons between children are made frequently. Informally, parents compare the physical and psychological development of their infants with that of older children they know or children of their own. Often, parents in BiRTHS would express to me how concerned they were about the apparent developmental delay of one or both twins compared with other (usually singleton) children they had seen. During formal assessments, whether at school or in a professional developmental health setting, children are compared with a control or norm group that is considered to develop normally for their age. Aside from physical development, developmental skills in the field of psychology can fall roughly into four categories: cognitive, communicative, social and emotional, and psychomotor development.

Cognitive development[86] is the ability to learn (e.g. exploring the environment during infancy) and problem solve (e.g. putting toys in a box). These skills are closely related to the frontal lobe of the brain and are sometimes referred to as executive functions. An example of a common test, which assesses these skills, is the intelligence test. *Communicative development*[87] is the ability to appropriately use and understand language. In infancy, this could be recognising simple words for colours and animals. At school age, correct use of full sentences might be expected.

Social and emotional development[88] refers to the ability to interact with others (e.g. smiling when played with) and to understand social rules (e.g. playing simple games). Finally, *psychomotor development*[89] is a combination of gross (e.g. sitting independently) and fine motor (e.g. picking up a small toy) skills. Most of these skills will become intrinsic later in life. For example, there is no need for us to think about how to walk or how to sit anymore.

Children are expected to have reached developmental milestones within certain age windows. For example, most children will have learned how to walk independently between 12 and 24 months[90]. Guidelines like these are used as a basis for developmental assessment tools, including the Ages and Stages Questionnaire (ASQ-3)[91, 92], Parents Evaluations of Developmental Status[92, 93], Bayley Scales of Infant Development[94] and Griffiths Mental Development Scales[95]. Generally, normative groups for formal assessments, such as these, consist of healthy singletons. Sutcliffe and Derom (2006)[96] addressed the question whether twins can be directly compared with singletons. Even when otherwise physically healthy, twins are likely to be born at earlier gestations and with lower birth weights. These two factors have been related to developmental delay and later cognitive skills as introduced in §1.6 and further defined below. Not only is it important for health care professionals and researchers to learn more about the development of twins, it is essential for parents to be provided with more twin-specific information. The following paragraphs describe the current knowledge of development and delays in the four developmental categories.

4.2.1 Cognitive development

Previous studies of intelligence quotients (IQ) of children between 2 and 15 years[97-99] have concluded that twins do indeed have lower IQ scores compared with singletons of the same age. However, a Dutch study of twins and their singleton siblings found no differences in IQ scores at adult age[100]. Shorter gestations, lower birth weights[54] and lower socioeconomic status[5, 56, 101] have been previously related to lower IQ scores in both singletons as well as twins. Aside from IQ, general developmental delay at the age of 2 years has been related to low Apgar scores (<7)[36, 102]. Little is known about how the aforementioned birth characteristics affect basic developmental skills in twins. Because basic developmental skills mediate cognitive abilities at older ages[103], it is important to investigate risk factors for developmental delay at the earliest possible stage to prevent any further delays at school age.

4.2.2 Communicative development

Low birth weight[54] and being male[104] have been found to have a significant negative effect on language development in singletons. However, Rutter et al. (2003)[105] suggest that obstetric and perinatal factors do not play a significant role in the apparent mild language delay (as suggested with regard to cognitive ability) of twins and emphasise the need to look for other potential causes. In her review, Thorpe (2006)[106] states that twins – boys in particular – have a higher risk of mild language delays, which normally disappear in middle childhood. Finally, she concludes that postnatal environmental factors are most likely to be the cause of language delay in twins. Socioeconomic status has not been found to have an effect on communicative development in singletons at 18 months[104], while a large twin study has found it to significantly affect verbal skills at 3 and 4 years[107]. Maternal age has been suggested to be a mediator of child development[108]. There seems to be a diversity of possible risk factors for communicative delays in twins. Similar to cognitive development, little is known about communicative skills in early infancy of twins. Because early communicative skills are an indicator of language acquisition[109, 110], it is important to determine any risk factors for developmental delay. Therefore, the effect of obstetric, perinatal and postnatal factors on communicative development in early infancy needs to be clarified, and the influence of maternal age needs to be further investigated.

4.2.3 Social and emotional development

In studies of singletons, social and emotional problems have been related to low birth weight, gestational age[55], lower socioeconomic status[56, 101] and parenting style[101]. Older maternal age seems to be a protective factor for problem behaviour in both singletons and twins[111]. Additionally, it has been suggested that children with general developmental delay (delays in at least 2 developmental areas) show less activity participation compared with their peers due to impaired social and motor skills[112]. In order to prevent problems in the social and emotional domain, and subsequent issues in social situations, it is important to better understand the effect of the abovementioned risk factors on social and emotional development at a very early age. The little available information about the social and emotional development of twins warrants for further research, especially because low birth weight, shorter gestations and older maternal age are particularly prevalent in twin pregnancies (Chapter 1).

4.2.4 Psychomotor development

Motor skills have been argued to be the basis for the development of communicative skills[113]. Twins with low birth weight have shown significant motor delays at 9 months and 2 years[54], and achieve motor milestones (e.g. rolling over and walking) at a later age compared with singleton children[114]. According to singleton research, socioeconomic status[56, 115], low birth weight[116] and gestational age[55, 117, 118] significantly influence motor skills. Preterm infants with appropriate weight-for-gestation seem to have different gross motor developmental trajectories compared with term infants in the first 18 months of life[60]. Earlier attainment of gross motor milestones has been associated with better performance on categorisation scales during assessments of executive functioning in adulthood[119]. Furthermore, it has been suggested that fine motor skills at 9, 18 and 24 months are strong predictors of later intellectual functioning[118] and general academic achievements at school age[59, 117]. Research also suggests that motor development may be more sensitive to prematurity than other developmental skills[58]. It is important to investigate its effect on motor skills in twins, who are generally born premature compared with singletons.

4.2.5 Rationale of this chapter

According to the abovementioned studies, low birth weight, shorter gestations, low socioeconomic status, older maternal age, parenting style, and low Apgar scores are related to the delay of cognitive, communicative, social-emotional and psychomotor skills in singletons. Most of these risk factors are also related to twin pregnancies (Chapter 1), suggesting that twin pregnancies could be an additional risk factor for developmental delays. Although some studies report catch-up of motor skills in preterm children within the first 2 years[57, 60] and of communicative skills in middle childhood[106], none (to my knowledge) studied delays in communicative and social-emotional domains. Because previous studies have mainly focused on risk factors for developmental delay after the first 2 years and the basic developmental skills are important for later cognitive abilities[59, 103, 117, 118], there is a gap of knowledge in the published literature, especially in the field of twin research.

4.2.6 Aims of this chapter

This chapter aims to:

- describe developmental trajectories of healthy twins between the ages of 3 and 24 months.
- explore the age at which twins catch-up to existing developmental singleton-based standards.
- investigate the effect of birth weight, maternal age and Apgar score on developmental skills in the first 2 years in order to determine whether these factors have similar effects as have been found in most previous research or no effect at all as reported by Rutter (2003)[105].

The term 'catch-up' in this chapter is defined as age-appropriate performance on developmental assessment of children, who initially showed developmental delays compared with their peers.

4.3 Methods

4.3.1 Study design and participants

Participants were part of the Birmingham Registry for Twin and Heritability Studies (BiRTHS)[120]. Pregnant mothers, who were between 12 and 28 weeks of gestation, were recruited from specialist multiples clinics in three Birmingham (UK) hospitals between 4th August 2008 and 15th July 2011. Upon joining the registry, parents were asked to complete a questionnaire with which demographic, lifestyle and social support details were collected. After the twins were born, follow-up questionnaires about the twins' development were sent to parents at 3, 6, 9, 12, 18 and 24 months. Finally, maternal medical history and delivery details were extracted from maternal medical records.

For the purpose of the analyses described in this chapter, families were selected from the BiRTHS cohort if delivery details were available and at least one of the six follow-up questionnaires were completed.

4.3.2 Materials

Maternal medical records provided data on gender, Apgar scores and birth anthropometry. Maternal age and ethnic background were extracted from the antenatal parental questionnaire. Developmental skills at 3, 6, 9, 12, 18 and 24 months were assessed with the ASQ-3[91, 92], a standardised screening tool for developmental delays in communicative, gross motor, fine motor, problem solving and personal-social skills (Chapter 2).

Scoring the ASQ-3

Each subscale of the ASQ-3 consists of six questions, which parents answer with 'yes', 'sometimes' or 'not yet'. These answers correspond with scores of 10 for 'yes', 5 for 'sometimes' and 0 for 'not yet', resulting in a range of scores between 0 and 60 for each subscale. Clinical cut-off scores are provided with the ASQ-3 set and based on a norm sample of healthy singletons, who were born at 40 weeks[83]. Based on the clinical cut-off scores, there are three categories in which a child can be placed: 'normal development' (above -1SD), 'cause for concern and further monitoring needed' (between 1 and 2 standard deviations below the mean) and 'referral for further developmental assessment needed' (more than 2 standard deviations below the mean).

4.3.3 Statistical analyses

Descriptive data

All analyses were performed in STATA 11 (StataCorp, 2009)[85]. Raw ASQ-3 scores were converted into z-scores using the norm sample mean, -1SD (1 standard deviation below mean) and -2SD (2 standard deviations below mean) cut-off scores and their given standard deviations for each questionnaire. T-tests were performed to investigate differences between twins' raw ASQ-3 scores and scores of the singleton norm sample.

Developmental trajectories

For this longitudinal approach to investigate initial developmental delays and catch-up, exploratory analyses showed nonlinear patterns for developmental skills over time. Therefore, multilevel spline regression models[121] were used in which repeated measures within each person and relatedness of twins were included as random effects in nested levels. Confounders that were included in the fixed part of the model were gestational age at birth, gender and maternal age. Predicted values from these models were used to graphically display the nonlinear developmental trajectories for each ASQ-3 subscale.

Determinants of developmental skills

Cross-sectional multilevel linear regression analyses were performed to investigate the associations between birth weight, maternal age and Apgar scores at 5 minutes on the ASQ-3 subscales at each follow-up. Although twins were considered individuals in these analyses, their relatedness was taken into account by including this in a nested level as a random effect. Adjustments for gestational age at birth and gender were included in the fixed portion of the model, and results were considered statistically significant at $\alpha=0.01$ due to multiple testing.

4.4 Results

4.4.1 Participants

A total of 152 healthy individual twins from BiRTHS (Chapter 2-3) were included. Only one follow-up was available for 33 twins (22%), two follow-ups for 18 twins (12%), three follow-ups for 43 twins (28%), four follow-ups for 28 twins (18%), five follow-ups for 26 twins (17%) and parents for 4 twins (3%) completed all six follow-up questionnaires.

The median gestational age at birth was 37 weeks, ranging from 26 to 39 weeks. Mothers' average age at delivery was 31.9 years (standard deviation (SD)=5.5). 87% of parents were White. Mean birth weight was 2.3 kilograms (kg; SD=0.5) with median Apgar score of 9 (range: 4-10).

4.4.2 Descriptive data

Significant differences with the singleton norm group were present for mean scores on gross motor, problem solving and personal-social subscales at 3, 6, 9 and 12 months ($p<0.001$; Table 4.1). Scores on the communication and fine motor subscales were significantly lower than the norm group mean scores at 3, 6 and 9 months ($p<0.001$). Furthermore, twins' performance on fine motor and personal-social skills at 3 months, and gross motor skills at 3 and 6 months, fell below the -1SD cut-off score ($p<0.001$). Problem solving skills at 3 months were significantly worse than the -2SD cut-off score (t=-2.43, $p=0.01$).

Table 4.1 Means and z-scores for ASQ-3 subscales at each follow-up between 3 and 24 months.

	Communication	Gross motor	Fine motor	Problem solving	Personal-social
3 months (N = 66)					
Mean (SD)	42.82 (13.61)*	40.15 (11.67)*	28.47 (14.28)*	30.46 (14.97)*	35.31 (14.97)*
z-score (SD)	-1.18 (1.54)	-1.79 (1.44)	-2.11 (1.30)	-2.48 (1.59)	-1.77 (1.60)
6 months (N = 96)					
Mean (SD)	46.00 (11.25)*	29.74 (14.74)*	38.75 (17.49)*	42.97 (15.54)*	36.35 (16.25)*
z-score (SD)	-0.30 (1.17)	-1.36 (1.26)	-0.86 (1.47)	-0.66 (1.37)	-1.04 (1.42)
9 months (N = 90)					
Mean (SD)	35.83 (14.65)*	29.39 (16.74)*	47.00 (15.19)*	41.50 (16.83)*	39.78 (13.34)*
z-score (SD)	-0.22 (1.19)	-1.20 (1.16)	-0.51 (1.45)	-0.77 (1.62)	-0.23 (1.13)
12 months (N = 100)					
Mean (SD)	44.15 (14.32)	36.65 (21.73)*	50.50 (12.30)	45.66 (14.70)*	43.15 (14.71)*
z-score (SD)	0.07 (1.04)	-0.93 (1.53)	-0.19 (1.39)	-0.31 (1.36)	-0.22 (1.23)
18 months (N = 67)					
Mean (SD)	40.30 (15.05)	54.03 (12.92)	53.06 (10.37)	44.40 (12.81)	46.04 (11.20)
z-score (SD)	-0.14 (1.03)	-0.16 (1.43)	0.07 (1.14)	-0.16 (1.26)	-0.18 (1.08)
24 months (N = 45)					
Mean (SD)	51.44 (14.45)	54.22 (12.48)	52.00 (9.32)	46.67 (13.78)	46.67 (11.92)
z-score (SD)	0.02 (1.11)	-0.06 (1.50)	0.04 (1.13)	-0.28 (1.40)	-0.46 (1.22)

Raw score for all ASQ-3 subscales range from 0 to 60, z-scores are based on singleton population means and standard deviations for each age and subscale. Significant differences with the norm scores are displayed with *p<0.001.
ASQ-3=Ages and Stages Questionnaires (3rd edition), N=sample size, SD=standard deviation.

4.4.3 Developmental trajectories

Figure 4.1 shows the developmental trajectories based on median ASQ-3 z-scores, adjusted for gender, gestational age at birth and maternal age. As described earlier in the cross-sectional descriptive data, it confirms overall improvement in communication, gross motor, fine motor, problem solving and personal-social skills between 3 and 24 months. Scores at 3 months were well below -1SD for all subscales, except for communication. Twins caught up to the singleton norm group by the age of 6 months on fine motor and problem solving skills. Catch-up for personal-social and gross motor skills was not seen until 9 and 18 months, respectively.

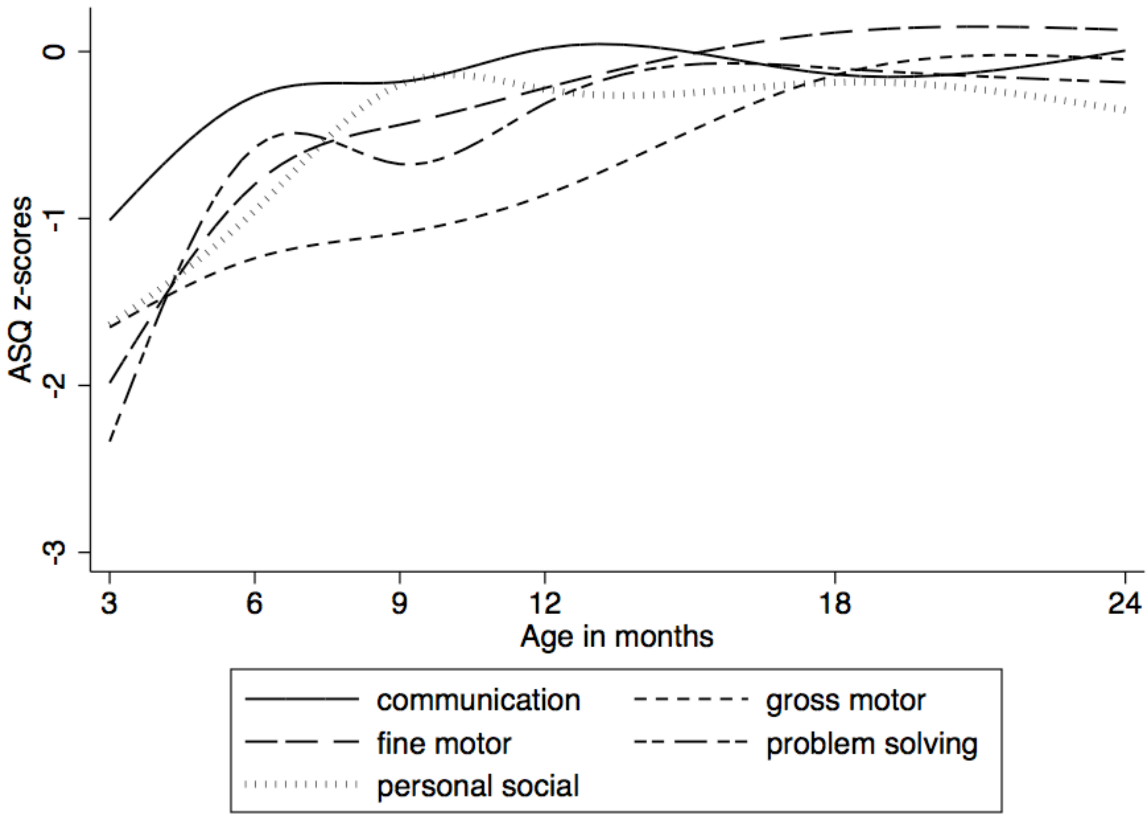


Figure 4.1 Developmental trajectories of healthy twins from 3 to 24 months, as assessed by the Ages and Stages Questionnaires (3rd edition). Data are adjusted for gestational age at birth, gender and maternal age.

When stratified by birth weight, Apgar score, gender and maternal age categories (above or below mean/median), it was noticeable that even though no statistically significant differences were found in mean scores between these groups, children with lower birth weight, lower Apgar scores and higher maternal age generally had lower scores on all subscales throughout the first two years (see Appendix V for graphs).

4.4.4 Determinants of developmental skills

Table 4.2 describes the effects of birth weight, Apgar score and maternal age on developmental skills. Birth weight had no significant effect on developmental skills at $p=0.01$, although there was a positive trend for higher birth weight and 6-month personal-social skills ($p=0.04$).

A point increase in Apgar score was associated with a 0.81 increase in gross motor skills at 18 months ($p<0.001$) and a 0.90 increase at 24 months ($p<0.01$). Similar trends were found for fine motor and problem solving at 24 months ($p=0.02$). Older maternal age, on the other hand, seemed to have a predominantly negative effect on developmental skills. A one-year increase in maternal age was associated with decreases on communication z-scores of 0.08 at 9 months ($p<0.01$), 0.06 at 12 months ($p<0.001$), 0.05 at 18 months ($p=0.04$) and 0.06 at 24 months ($p=0.01$). Similarly, older maternal age decreased problem solving by 0.09 at 9 months ($p=0.01$) and by 0.07 at 24 months ($p=0.01$). Personal-social skill z-scores decreased by 0.07 at 9 months ($p<0.01$) for each year's increase in maternal age. There were negative trends for maternal age and 18-month communication, 12-month gross motor, 9- and 12-month fine motor, and 3- and 12-month personal-social skills ($p=0.04$).

Table 4.2 Increase in mean ASQ-3 z-scores for each additional kilogram (kg) in birth weight, year in maternal age and point in Apgar score, displayed for each subscale at each follow-up between 3 and 24 months.

	3 months (N = 66) Coef. (95% CI)	6 months (N = 96) Coef. (95% CI)	9 months (N = 90) Coef. (95% CI)	12 months (N = 100) Coef. (95% CI)	18 months (N = 67) Coef. (95% CI)	24 months (N = 45) Coef. (95% CI)
Communication						
Birth weight (kg)	0.70 (-0.20 - 1.61)	0.18 (-0.42 - 0.77)	0.40 (-0.17 - 0.98)	-0.22 (-0.76 - 0.33)	0.24 (-0.44 - 0.91)	0.31 (-0.65 - 1.27)
Maternal age (years)	-0.02 (-0.10 - 0.06)	-0.01 (-0.05 - 0.04)	-0.08 (-0.13 - -0.03)**	-0.06 (-0.1 - -0.03)***	-0.05 (-0.09 - 0.00)*	-0.06 (-0.12 - -0.01)**
Apgar at 5 minutes	-0.48 (-0.99 - 0.03)	-0.15 (-0.42 - 0.11)	0.04 (-0.13 - 0.40)	0.21 (-0.02 - 0.43)	0.17 (-0.13 - 0.46)	0.26 (-0.21 - 0.73)
Gross motor						
Birth weight (kg)	0.25 (-0.57 - 1.08)	0.35 (-0.31 - 1.00)	0.35 (-0.24 - 0.93)	0.00 (-0.81 - 0.82)	0.18 (-0.61 - 0.97)	0.32 (-0.89 - 1.53)
Maternal age (years)	-0.02 (-0.10 - 0.05)	0.03 (-0.02 - 0.08)	-0.05 (-0.10 - 0.01)	-0.06 (-0.11 - -0.01)**	-0.05 (-0.10 - 0.00)	-0.05 (-0.12 - 0.03)
Apgar at 5 minutes	0.19 (-0.27 - 0.66)	-0.08 (-0.37 - 0.22)	0.08 (-0.28 - 0.45)	0.14 (-0.20 - 0.48)	0.81 (0.51 - 1.11)***	0.90 (0.34 - 1.46)**
Fine motor						
Birth weight (kg)	-0.03 (-0.78 - 0.72)	0.35 (-0.33 - 1.04)	0.15 (-0.51 - 0.80)	0.14 (-0.57 - 0.85)	0.23 (-0.51 - 0.97)	-0.16 (-0.94 - 0.63)
Maternal age (years)	-0.05 (-0.11 - 0.02)	-0.03 (-0.09 - 0.02)	-0.06 (-0.12 - 0.00)*	-0.05 (-0.09 - 0.00)*	-0.05 (-0.09 - 0.00)	-0.05 (-0.10 - 0.00)*
Apgar at 5 minutes	0.15 (-0.27 - 0.58)	-0.01 (-0.32 - 0.29)	0.15 (-0.26 - 0.55)	0.20 (-0.09 - 0.48)	0.09 (-0.24 - 0.42)	0.45 (0.07 - 0.83)*
Problem solving						
Birth weight (kg)	-0.33 (-1.21 - 0.54)	0.27 (-0.33 - 0.87)	0.32 (-0.39 - 1.04)	-0.01 (-0.77 - 0.74)	-0.13 (-0.95 - 0.69)	0.26 (-0.75 - 1.26)
Maternal age (years)	-0.03 (-0.12 - 0.05)	-0.03 (-0.08 - 0.02)	-0.09 (-0.15 - -0.02)**	-0.03 (-0.08 - 0.01)	-0.03 (-0.09 - 0.02)	-0.07 (-0.13 - -0.01)**
Apgar at 5 minutes	0.37 (-0.13 - 0.86)	0.03 (-0.24 - 0.30)	-0.10 (-0.54 - 0.33)	-0.07 (-0.39 - 0.24)	0.08 (-0.29 - 0.44)	0.50 (0.02 - 0.98)*
Personal-social						
Birth weight (kg)	-0.32 (-1.22 - 0.58)	0.67 (0.02 - 1.32)*	0.23 (-0.29 - 0.75)	-0.18 (-0.82 - 0.46)	-0.19 (-0.97 - 0.60)	0.52 (-0.45 - 1.49)
Maternal age (years)	-0.09 (-0.18 - -0.01)*	-0.02 (-0.07 - 0.03)	-0.07 (-0.11 - -0.02)**	-0.04 (-0.08 - 0.00)*	-0.02 (-0.06 - 0.03)	-0.05 (-0.11 - 0.00)
Apgar at 5 minutes	0.42 (-0.08 - 0.93)	-0.01 (-0.31 - 0.30)	0.08 (-0.24 - 0.41)	0.18 (-0.09 - 0.45)	-0.05 (-0.39 - 0.30)	0.10 (-0.40 - 0.60)

All results are adjusted for gestational age at birth and gender. *p<0.05, **p<0.01, ***p<0.001.
ASQ-3=Ages and Stages Questionnaires (3rd edition), Coef. (95% CI)=regression coefficient (95% confidence interval).

4.5 Discussion

In this chapter, I have demonstrated that healthy twins score below the normal range on a singleton-based assessment in the first year of life, even when prematurity was taken into account. Furthermore, the findings suggest that maternal age, as opposed to birth weight and 5-minute Apgar scores, mostly influences developmental skills in infancy.

4.5.1 Twin-singleton differences

As expected, twin-singleton differences up to 9 months were found on all domains of the ASQ-3. However, some studies have found that twin-singleton differences increase with age[105, 122, 123], while I found the opposite. I did not find statistically significant gender differences on communicative performance[104, 106]. Furthermore, studies of older twins found within pair differences in developmental assessments[122, 123], which were not found in the current study. Differences between the age of the sample in this study and previous studies could explain these findings. Follow-up of the current sample would be needed to further explore this possibility.

4.5.2 Developmental trajectories

The speed with which twins caught up with singletons varied per skill as well as age. Performance on the communication subscale was the most stable over time, while scores for gross motor skills were one of the last to reach a plateau. This could have been expected, considering the vulnerability of psychomotor skills as described in §4.2.4 and findings of low stability of motor skills from birth up to school age[124, 125]. Catch-up on all subscales, except for gross motor skills, occurred before the age of 9 months, which is earlier than previous studies have reported[60, 106]. It is most likely that this is due to the assessment in the studies, which may measure slightly different aspects of each domain. Nevertheless, this study seems to agree that healthy twins cannot be simply considered comparable to healthy singletons or ‘at-risk’ infants (e.g. born at <33 weeks gestation and <1500 grams birth weight) and seem to follow a developmental path which is comparable to the ‘low-risk’ group consisting of singletons born 33-37 weeks with normal birth weight and Apgar score >7, as describe by Gasson and Piek (2003)[57]. Also, it seems that even within the group of healthy twins in BiRTHS, a distinction might be made between ‘at-risk’ and ‘low-risk’ infants. It would therefore be advisable for healthy twins to be considered as having a higher risk of developmental delay within the first 2 years compared with healthy singletons. However, comparisons with a matched singleton control group and ‘at-risk’ twins are needed to provide more definitive conclusions. Also, considering the findings from this study and aforementioned studies, it is likely that developmental skills have not fully stabilised by the age of 24 months and further follow-up is needed to explore how these skills develop beyond infancy into toddlerhood and early childhood.

4.5.3 Determinants of developmental skills

Overall, older maternal age seemed to have a negative effect on developmental skills within the first 24 months. This contradicts findings from previous studies in which these effects disappear after the first year, as children have caught up[57] and where maternal age has been found to be a protective factor[111]. The lack of significant findings at younger ages may be due to a delayed effect and the influence of the proposed predictor (maternal age in this case) might become more apparent after the first two years, as reported by Goyen and Lui (2002)[61]. Older mothers are more likely to return to work within the first year after birth[126], and older maternal age has been indirectly related to delayed motor and social development[126], as well as cognitive development at school age[127, 128]. Maternal age as a mediator for factors, such as mother-child interaction[129], educational level and maternal occupational commitments[126], could explain the contradictory findings in the current study. The effect of maternal age needs to be further investigated by including postnatal maternal and mother-child factors.

Higher Apgar scores were advantageous for gross motor skills at 18 and 24 months. Again, this contradicts findings from previous studies where effects had been found at younger ages[57] and could be due to delayed effects, which would present themselves later in childhood[61]. Compared with maternal age, Apgar score did not seem to have much influence on developmental skills. This could be because there is simply no effect to be found, or that there might be other perinatal factors that mediate the influence of Apgar scores[130, 131].

Also contrary to previous studies[54, 116], but in line with Rutter et al. (2003)[105], results from the current study show that birth weight has no or little effect on developmental scores throughout the first 2 years. It is possible that this is because the current sample consisted of younger children, compared with the aforementioned studies, and that effects will only become noticeable at early adolescence[132].

4.5.4 Limitations

It is important to replicate the findings presented in this chapter with a sample in which follow-up is more complete. Although sample size varied at each age, there were no significant differences in average and range for birth weight, gestational age at birth and maternal age at each follow-up. Nevertheless, a larger sample and a singleton control group would be desirable to confirm the results in this chapter and to investigate whether there is indeed a twin-singleton difference in predictors of developmental skills.

Further follow-up is also needed to determine within-twin pair difference beyond infancy and the effect of environmental factors in childhood, such as twin-twin interaction[123], type of childcare and parent-infant interaction[123, 129]. Particularly communicative skills could be affected by parent-child and twin-twin interaction that is specific to raising young same-aged children[105].

Ethnicity was not included in my analyses, as the addition of ethnicity did not significantly improve the regression model. However, previous studies have found that ethnicity influences parents' child rearing choices, which in turn have an effect on child development[5, 129, 133]. It seems that ethnicity might indirectly influence child development through the abovementioned environmental factors. As with twin-singleton and within twin pair differences, these environmental influences could emerge later in life. It would be recommendable for future studies to include ethnicity and environmental factors as potential predictors of developmental skills.

4.5.5 Conclusion

Although the data in the current study did not allow for longitudinal analyses, healthy twins did score below the normal range on current singleton norms for cognitive, communicative, social and emotional, and psychomotor development at every follow-up in the first year of life.

Considering the previous literature on the development of twins compared with singletons, my finding that twins are delayed in early infancy seems to be reasonable. Whether these results are comparable to preterm singletons, or whether there are twin-specific issues involved, should be further investigated in a study that uses a matched singleton control group. Nonetheless, twins should be considered at a higher risk for developmental delay and those who formally assess the development of twins at this early age should be aware of this when reporting their findings to parents. Birth weight and Apgar scores do not seem to be as influential as maternal age at this stage, but may become more important later in life. Similarly, further investigation is needed into environmental influences on child development as they emerge at older ages.

Results should be confirmed by using more comprehensive assessments of child development as well as possible influential factors. Moreover, a longer follow-up period and a larger, more complete dataset are needed to further investigate long-term effects of perinatal, maternal and environmental factors on child development, and determine when developmental skills fully stabilise after 24 months.

CHAPTER 5

ANTENATAL GROWTH AND DEVELOPMENTAL SKILLS IN EARLY INFANCY

"She said that I am not feeding my twins, because they're not growing enough and they haven't attempted crawling yet." – BiRTHS parent about a health visitor

5.1 Abstract

Background: Antenatal head circumference is suggested to be a predictor of development in singletons. Antenatal head circumference is known to be smaller in twins, potentially increasing the risk of developmental delays, yet this has not yet been investigated. I aimed to investigate the effect of head circumference size at on twins' development in infancy.

Methods: Developmental skills of 117 individual twins from the Birmingham Registry for Twin and Heritability Studies were assessed with the Ages and Stages Questionnaires at 3, 6, 9, 12, 18 and 24 months, for which z-scores were calculated. Head circumference was obtained from antenatal ultrasound scans. Multilevel regression analyses, adjusted for chorionicity, maternal age and gestational age at birth, were performed using head circumference at 20, 28, 33 and 36 weeks gestation, and head circumference growth between 20-27, 28-32 and 33-36 weeks gestation.

Results: Head circumference size before 36 weeks gestation was not associated with developmental skills at any follow-up age. A 1-millimetre increase above average 36-week head circumference and 33-36 week head circumference growth were associated with decreased gross motor and personal-social skills (p<0.01).

Conclusions: Findings in this chapter contradict previous studies. It is possible that 30 weeks gestation (in previous studies) was not sufficiently late in pregnancy to observe any negative associations with developmental skills. It is also possible that negative associations between antenatal head circumference with developmental skills can be explained by insufficient antenatal pruning of synapses.

5.2 Introduction

In Chapter 4, I described the negligible effect of birth weight on early development in a twin sample from BiRTHS. This contradicts previous findings of birth weight effects on later cognitive development, as outlined in §4.1. In Chapter 1, I explained that antenatal growth of twins is known to decrease compared with that of singletons from 28-32 weeks onwards[24, 134-136], which (together with shorter gestations) generally results in lower birth weights compared with healthy singletons[49]. In infancy, the effect of birth weight on developmental skills may be obscured by a child's antenatal wellbeing[137]. Together with vital functions such as heart rate, antenatal growth is an important indicator of antenatal wellbeing. Although many studies have approximated the relationship between antenatal growth and developmental skills by using birth weight (Chapter 4), the actual relationship has not yet been investigated much.

5.2.1 Antenatal growth measurements

As described in Chapter 2, antenatal growth in twins is measured by ultrasound scan at regular intervals: every 2 weeks from booking (~12 weeks) onwards in monochorionic pregnancies and every month from 20 weeks gestation onwards in dichorionic pregnancies. Standard measurements are biparietal diameter, head circumference, femur length and abdominal circumference.

Biparietal diameter and abdominal circumference in twin pregnancies show significant decreases in growth after about 32 weeks compared with singletons[10, 138]. Growth of head circumference in twins decreases from approximately 26 weeks onwards[139]. Finally, femur length throughout pregnancy is similar for twins and singletons[138, 140]. The growth difference from singletons[24, 134-136] makes twins an interesting study population as they could be at higher risk for developmental delays compared with singletons.

The growth measurements, which I have described above, are normally used to estimate foetal weight. However, because twins are likely to fall below the 10th centile for singleton birth weights in the last trimester[141, 142], the conventional formulae for estimated foetal weight are not very reliable in multiple pregnancies[143]. Therefore, estimated foetal weight will not be included in this chapter.

5.2.2 Antenatal growth and developmental skills

To my knowledge, no evidence has been previously found for the relationship between developmental skills and femur length or abdominal circumference. Furthermore, only four studies looked into the relationship between antenatal growth measurements and postnatal development in singletons, with conflicting results[144-147]. It remains unclear whether growth before or from mid-pregnancy onwards is more important in relation to postnatal development. In a study of healthy singletons, Walker et al. (2007)[144] found a positive relationship between larger head circumference at 14 weeks and better reasoning ability at school age, while Harvey et al. (1982)[145] found that decreased head growth prior to 26 weeks of gestation was related to general cognitive delays and motor impairments at 5 years. Additionally, no relationship between reasoning ability at school age with head circumference at 25 or 35 weeks of gestation[144] has been found, nor a relationship between head circumference at 18 weeks gestation and intelligence at age 9[147]. The Generation R Study, a large Dutch cohort, made a distinction between *size* and *growth*. They found that both *size* and *growth* – particularly the ratio between abdominal circumference and head circumference – from mid-pregnancy onwards were positively related to neuromotor development in the first three months[148]. Using the same cohort, Henrichs et al. (2010)[146] found that increased head *growth* from mid-pregnancy onwards reduces the risk of overall developmental and social delays at the age of one. The contradictory findings in the aforementioned studies may be due to the age at which the children were assessed. However, it is also possible that antenatal *size* has a different effect on postnatal development compared with antenatal *growth*; perhaps there are gestational windows in which growth is particularly important for postnatal development, similar to critical age windows in childhood[89, 149], which cannot be identified when only looking at size.

5.2.3 Rationale of this chapter

Because the basic developmental skills are important for later cognitive functioning[103], it is essential to better understand the effect of antenatal size and growth on developmental skills from the earliest possible age onwards. Twins could be at higher risk for developmental delays compared to singletons as head circumference growth of twins decreases from approximately 26 weeks onwards[139]. Furthermore, maternal age in twin pregnancies is often older[13] and has been associated with child development[108, 111] as well as antenatal growth[150], potentially increasing twins' risks of developmental delays even further.

In this chapter, I will only make use of the antenatal head circumference measurements obtained from the ultrasound scans, as there is no evidence that femur length or abdominal circumference would be related to psychological development at all and biparietal measurements were very incomplete. I did not include the ratio between abdominal circumference and head circumference in the current study, as the mean ratio within the BiRTHS cohort was 0.91 with a standard deviation of 0.04 and would therefore not likely be useful in statistical analyses.

5.2.4 Aim of this chapter

This chapter aims to:

- describe the relationship between antenatal head circumference *size* and *growth* and developmental skills in the infancy period of twins.

5.3 Methods

5.3.1 Study design and participants

Similar to Chapter 4, participants were part of the Birmingham Registry for Twin and Heritability Studies (BiRTHS)[120] and the same clinical records, antenatal and follow-up questionnaires were used.

For the purpose of the analyses described in this chapter, families were selected from the BiRTHS cohort if antenatal ultrasound details, maternal details and at least one of the six follow-up questionnaires were completed.

5.3.2 Data

Details on maternal age and ethnic background were assessed by means of antenatal questionnaires. Maternal obstetric details, such as parity, gestational age at delivery and chorionicity were extracted from maternal medical notes. Developmental skills at 3, 6, 9, 12, 18 and 24 months were assessed with the Ages and Stages Questionnaires (ASQ-3)[91, 92]. A detailed description of this questionnaire can be found in Chapters 2 and 4.

Antenatal head circumference measurements were obtained from ultrasound reports. Based on scan protocol at the hospitals[20] and a review by Lumley (2003)[151], ultrasound data were categorized into three age window, in which growth was likely to be most linear: 20-27 weeks, 28-32 weeks and 33-36 weeks of gestation. Growth was defined as absolute growth in millimetres (mm) within each age window. From these age windows, I also used size at 20, 28, 33 and 36 weeks to determine their effects on developmental skills.

5.3.3 Statistical analyses

Descriptive data

All analyses were performed in STATA 11[85]. Similar to the analyses in Chapter 4, ASQ-3 scores on all subscales were converted into z-scores using the means and standard deviations, which were provided for the norm sample consisting of healthy singleton children born at 40 weeks[83]. Subsequent analyses were performed using these z-scores. T-tests were performed to determine any significant difference in ASQ-3 z-scores between our sample and the norm group. Wilcoxon signed-rank tests were performed to explore any growth differences across the age windows and between chorionicity.

The association of antenatal head circumference with developmental skills

Antenatal growth measurements were interpolated if scans were not available for the exact cut-off points for each age window. Outliers, defined as <1st centile or >99th centile for each measurement, were not included in the analyses (N=1). Multilevel linear regression analyses were performed to study the effect of relative antenatal size at each measurement and antenatal growth in each age window on communication, motor, problem solving and personal-social skills. Although twins were considered individuals in these analyses, their relatedness was taken into account by including this in a nested level as a random effect. In the fixed part of the model, I adjusted for gestational age at birth and maternal age. Based on previous literature[55, 150] and findings in Chapter 4, I investigated the effect adding maternal age to the regression model and found that the regression coefficient changed by >10%, justifying inclusion as a potential confounder. Regression models for head circumference size at 20 and 36 weeks and growth in 33-36 weeks gestation were further adjusted for chorionicity, following significant between-twin pair differences in antenatal head measurements. Additionally, models for antenatal growth were also corrected for foetal size at the beginning of each age window. Results were considered statistically significant if *p*-values were smaller than 0.01, due to multiple testing.

5.4 Results

5.4.1 Participants

One hundred and seventeen individual twins were included, of which 52 (44%) had only one set of completed ASQ-3 questionnaires, 12 (10%) had two completed sets, 24 (21%) had three sets, nine (8%) had four sets, four (3%) had five completed sets and 16 (14%) completed all six of the ASQ-3 questionnaires. Sample size at each specific follow-up age can be found in Table 5.2

Twins were born at a median age of 36 weeks (range: 28-39) with a mean birth weight of 2.4 kilograms (kg; standard deviation (SD)=0.5). Mothers' average age at delivery was 31.4 years (SD=4.7). 93% of parents were White and 52% of twins were male.

5.4.2 Descriptive data

Antenatal growth

Table 5.1 provides an overview of ultrasound measurements at four gestations and antenatal growth in three age windows. Overall, head circumference growth decreased from 9.4mm/week in 20–27 weeks to 8.9mm/week in 28–32 weeks and 7.6mm/week in 33–36 weeks.

Table 5.1 Mean head circumference size at 20, 28, 33 and 36 weeks, and absolute mean growth between 20-27, 28-32 and 33-36 weeks for twins included in the analyses.

	Mean size (SD)		Mean growth (SD)
20 weeks	172.7 (6.8)	20 - 27 weeks	75.2 (9.8)
28 weeks	249.4 (8.0)	28 - 32 weeks	44.5 (9.6)
33 weeks	296.5 (12.4)	33 - 36 weeks	30.2 (13.5)
36 weeks	327.1 (21.7)		

SD = Standard deviation. All measurements are in millimetres.

Developmental skills

Mean scores and z-scores on all ASQ-3 subscales can be found in Table 5.2. Results were very similar to findings presented in Chapter 4. Twins performed significantly worse than the singleton norm group on all subscales (p<0.01). They caught up on different skills at various ages up to 24 months, except for personal-social skills, which remained worse compared with the norm group (t=-2.80, p<0.01).

Table 5.2 Means and standard deviations of raw ASQ-3 scores and their corresponding z-scores at all follow-up ages for twins included in the analyses.

	Communication	Gross motor	Fine motor	Problem solving	Personal social
3 months (N=50)					
Mean (SD)	40.6 (14.5)**	40.5 (11.3)**	27.8 (13.7)**	30.3 (14.4)**	35.2 (14.5)**
z-scores (SD)	-1.3 (1.6)	-1.7 (1.4)	-2.2 (1.2)	-2.5 (1.5)	-1.8 (1.5)
6 months (N=82)					
Mean (SD)	45.8 (11.3)*	30.5 (15.1)**	39.3 (17.2)**	42.8 (15.2)**	36.2 (16.2)**
z-scores (SD)	-0.3 (1.2)	-1.3 (1.3)	-0.8 (1.4)	-0.7 (1.3)	-1.1 (1.4)
9 months (N=77)					
Mean (SD)	36.3 (15.3)	27.7 (16.7)**	46.9 (14.9)**	41.4 (16.2)**	39.5 (13.7)
z-scores (SD)	-0.2 (1.2)	-1.3 (1.2)	-0.5 (1.4)	-0.8 (1.6)	-0.2 (1.2)
12 months (N=79)					
Mean (SD)	43.5 (14.4)	36.3 (20.9)**	50.7 (12.4)	45.0 (15.2)*	42.5 (14.8)
z-scores (SD)	0.2 (1.0)	-1.0 (1.5)	-0.2 (1.4)	-0.4 (1.4)	-0.3 (1.2)
18 months (N=56)					
Mean (SD)	38.7 (15.7)	54.0 (12.8)	52.4 (11.5)	43.3 (14.0)	44.5 (12.9)
z-scores (SD)	-0.2 (1.1)	-0.2 (1.4)	0.0 (1.3)	-0.3 (1.4)	-0.3 (1.2)
24 months (N=38)					
Mean (SD)	48.6 (16.9)	54.1 (12.2)	51.3 (9.2)	46.4 (13.6)	45.9 (12.4)*
z-scores (SD)	-0.2 (1.3)	-0.1 (1.5)	-0.1 (1.1)	-0.3 (1.4)	-0.5 (1.3)

Z-scores were calculated by using the means and standard deviations (SD) for the singleton norm group provided with the ASQ-3. Significant differences with singleton norm scores are displayed with *p<0.01, **p<0.001.

5.4.3 The association of antenatal head circumference size with developmental skills

Head circumference size before 36 weeks gestation was not associated with developmental skills at any follow-up age. Furthermore, increased head circumference at 36 weeks was only associated with significant changes in gross motor and personal-social z-scores (Figure 5.1). Larger 36-week head circumference was significantly associated with gross motor z-score decreases of -0.03 (95% CI -0.06--0.01) at 3 months, -0.02 (95% CI -0.03-0.00) at 12 months and -0.02 (95% CI -0.03--0.01) at 18 months. Also, larger head circumference at 36 weeks gestation was significantly associated to a -0.04 (95% CI -0.07--0.02) decrease in personal-social z-scores at 3 months.

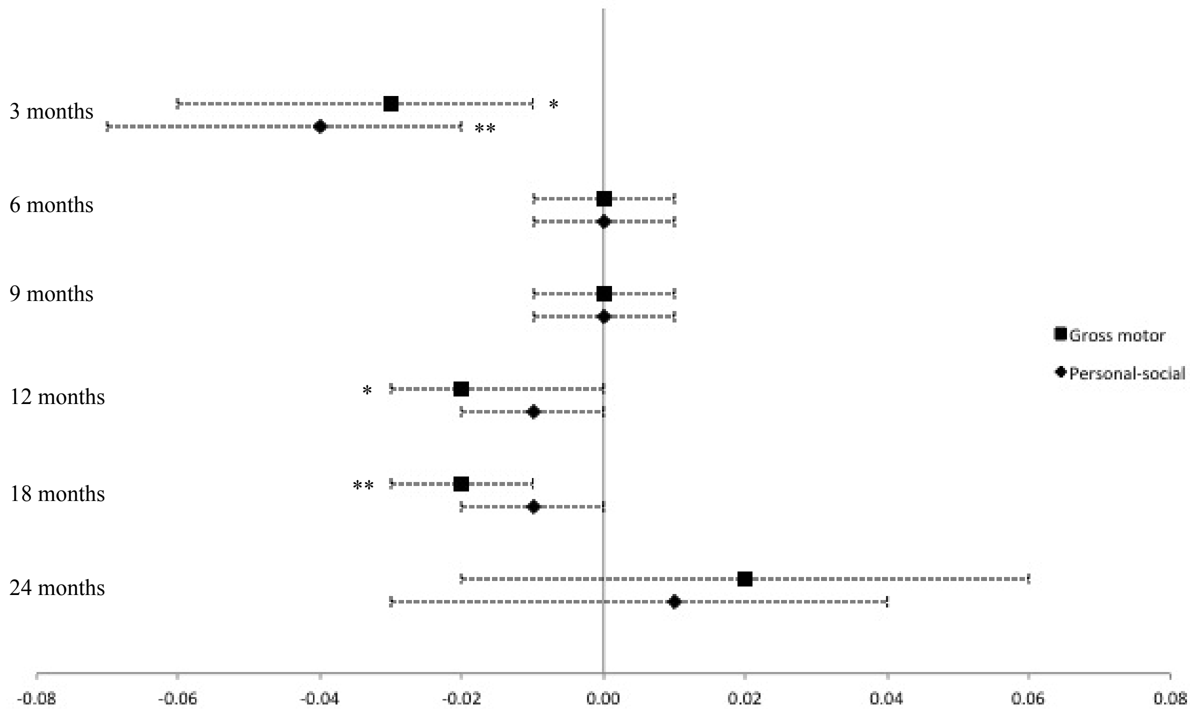


Figure 5.1 Change in postnatal gross motor and personal-social z-scores for each millimetre increase in head circumference size at 36 weeks.
*$p<0.01$, **$p<0.001$.

5.4.4 The association of antenatal head circumference growth with developmental skills

The association of head circumference *growth* with gross motor and personal-social skills is graphically displayed in Figure 5.2. Head growth in 20-27 weeks was not significantly associated with developmental skills at any follow-up age. Increased head growth in 28-32 weeks significantly decreased fine motor z-scores at 12 months by -0.02 (95% CI -0.03-0.00; result not shown). Finally, increased growth between 33 and 36 weeks was related to gross motor z-score decreases of -0.04 (95% CI -0.08--0.01) at 3 months and -0.03 (95% CI -0.05--0.01) at 18 months. Increased head circumference growth in 33-36 weeks was also associated with a personal-social z-score decrease of -0.05 (95% CI -0.09--0.02) at 3 months. Full detailed tables of all results on which Figure 5.1 and Figure 5.2 are based can be found in Appendix VI.

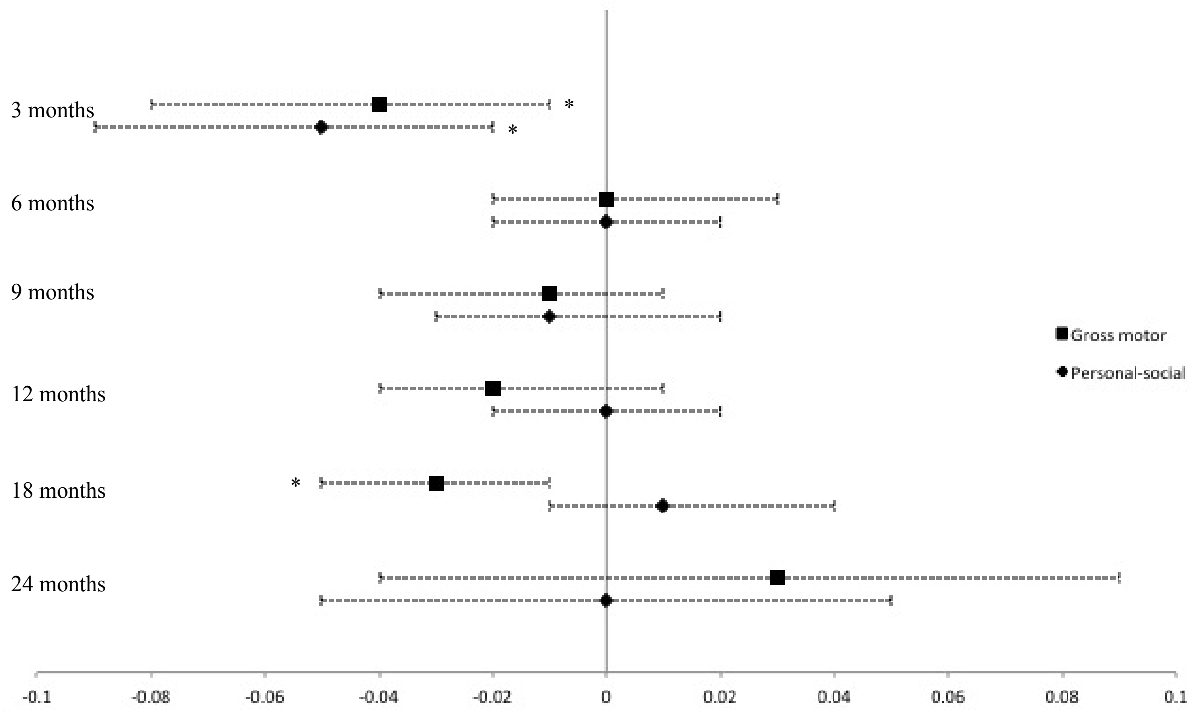


Figure 5.2 Change in postnatal gross motor and personal-social z-scores for each millimetre increase in head circumference growth in 33-36 weeks.
**p*<0.01, ***p*<0.001.

5.5 Discussion

Findings from this study confirm the importance of antenatal head size and growth in relation to postnatal developmental skills. Furthermore, it seemed that the relationship between developmental skills and antenatal head circumference (size and growth) was most evident at 36 weeks and in the age window between 33 and 36 weeks. Also, mainly gross motor skills, followed by personal-social and fine motor skills seemed most affected by antenatal head measurements, while communication and problem solving skills were not.

5.5.1 The association of antenatal head circumference with developmental skills

All statistically significant associations of head circumference size and developmental skills, with the exception of fine motor skills and head circumference growth, occurred in late pregnancy (>33 weeks) and were negative. This contradicts previous singleton studies in which positive associations have been found[144-146] and could be related to differences in antenatal growth, as growth in twin pregnancies is known to decrease from 28-32 weeks gestation onwards compared with singletons[146]. However, there is no evidence to support this as no previous twin studies have investigated the relationship between antenatal head circumference and developmental skills. It is more likely that findings in this chapter differ due to the use of measurements at later gestations compared with previous studies. The definition of 'late pregnancy' in aforementioned studies was around 30 weeks, while BiRTHS allowed for data at even later gestations (up to 36 weeks) and relatively closer to birth considering that median gestational age in the study was 36 weeks. It is possible that 30 weeks gestation was not sufficiently late in pregnancy to observe any negative associations with developmental skills. Therefore, negative associations might have been observed in singleton populations if head circumference at gestations beyond 30 weeks was available. Although preterm delivery has been previously related to unfavourable intrauterine conditions (e.g. decreased growth)[152] and previous studies have had samples of children born >37 weeks, this is unlikely to explain the negative association between antenatal growth and developmental skills within the BiRTHS sample, as the sample consisted of twins without any severe health or growth issues. Increased antenatal head circumference growth in late pregnancy could be related to catch-up growth, whereby the quickest growth should be observed in children with small head circumferences. However, this is unlikely to have been an issue in this study, as analyses were adjusted for size at the beginning of each age window and no evidence of antenatal catch up growth has been previously reported.

Communicative, fine motor and problem solving skills between 3 and 24 months did not seem to be affected by antenatal head size at any gestation. This finding seems to agree with previous studies, in which decreased size has not been related to communicative and fine motor skills, but has been related to worse performance in reasoning and social domains[144, 146]. Infants learn new gross motor and personal-social skills more rapidly compared with communicative, fine motor and problem solving skills[90, 153]. It might therefore be more beneficial for twins to focus on skeletal and muscular development in late pregnancy, so that gross motor functions can be optimal in early infancy and further developmental delays in other areas that rely on these basic skills can be minimised. Following this, it might be possible that changes in infant communicative, fine motor and problem solving skills are not large enough to detect an effect in a regression analysis. A further explanation for the lack of associations with communication skills could be that is might be influenced by interactive postnatal factors that I did not investigate, such as parent-child and twin-twin interaction[123], and not so much by antenatal conditions. Further research is necessary to explore these options.

5.5.2 Limitations

The relationship between antenatal head circumference and developmental skills should be further investigated in a longitudinal model, in which adjustments can be made for a child's developmental trajectory. Also, the relatively small sample and incomplete postnatal follow-up may have had an impact on the interpretation of the results. I may not have been able to detect associations when they should have been there, or found associations that were significant, but had a large confidence interval. This is particularly noticeable at 24 months, where data was available for only 38 children. A more complete dataset would be desirable in future studies.

As mentioned earlier, 'asymmetrical' growth (defined as abdominal circumference/head circumference ratio), whereby the head is disproportionate to the abdomen, has been found to be a good predictor of early neuromotor delays in singletons[148]. Therefore, no meaningful analyses were possible with this sample, but asymmetrical growth could be considered in a more varied sample of twins.

It is also important to assess the relationship between postnatal growth and developmental skills in order to provide a complete overview of how growth affects neurocognitive development. This will be done in Chapter 6. Similar to the previous chapter's limitations, further follow-up is also needed to determine within-twin pair difference beyond infancy and the effect of environmental factors in childhood, such as twin-twin interaction[123], type of childcare and parent-infant interaction[123, 129].

5.5.3 Conclusion

To my knowledge, no previous study has looked into antenatal growth in specific age windows up to 36 weeks, nor has there been previous research in this area with regard to twins. I found that antenatal head circumference in late pregnancy mainly seems to have an influence on gross motor skills in infant twins. The results from this chapter should be interpreted with caution, due to the small and incomplete dataset. However, the current study does provide promising results and should be considered a stepping-stone for further research into the effects of antenatal growth on early twin development. More research is particularly needed with regard to postnatal covariates as children grow up.

CHAPTER 6

POSTNATAL ANTHROPOMETRY AND DEVELOPMENTAL SKILLS IN EARLY INFANCY

"She has always been the smaller one, even at birth. Compared with her twin brother she looks a year younger because of her size." – BiRTHS parent about her toddler.

6.1 Abstract

Background: Previous studies in singletons have suggested increased physical growth in small infants may be related to developmental delays. However, the relationship between postnatal anthropometry and developmental skills of infant twins remains unclear. I therefore investigated this in a sample of healthy twins.

Methods: Developmental skills of 134 individual twins from the Birmingham Registry for Twin and Heritability Studies were assessed with the Ages and Stages Questionnaires (ASQ-3) at 3, 9, 12, 18 and 24 months. Weight, length and head circumference were measured at birth and each follow-up age. Multilevel regression analyses, adjusted for gestational age at birth and gender, were performed.

Results: A 1-kilo increase above mean weight in the second year was significantly associated with decreased concurrent ASQ-3 z-scores up to -0.11 (p<0.01), while a centimetre increase above mean head circumference was associated with z-score increases up to 0.06 (p<0.01). Weight gain and head circumference growth in 3-9 months were associated with decreases in developmental performance at 24 months.

Conclusions: There is indeed a twin-singleton difference in postnatal growth and development. The findings provide evidence in support of the hypothesis that an early focus on physical growth may impair later developmental performance. The current findings also suggest that optimal postnatal size and growth may be necessary to avoid negative developmental outcomes in infancy.

6.2 Introduction

As described in Chapter 5, there may be a relationship between antenatal head measurements and developmental skills in infancy. However, I did not find effects of antenatal head circumference on all domains of developmental skills; gross motor and personal-social skills were mostly affected by antenatal growth, while communicative, fine motor and problem solving skills were not significantly affected. Furthermore, only few effects were found within the first 6 months of postnatal follow-up. This could be explained by the influence of postnatal factors, including postnatal growth. Previous studies have suggested increased physical growth in children who are small at birth may be related to developmental delays[55, 154, 155]. However, this hypothesis has not yet been tested in early infancy. More specifically, the relationship between postnatal anthropometry and developmental skills in the first two years of life has not been studied in healthy infant twins. The following paragraph describes growth measurements in the BiRTHS postnatal clinic and their relationship to developmental skills.

6.2.1 Postnatal growth patterns

Postnatal growth in infancy is generally characterised by a rapid growth velocity during the first three months of life, followed by slowly decreasing growth velocity thereafter[156, 157]. Most growth standards that are currently in use, such as the World Health Organisation growth standards and the British 1990 Growth Reference[156], are based on singleton children. However, evidence suggests that premature infants, small-for-gestational-age children and twins do not follow the same growth curves as singletons born at term[157, 158]. Furthermore, much like antenatal growth, twins' postnatal growth differs so much from that of singletons[49], that some recommend the use of twin-specific postnatal growth references in the first 2.5 years of life[48].

It has been shown that twins reach average height (compared with the general population) between the ages of 4[159] and 18 years[47]. However, head circumference and weight were not average compared with singleton standards at the age of 4[159] and Estourgie-van Burk et al. (2010) found that twins remained lighter at the age of 18 years[47].

6.2.2 Postnatal growth and developmental skills

Similar to antenatal anthropometry described in Chapter 5, there is increasing evidence that postnatal size and growth velocity are related to cognitive development. In particular, length and head circumference seem to be important in this respect[55, 160, 161]. In terms of concurrent developmental skills (at the same age as the anthropometric measurement), height of children at school age has been related to intelligence quotients (IQ)[66, 162]. Further, small head circumference at 6 years has been associated with significantly worse motor skills, adaptive behaviour and general academic achievements[154].

Postnatal growth velocity can also be used to predict developmental skills measured at an older age compared with the growth measurement. Aside from the relationship with concurrent developmental performance, head circumference has also been associated with cognitive performance at the age of 10[67]. Weight gain and linear growth during the first year of very preterm infants has also been related to better cognitive skills at 18 months[163]. However, it did not have an effect on developmental skills of monochorionic twins between 12 and 24 months[164]. The association of weight gain, height and head circumference growth with developmental skills in the entire infancy period of twins remains unknown.

6.2.3 Rationale of this chapter

As suggested by previous studies, early developmental delays are a consequence of increased physical growth[55, 154, 155]. However, previous studies have focused on samples of either very preterm[163] or term[67] singletons. Healthy twins, born around 37 weeks of gestation and without any congenital abnormalities that would cause developmental delays have yet not been investigated.

6.2.4 Aims of this chapter

This chapter aims to:

- describe twins' postnatal growth trajectories compared with current singleton growth references.
- determine the association between early postnatal growth and development in infant twins.

I made distinctions between concurrent and later developmental skills, as previous studies have suggested there may be different effects[67, 164]. Further, because there is no evidence that midarm circumference or skinfold thickness have an effect on developmental skills, I focussed only on general growth (approximated by weight), length and head circumference in this chapter.

6.3 Methods

6.3.1 Study design and participants

Similar to the previous chapters, I made use of participants in the Birmingham Registry for Twin and Heritability Studies (BiRTHS)[120]. Additionally to the clinical record forms and questionnaires, families were invited to attend a postnatal clinic appointment during which the twins were weighed and measured, coinciding with the follow-up questionnaires. For the purpose of the analyses described in this chapter, families were selected from the BiRTHS cohort if maternal details were available, at least one of the five follow-up questionnaires were completed and at least one postnatal clinic was attended.

6.3.2 Materials

Details on maternal age and ethnic background were extracted from antenatal questionnaires. Obstetric details, such as birth weight, gestational age at birth and mode of delivery were extracted from maternal medical notes. Communicative, gross motor, fine motor, problem solving and personal-social skills at 3, 9, 12, 18 and 24 months were assessed with the Ages and Stages Questionnaires (ASQ-3)[91, 92]. A detailed description of this questionnaire can be found in Chapters 2 and 4.

6.3.3 Growth measurements

Birth weight, which is routinely measured by midwives, was extracted from maternal delivery notes. Length measurements at birth, taken by hospital staff, are not reliable and head circumference is not routinely measured in all hospitals. Therefore, these two birth measurements were not included in the analyses described in this chapter.

In the BiRTHS postnatal clinics we measured weight (without clothes or nappies) in kilograms (kg) using SECA digital baby scales. Length and head circumference in centimetres (cm), were measured using the Rollameter 100 and re-usable Lasso-o measuring tapes from Harlow Healthcare, respectively. Detailed information about the measuring methods and reliability of the measurements can be found in Appendix III.

The effects of weight, length and head circumference at 3, 9, 12, 18 and 24 months on concurrent developmental skills were studied. Age windows were created based on these follow-up ages (0-3, 3-9, 9-12 and 12-18 months) to investigate the relationship between growth velocity in each age window and later developmental performance at 24 months. Growth velocity was defined as weight gain in grams per day and growth of length and head circumference in cm per day.

6.3.4 Statistical analyses

Descriptive data

All analyses were performed in STATA 11[85]. Similar to the analyses in Chapters 4 and 5, ASQ-3 scores on all subscales were converted into z-scores using the means and standard deviations provided for the norm sample[83]. Subsequent analyses were performed using these z-scores. T-tests were performed to determine any significant difference in ASQ-3 z-scores between our sample and the norm group. Between-twin pair differences in maternal age and gestational age at birth were also investigated with t-tests.

Growth trajectories

Postnatal anthropometric measurements were interpolated if data were not available within a 2-week margin below or above each follow-up age (e.g. between 2.5 and 3.5 months, similar to the ASQ-3 protocol as described in Chapter 2). Data were not extrapolated. Gender-specific standardized growth charts for weight, length and head circumference were created by using the 3rd, 50th and 97th centiles from the LMSgrowth add-in for Microsoft Excel[165] (based on the British 1990 Growth Reference[156]). Mean size of twins at birth, 3, 9, 12, 18 and 24 months were plotted on the derived singleton charts. The British 1990 Growth Reference[156] was further used to calculate standard deviation scores (SDS) to quantify twin-singleton differences.

The association of postnatal growth with developmental skills

Multilevel linear regression analyses were performed to study the association of postnatal weight, length and head circumference at each follow-up age with the communication, motor, problem solving and personal-social skills at concurrent ages (3, 9, 12, 18 and 24 months). Growth in each age window was used as a predictor of developmental skills at 24 months in separate multilevel linear regressions. Twins' relatedness was taken into account by including this as a random effect. All regression models were adjusted for gestational age at birth and gender. Additionally, models for growth were adjusted for size at the beginning of each age window. Results were considered statistically significant if *p*-values were smaller than 0.01, taking multiple testing into account.

6.4 Results

6.4.1 Participants

A total of 134 healthy individual twins from BiRTHS (Chapter 2-3) were included in the study. Only one follow-up was available for 32 twins (24%), two follow-ups for 52 twins 39%), three follow-ups for 35 twins (26%), four follow-ups for 14 twins (10%) and five follow-ups for 1 twin (1%). None of the parents completed all six follow-ups. Sample size for each specific follow-up can be found in Table 6.1.

Twins were born at a median gestational age of 37 weeks (range: 27-38), with a mean birth weight of 2.4 kilograms (kg; standard deviation (SD)=0.5). Mothers' average age at delivery was 32.9 years (SD=4.9). 81% of parents were White.

6.4.2 Descriptive data

Developmental skills

Mean scores and z-scores on al ASQ-3 subscales can be found in Table 6.1. Twins scored significantly worse than the singleton norm group on all subscales at 3 (p<0.001), and all subscales except for communication at 9 months (p<0.001). Scores remained lower than those of singletons for gross motor and problem solving at 12 months (p<0.01). Finally, personal-social skills were worse at 18 and 24 months (p<0.01).

Table 6.1 Means and standard deviations of raw ASQ-3 scores and their corresponding z-scores at all follow-up ages for twins included in the analyses.

	Communication	Gross motor	Fine motor	Problem solving	Personal-social
3 months (N=53)					
Mean (SD)	40.4 (14.2)**	40.5 (11.1)**	28.3 (13.2)**	30.2 (13.9)**	35.2 (14.3)**
z-score (SD)	-1.3 (1.6)	-1.7 (1.4)	-2.1 (1.2)	-2.5 (1.5)	-1.8 (1.5)
9 months (N=77)					
Mean (SD)	36.0 (14.9)	28.9 (17.2)**	46.9 (14.8)*	40.5 (16.5)**	39.1 (13.3)
z-score (SD)	-0.2 (1.2)	-1.2 (1.2)	-0.5 (1.4)	-0.9 (1.6)	-0.3 (1.1)
12 months (N=83)					
Mean (SD)	43.3 (13.9)	36.4 (21.6)**	49.7 (12.9)	43.5 (15.5)*	41.7 (14.6)**
z-score (SD)	0.0 (1.0)	-1.0 (1.5)	-0.3 (1.5)	-0.5 (1.4)	-0.3 (1.2)
18 months (N=56)					
Mean (SD)	38.8 (15.7)	53.5 (13.1)	51.9 (11.43)	42.9 (13.6)	44.5 (12.6)*
z-score (SD)	-0.2 (1.1)	-0.2 (1.4)	-0.1 (1.3)	-0.3 (1.3)	-0.3 (1.2)
24 months (N=38)					
Mean (SD)	49.0 (16.0)	53.8 (12.2)	51.7 (9.0)	46.3 (13.3)	45.8 (11.9)**
z-score (SD)	-0.2 (1.2)	-0.1 (1.5)	0.0 (1.1)	-0.3 (1.4)	-0.5 (1.2)

Z-scores were calculated by using the means and standard deviations (SD) for the singleton norm group provided with the ASQ-3. Significant differences with singtleton norm scores are displayed with *p<0.01, **p<0.001.

Postnatal growth

Growth trajectories for male and female twins can be found in Figure 6.1-Figure 6.2. In general, weight and head circumference followed the 10th centile up to about 6-9 months (SDS_{weight}=-1.39, $SDS_{head\ circumference}$=-1.61), after which increased growth velocity was observed. Weight remained within the normal range between the 3rd and 97th centile up to 24 months (SDS_{weight}=-0.19). Male head circumference surpassed the 97th centile after 18 months ($SDS_{head\ circumference}$=-0.54). Although, males and females were shorter compared with singletons at 3 months (SDS_{length}=-1.05), growth surpassed the 97th centile after 18 months and was 2 standard deviations (SD) above the singleton mean at 24 months.

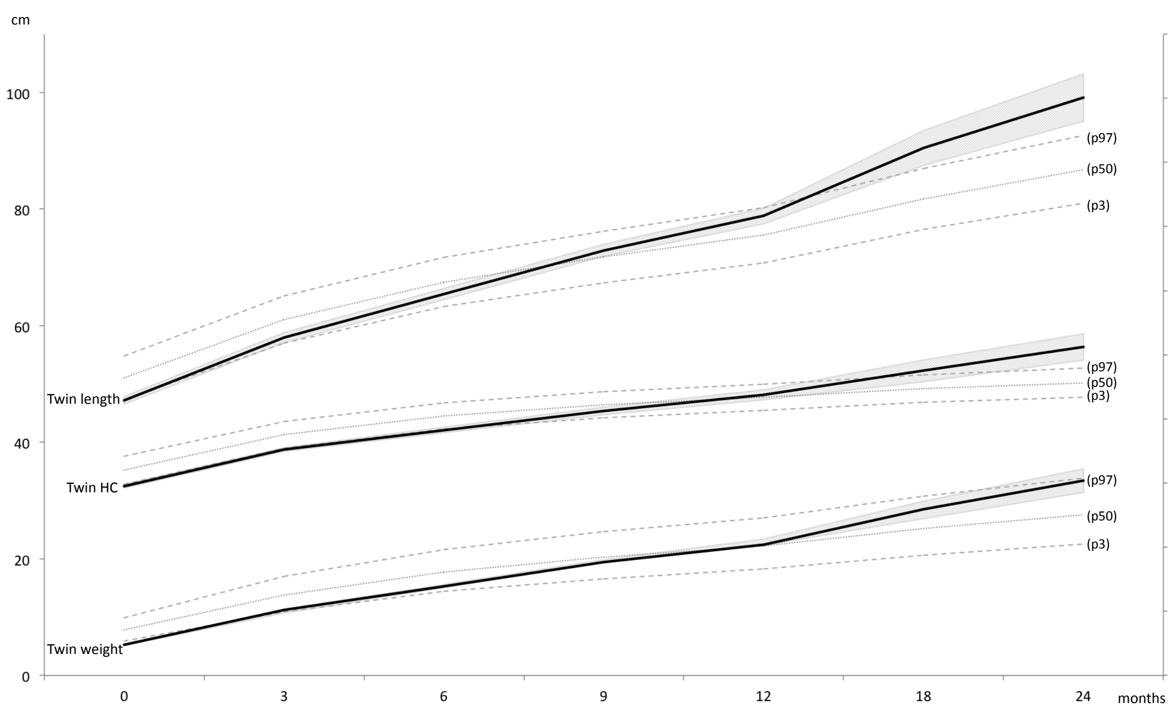


Figure 6.1 Average weight in kilograms (kg) and length and head circumference (HC) in centimetres (cm) of all included male twins from birth to 24 months, compared to current singleton growth references.

p3=3rd centile, p50=median, p97=97th centile (normal range between p3 and p97), 95% confidence interval shaded in grey. [Derived from the *British Growth Reference data for Excel*][156].

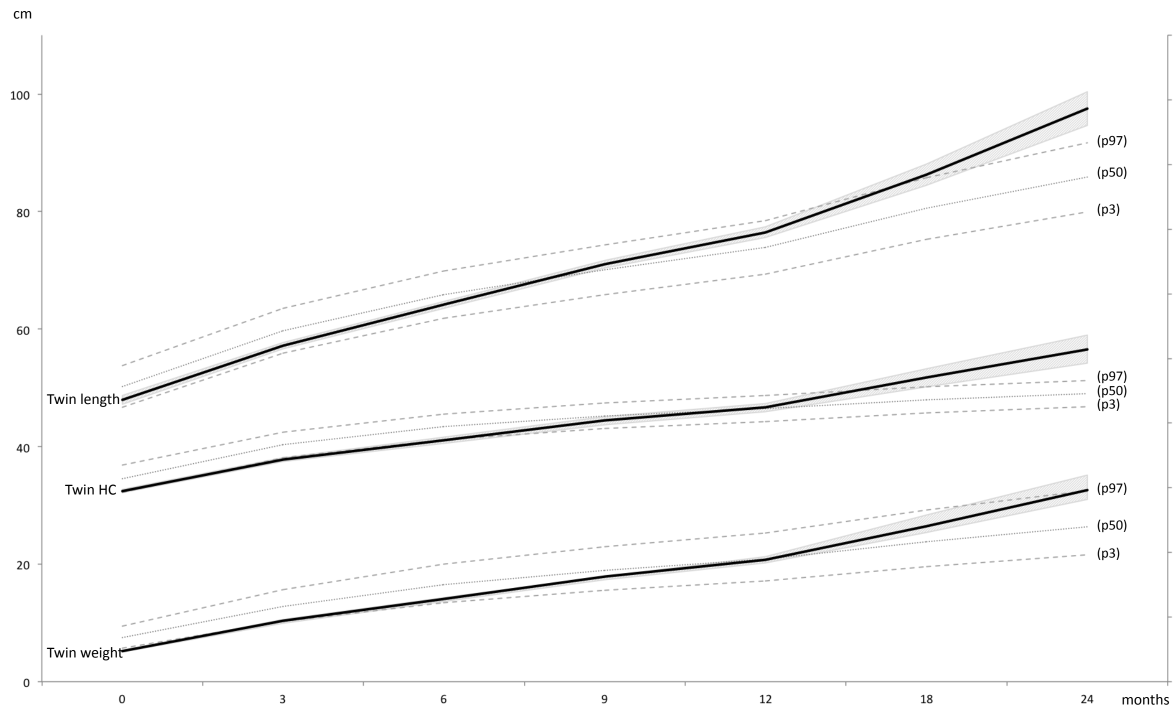


Figure 6.2 Average weight in kilograms (kg) and length and head circumference (HC) in centimetres (cm) of all included female twins from birth to 24 months, compared to current singleton growth references.

p3=3rd centile, p50=median, p97=97th centile (normal range between p3 and p97), 95% confidence interval shaded in grey. [Derived from the *British Growth Reference data for Excel*][156].

6.4.3 The association of weight, length and head circumference with concurrent developmental skills

Most associations of weight and concurrent developmental skills were negative. Only weight at 24 months had a statistically significant effect on communication skills at the same age (Table 6.2), whereby every additional kg above the average weight was associated with a decrease of 0.11 (95% confidence interval (CI) -0.19--0.02) in z-score. There was also a negative trend for increased weight and problem solving z-score at 12 months. Head circumference on the other hand was mostly positively associated with developmental skills or not at all. Fine motor z-scores at 12 months significantly increased by 0.06 (95% CI 0.02-0.12) with each cm above average head circumference at the same age. There was a trend for communication skills at 3 months to be positively affected by head circumference, whereas personal-social skills at 24 months were negatively affected. However, these results were not statistically significant (p<0.05). Similar trends were found for 3-month communication skills and 24-month personal-social skills in relation to increased length.

Table 6.2 The association of weight in kilogram (kg), length in centimetres (cm) and head circumference in cm with concurrent developmental skills.

	Weight (kg) Coef. (95% CI)	Length (cm) Coef. (95% CI)	Head circumference (cm) Coef. (95% CI)
Communication			
3 months	0.33 (-0.12 - 0.79)	0.21 (0.03 - 0.40)*	0.36 (0.04 - 0.79)*
9 months	-0.08 (-0.25 - 0.9)	0.00 (-0.05 - 0.05)	0.02 (-0.03 - 0.07)
12 months	-0.03 (-0.14 - 0.09)	0.00(-0.03 - 0.04)	0.01 (-0.05 - 0.06)
18 months	-0.07 (-0.17 - 0.04)	-0.02 (-0.05 - 0.01)	0.03 (-0.02 - 0.07)
24 months	-0.11 (-0.19 - -0.02)**	-0.01 (-0.04 - 0.01)	-0.01 (-0.04 - 0.02)
Gross motor			
3 months	-0.01 (-0.35 - 0.33)	-0.07 (-0.22 - 0.08)	0.00 (-0.25 - 0.25)
9 months	-0.04 (-0.22 - 0.14)	-0.01 (-0.06 - 0.05)	0.00 (-0.05 - 0.06)
12 months	0.09 (-0.07 - 0.25)	0.03 (-0.02 - 0.08)	-0.01 (-0.09 - 0.07)
18 months	0.07 (-0.05 - 0.20)	0.02 (-0.02 - 0.06)	0.02 (-0.04 - 0.07)
24 months	-0.02 (-0.10 - 0.07)	0.00 (-0.02 - 0.02)	0.02 (-0.01 - 0.05)
Fine motor			
3 months	0.11 (-0.25 - 0.47)	-0.01 (-0.18 - 0.16)	0.04 (-0.23 - 0.31)
9 months	-0.09 (-0.29 - 0.11)	-0.02 (-0.08 - 0.04)	0.00 (-0.07 - 0.06)
12 months	-0.07 (-0.22 - 0.09)	0.00 (-0.05 - 0.06)	0.00 (-0.07 - 0.07)
18 months	0.05 (-0.07 - 0.17)	0.00 (-0.04 - 0.04)	0.06 (0.02 - 0.12)**
24 months	-0.06 (-0.13 - 0.01)	-0.02 (-0.03 - 0.00)	-0.01 (-0.04 - 0.01)
Problem solving			
3 months	-0.27 (-0.74 - 0.19)	-0.05 (-0.24 - 0.15)	-0.15 (-0.50 - 0.19)
9 months	0.07 (0.16 - 0.29)	0.00 (-0.07 (0.06)	0.02 (-0.05 - 0.09)
12 months	-0.15 (-0.30 - 0.00)*	-0.04 (-0.09 - 0.01)	-0.01 (-0.09 - 0.06)
18 months	-0.07 (-0.19 - 0.06)	-0.03 (-0.07 - 0.01)	0.00 (-0.06 - 0.06)
24 months	-0.05 (-0.13 - 0.03)	-0.02 (-0.04 - 0.00)	-0.02 (-0.05 - 0.01)
Personal-social			
3 months	-0.15 (-0.58 - 0.28)	-0.04 (-0.19 - 0.11)	-0.14 (-0.45 - 0.17)
9 months	0.00 (-0.15 - 0.15)	0.01 (-0.04 - 0.05)	0.01 (-0.03 - 0.06)
12 months	0.06 (-0.09 - 0.20)	0.02 (-0.02 - 0.07)	0.04 (-0.03 - 0.10)
18 months	-0.09 (-0.21 - 0.03)	-0.02 (-0.06 - 0.01)	0.03 (-0.02 - 0.08)
24 months	-0.02 (-0.10 - 0.05)	-0.02 (-0.04 -0.00)*	-0.03 (-0.06 - 0.00)*

Coef. (95% CI)=Regression coefficient (95% Confidence interval).
Results have been adjusted for gestational age at birth. All significant results are displayed with *p<0.05 and **p<0.01.

6.4.4 The association of early postnatal growth with developmental skills at 24 months

There was a negative trend of increased growth velocity in most age windows and worse developmental skills at 24 months (Table 6.3). Mainly communication, fine motor and personal-social skills were affected. The following paragraphs describe statistically significant findings and trends.

An additional 1gram/day above average weight gain between birth and 3 months was associated with a -0.06 (95% CI -0.11--0.01) decrease in fine motor z-score. Above average weight gain between 3 and 9 months was associated with a -0.07 (95% CI -0.13--0.01) decrease in 24-month communication skills. Above average head circumference growth of an additional 1cm/day between 3 and 9 months was associated with a -0.04 (95% CI -0.07--0.02) decrease in personal-social z-score. Growth in length did not have statistically significant effects on 24-month developmental skills. However, there was a trend of growth in 3-9 months and decreasing personal-social z-scores.

Stratified analyses by growth rate (above/below median) confirmed these findings (see Appendix VII). Furthermore, results suggested that fine motor, problem solving and personal-social skills were negatively associated with increased growth in length and head circumference between 12 and 18 months, while positively associated with above average weight gain in the same age window.

Table 6.3 The association of growth in 0-3, 3-9, 9-12 and 12-18 month with developmental skills at 24 months.

	Communication Coef. (95% CI)	Gross motor Coef. (95% CI)	Fine motor Coef. (95% CI)	Problem solving Coef. (95% CI)	Personal-social Coef. (95% CI)
Weight (gram/day)					
0 - 3 months	-0.04 (-0.09 - 0.02)	-0.01 (-0.08 - 0.07)	-0.06 (-0.11 - -0.01)**	-0.05 (-0.11 - 0.01)	-0.03 (-0.07 - 0.02)
3 - 9 months	-0.07 (-0.13 - -0.01)**	0.01 (-0.09 - 0.11)	-0.03 (-0.09 - 0.02)	-0.03 (-0.10 - 0.04)	-0.04 (-0.10 - 0.01)
9 - 12 months	-0.01 (-0.07 - 0.05)	0.03 (-0.06 - 0.12)	0.02 (-0.04 - 0.08)	-0.01 (-0.08 - 0.07)	0.04 (-0.02 - 0.11)
12 - 18 months	-0.01 (-0.10 - 0.08)	0.02 (-0..09 - 0.14)	0.33 (-0.07 - 0.74)	0.03 (-0.06 - 0.12)	0.05 (-0.04 - 0.13)
Length (cm/day)					
0 - 3 months	-	-	-	-	-
3 - 9 months	-0.01 (-0.03 - 0.02)	0.02 (-0.03 - 0.06)	-0.02 (-0.04 - 0.01)	-0.01 (-0.04 - 0.03)	-0.03 (-0.05 - 0.00)*
9 - 12 months	-0.01 (-0.03 - 0.01)	0.00 (-0.03 - 0.03)	-0.01 (-0.03 - 0.01)	-0.02 (-0.04 - 0.01)	-0.01 (-0.03 - 0.01)
12 - 18 months	0.00 (-0.03 - 0.03)	0.00 (-0.04 - 0.04)	0.01 (-0.01 - 0.04)	-0.01 (-0.04 - 0.02)	0.00 (-0.03 - 0.02)
Head circumference (cm/day)					
0 - 3 months	-	-	-	-	-
3 - 9 months	0.01 (-0.02 - 0.03)	0.02 (-0.03 - 0.06)	-0.02 (-0.04 - 0.01)	-0.01 (-0.04 - 0.03)	-0.04 (-0.07 - -0.02)***
9 - 12 months	-0.01 (-0.03 - 0.02)	0.00 (-0.06 - 0.06)	0.01 (-0.02 - 0.05)	-0.01 (-0.05 - 0.03)	0.03 (-0.01 - 0.06)
12 - 18 months	0.00 (-0.04 - 0.04)	-0.01 (-0.08 - 0.05)	0.01 (-0.03 - 0.06)	-0.03 (-0.07 - 0.02)	0.01 (-0.04 - 0.06)

Coef. (95% CI)=Regression coefficient (95% Confidence interval).
Results have been adjusted for gestational age at birth and size at the beginning of each age window. All significant results are displayed with *p<0.05, **p<0.01 and ***p<0.001.

6.5 Discussion

This study showed that increased weight in the second year was associated with decreased concurrent developmental performance, while increased head circumference in the second year was generally related to increased developmental scores. Above average growth rates for weight and head circumference between birth and 9 months were associated with worse developmental performance at 24 months. These findings provide evidence in support of the hypothesis that an early focus on physical growth may impair later developmental performance[55, 154, 155].

6.5.1 Twin-singleton differences

As expected, twins' weight, length and head circumference was below average compared with singletons. An increased growth rate was present in twins from 3 months onwards, leading to twins surpassing the normal range for singleton length and head circumference[156] with up to 2SD by the age of 24 months. This could be a sign of catch-up growth[166]. The large twin-singleton difference at 24 months could also be due to the relatively small number of twins who were measured at that age. Nonetheless, these findings support the need for twin-specific growth standards.

Similar to the previous chapters, there were signs of developmental delay in the first year and catch-up to singleton standards by 12 months on most subscales. Further follow-up is needed to determine any twin-singleton differences beyond infancy[61, 123].

6.5.2 The association of weight, length and head circumference with concurrent developmental skills

Very few effects were found for weight, length and head circumference on concurrent developmental skills. Interestingly, length and head circumference up to 18 months seemed to have positive effects on developmental skills, whereas negative associations were found at 24 months. There are indications of early structural brain differences between twins and singletons[167], which could be related to these different associations with developmental skills. However, these structural differences have only been found in the neonatal period and not in later childhood[168]. It is therefore possible that the relationship between postnatal anthropometry (particularly head circumference) and developmental skills in infancy is 'unstable'. Finally, it seems that gross motor skills are more affected by antenatal head size and growth (Chapter 5) and not at all by postnatal size. It is possible that sensitive periods for motor development[169] include very early or even antenatal periods.

6.5.3 The association of early postnatal growth with developmental skills at 24 months

In contrast to the relationship between size and concurrent developmental skills in the previous paragraph, growth seemed to have a more uniform effect on developmental skills at 24 months. Increased growth up to 9 months was mostly negatively associated with developmental skills at 24 months. The period between birth and 3 months could not be investigated for length and head circumference due to the unreliability of measurements at birth, but the findings do suggest that early postnatal growth may have a negative effect on later developmental skills.

These findings contradict a previous study, in which poor head growth in infancy was associated with poor neurocognitive outcomes in preterm infants[170]. The different findings could be due to different sample characteristics or the large age window that was used by Cheong et al. (2008)[170] in which growth was measured. Another explanation is the timing of peak growth velocity[171, 172], which could not be investigated with the current data as there were no measurements between birth and 3 months when several growth spurts are likely to occur[158, 173, 174].

Similar to size, gross motor skills were not affected by postnatal growth, adding evidence to the suggestion that antenatal and early infancy periods may be more important for this developmental domain.

6.5.4 Limitations

In order to confirm these findings in infancy, a more complete follow-up would be necessary. In particular, data at 24 months was very limited in this study sample. The incompleteness of the data was also the reason for interpolation of the growth data, for which linear growth between each follow-up was assumed. This could have disguised otherwise detectable growth spurts. This may have had consequences for the *growth* analyses in particular. A more complete dataset would be needed to adequately investigate the possible consequences. However, as previous studies have hypothesised that increased physical growth may negatively affect cognitive skills in childhood, it may be more likely that similar effects can be found in infancy (like the negative trends in this chapter). Another interesting question to address in future studies could be the detection of minor growth spurts in infancy and their effects on developmental skills. For this, measurements at shorter intervals would be needed. As in previous chapters, further follow-up is also needed to determine within-twin pair difference beyond infancy and the effect of environmental factors in childhood, such as twin-twin interaction[123], type of childcare and parent-infant interaction[123, 129], and ethnicity[5, 129, 133].

6.5.5 Conclusion

This study should be considered as a pilot, in which I found that there may indeed be a twin-singleton difference in growth during the infancy period. Furthermore, it seems that developmental skills in twins may be negatively affected by early focus on physical growth, as suggested in previous studies. The results suggest that size is not a very good predictor of concurrent developmental skills, while early growth seems to play a bigger role in developmental performance at 24 months. However, this should be interpreted with caution, as I did not have data for many children at 24 months. Further research with a more complete and larger dataset is needed to provide more information regarding the relationship between postnatal growth and development.

CHAPTER 7

MATERNAL OCCUPATION AND PREGNANCY OUTCOMES

"Hospital admission is out of the question, because of my job. So I will come to the hospital for check-ups every morning before I go to work instead." – BiRTHS parent

7.1 Abstract

Background: Previous research in singleton pregnancies suggested that maternal occupations with high levels of physical activity and psychological strain may be harmful and could result in preterm delivery and low birth weight. I investigated effects of maternal occupational physical activity and psychological strain on twin pregnancy outcomes, as mothers of multiples may be at higher risk for preterm birth and low birth weight.

Methods: Birth weight, gestational age, Apgar score, delivery mode and neonatal ward admission details were obtained from hospital records of 197 individual twins in the Birmingham Registry for Twin and Heritability Studies. Maternal occupational physical activity and psychological strain were converted into metabolic equivalents from a parent-completed questionnaire. Multilevel spline regression models[121] were used to investigate the associations of occupational physical activity and psychological strain with birth weight, length of gestation, mode of delivery and neonatal wellbeing.

Results: Higher work-related psychological strain significantly increased gestational length by four days (p=0.03). I found no significant association of maternal occupational physical activity with perinatal outcomes. However, there was a trend for decreased birth weight in children born to women with jobs that involved >175 metabolic equivalents per week.

Conclusions: The current findings suggest that there may be a relationship between maternal occupation and pregnancy outcomes. However, due to methodological limitations and incomplete data, it is not possible to provide more conclusive suggestions for clinical practice and I suggest more research is needed.

7.2 Introduction

Increasingly more women have joined the paid employment workforce in the past few decades, from less than 50% of women in 1960 to 70% in 2001[175]. According to figures from the Office for National Statistics, female employment rates were 66% at the end of the BiRTHS recruitment period in July 2011. This was lower than employment rates a decade earlier, but could be due to the high number of redundancies in recent years[176]. Nevertheless, those who decide to have children will need to strike a balance between any potential risks from paid employment and a healthy pregnancy.

Based on previously identified occupational risk factors, such as exposure to hazardous environments[177] and strenuous activities[178], some advise mothers to decrease their level of physical activity or work in a safer environment during pregnancy[179]. However, it is difficult to determine what can be considered too strenuous, as this will vary for each individual. Furthermore, very little is known about psychological strain or job stress, which can also affect pregnancy outcomes[180]. In this chapter I will focus on the distinction between physical activity and psychological strain, and their individual contributions to twin pregnancy outcomes.

7.2.1 Association of occupational physical activity with perinatal outcomes

Due to the various factors that play a role in how physical activity affects pregnancy and its outcomes, findings in previous studies are incongruent. However, there is a general hypothesis of a U-shaped relationship between physical activity and pregnancy outcomes, whereby too little or too much physical activity can result in low birth weight or preterm birth[178, 181]. Physical activity is encouraged in all women and should be advised to fit with their individual fitness levels[179, 182-184], while exercise during complicated pregnancies should be managed conservatively[185]. Bonzini et al.(2009)[43] further specified that trunk bending activities, in particular, during the third trimester form an increased risk for preterm labour. However, there is evidence that suggests it is not the type of activity, but in fact the duration that is related to the decrease in birth weight[183]. Similar to general and leisure time physical activity, studies on occupational physical activity and birth outcomes show that more strenuous jobs are related to preterm birth[186], low birth weight[44, 181], intrauterine growth restriction[183] and non-elective caesarean section[187].

Preterm birth and low birth weight have been related to unfavourable health outcomes[52, 188, 189] and cognitive delays later in life[36]. Delivery by caesarean section has been linked to common infectious diseases and respiratory tract infections[37]. Previous studies give a good outline of the effect of maternal physical activity on pregnancy outcomes in singletons. However, to my knowledge, no studies have been conducted to investigate the impact of maternal occupation on twin pregnancies, which are usually more complex and often result in lower birth weights, preterm labour and neonatal hospital admissions[190] (also Chapter 1). It is therefore possible that mothers of twins are at an increased risk of experiencing negative effects of physical exercise during pregnancy when compared with mothers of singletons.

7.2.2 Definitions of occupational physical activity

Aside from the individual-dependent effect of physical activity, the previous paragraph outlined that definitions of physical activity vary from a simple 'yes/no' answer to 'number of hours per day' and even further categorisation of activities (i.e. light activity vs. moderate activity). Domingues et al. (2009)[179] further emphasized these issues in a review that spanned research between 1987 and 2007. This increases the difficulty in interpreting results from various studies.

In this chapter I propose the use of metabolic equivalents (METs) as an alternative to quantify the intensity physical activity. It is defined as the expenditure of 1 kilocalorie per kilogram per hour, based on the metabolism of an average person. METs are roughly classified as light (<3 METs), moderately intense (3-6 METs) and vigorous (>6 METs), whereby an activity of 6 METs is twice as intense as an activity of 3 METs[191].

7.2.3 Association of occupational psychological strain with perinatal outcomes

A quantifiable measure of occupational strain can be defined as the relationship between demands of the job (occupational demand) and how much (occupational) control the employee feels they have over the outcome of a task[192]. A person who needs to meet high demands, but has very little control will feel a higher psychological strain compared with a person who has complete autonomy in decision-making and task completion.

As with occupational physical activity, the effect of psychological strain seems very subjective. Mothers with low job satisfaction and high job strain have been found more likely to deliver babies that are premature[6, 179] with lower birth weights[180] and smaller head circumferences[43]. Occupational demand on its own has not been significantly related to a higher risk of preterm birth[186]. The effect of occupational psychological strain on twin pregnancies is unknown. However, mothers of twins are more likely to experience psychological stress[45] and have a higher risk of postnatal depression[193]. Similar to physical activity, it is possible that psychological strain will have a more negative effect on twin pregnancy outcomes.

7.2.4 Rationale of this chapter

In Chapters 1 and 4, I have described the effect of 5-minute Apgar score, gestational age and birth weight on postnatal development. Although I have not found an effect of birth weight on developmental skills in infancy, studies of older children have found such an effect[54, 116].

There is a need to identify and manage risk factors (including maternal occupation) as early as possible, so that children can be given the best possible start to life and cognitive development. Previous studies in singleton pregnancies have already identified certain occupational risk factors (e.g. physical activity) related to pregnancy outcomes such as birth weight[44, 180, 181], gestational length[178, 181, 186], Apgar score[36] and delivery mode[187]. Furthermore, negative pregnancy outcomes could lead to neonatal hospital admission, which puts infants at risk of contracting hospital-acquired infections[194] and has even been found to alter brain development patterns[9]. However, the relationship between occupational physical activity and psychological strain and twin pregnancy outcomes remains unclear.

7.2.5 Aims of this chapter

This chapter aims to:

- investigate how maternal work-related physical activity affect birth weight, gestational length, 5-minute Apgar score, delivery mode and neonatal hospital admission in twin pregnancies.
- identify how maternal work-related psychological strain affect birth weight, gestational length, 5-minute Apgar score, delivery mode and neonatal hospital admission in twin pregnancies.

7.3 Methods

7.3.1 Study design and participants

Participants were part of the Birmingham Registry for Twin and Heritability Studies (BiRTHS)[120]. In addition to maternal medical records and antenatal questionnaires, neonatal records were checked for any neonatal ward admissions. For the purpose of this analysis, families were selected from the BiRTHS cohort if delivery and maternal employment details were available.

7.3.2 Data

Maternal age, parity, twins' gender, birth weight, Apgar scores, gestational age at birth and delivery mode were extracted from maternal medical notes and delivery records. Neonatal admission records were searched for all twins in BiRTHS.

Maternal occupation and job title were extracted from the antenatal parental questionnaire (Appendix A-II.i-A-II.ii) and were classified into major categories according to the International Standard Classification of Occupations (ISCO-88)[195]. I identified relevant activities according to job descriptions in each of the major categories of the ISCO-88 and calculated METs for these activities from the Compendium of Physical Activities[191]. The derived average METs (Appendix Table VIII.i) were multiplied by the number of working hours per week or 40 hours for full-time jobs. The major categories of the ISCO-88 were also converted into perceived psychological demand, control and strain as suggested in the classification by de Smet et al. (2005)[192].

7.3.3 Statistical analyses

Descriptive data

As described in Chapter 1, mono- and dichorionic pregnancies differ from each other in terms of birth weight, gestational length, delivery mode and neonatal admissions. Therefore, t-tests were performed to investigate differences between any of these outcomes in mono- and dichorionic twins, in order to determine how chorionicity should be used in further analyses. Wilcoxon's rank-sum test was used to test for any differences in neonatal admission.

Additionally, birth weight was converted into standard deviation scores (SDS) using the British 1990 Growth Reference[156] for singleton children to determine whether twins differed from singletons.

Multilevel spline regression analyses – continuous variables

Because the relationships between maternal occupation and the outcome variables were not linear, multilevel spline regression models[121] were used to investigate the associations of occupational physical activity and psychological strain with twins' birth weight, gestational age at birth and 5-minute Apgar scores. Although twins were considered individuals in these analyses, their relatedness was taken into account by including it as a random intercept. Adjustments for maternal age and parity were included in the fixed portion of the model. Birth weight models were additionally adjusted for gestational age at birth. Predicted mean values from these models were used to graphically display the nonlinear relationships between occupational physical activity and estimated birth weight, gestational age and Apgar score.

Multilevel logistic spline regression analyses – categorical variables

Multilevel logistic spline regression analyses were performed to investigate the effects of occupational physical activity and psychological strain on delivery mode and neonatal admission. Delivery mode was categorised as 'no emergency caesarean' and 'emergency caesarean' to estimate any major unforeseen problems at birth. Neonatal admission was simply categorised as 'yes' or 'no'. Again, the relatedness of twins was incorporated as a nested level in the model and the fixed portion of the model included adjustments for maternal age, parity and gestational age (birth weight models only). Results were presented graphically as estimated mean probabilities of a delivery by emergency caesarean and neonatal admission. All analyses were performed in STATA 11 (StataCorp, 2009)[85], and results were considered statistically significant at α=0.05.

7.4 Results

7.4.1 Participants

A total of 197 twins from BiRTHS were included, of which 53% were male. Average birth weight was 2.4 kilograms (kg, standard deviation (SD)=0.5) and twins were born at a median of 37 weeks gestation (range: 27-39), with a median Apgar score of 9 (range: 5-10). Nearly a third of the infants (31%) were admitted to a neonatal ward for special care. Average maternal age at delivery was 31.6 years (SD=5.0) and 48% did not have older children (primiparous). 38% of mothers were admitted to hospital at least once during their pregnancy. The majority (77%) of mothers were employed in regular paid work and 81% was White.

7.4.2 Descriptive data

Adjusted birth weight for monochorionic and dichorionic twins was below the singleton mean (birth weight SDS_{MC}=-0.90 and birth weight SDS_{DC}=-0.60). Significant differences were found for birth weight ($t=-5.52$, $p<0.001$) and neonatal hospital admission ($z=2.79$, $p=0.01$) between mono- and dichorionic twins. Therefore, birth weight and neonatal hospital admissions analyses were additionally adjusted for chorionicity. After stratification by delivery mode, we found significantly shorter gestations ($p<0.001$) in those who had an emergency caesarean section. Analyses of gestational age score were therefore additionally adjusted for delivery mode.

7.4.3 Occupational determinants of perinatal outcomes

Multilevel spline regression analyses

After stratification by total MET per week (above/below median), 5-minute Apgar scores were 0.2 points higher in children born to mothers with below-median activity levels (p=0.05). No other statistically significant associations were found between occupational physical activity and birth weight and gestational age. However, occupational physical activity did seem to have a negative effect on particularly birth weight when exceeding 175 METs/week (Figure 7.1), which is equivalent to about 11.5 hours of light activity or 6 hours of moderate-intensity activity per work day[196]. The effects did not reach statistical significance, although there was a positive trend for birth weight (p=0.06) and gestational age when physical activity exceeded 175 METs/week (p=0.07).

After stratifying by total strain per week (above/below median), gestations of mothers with higher levels of perceived strain were approximately four days longer than gestations of mothers, who perceived lower levels of strain (p=0.03;Figure 7.1). No other effects of psychological strain on estimated birth weight, gestational age or Apgar score were found. Appendix Table VIII.ii provides accompanying results for these figures.

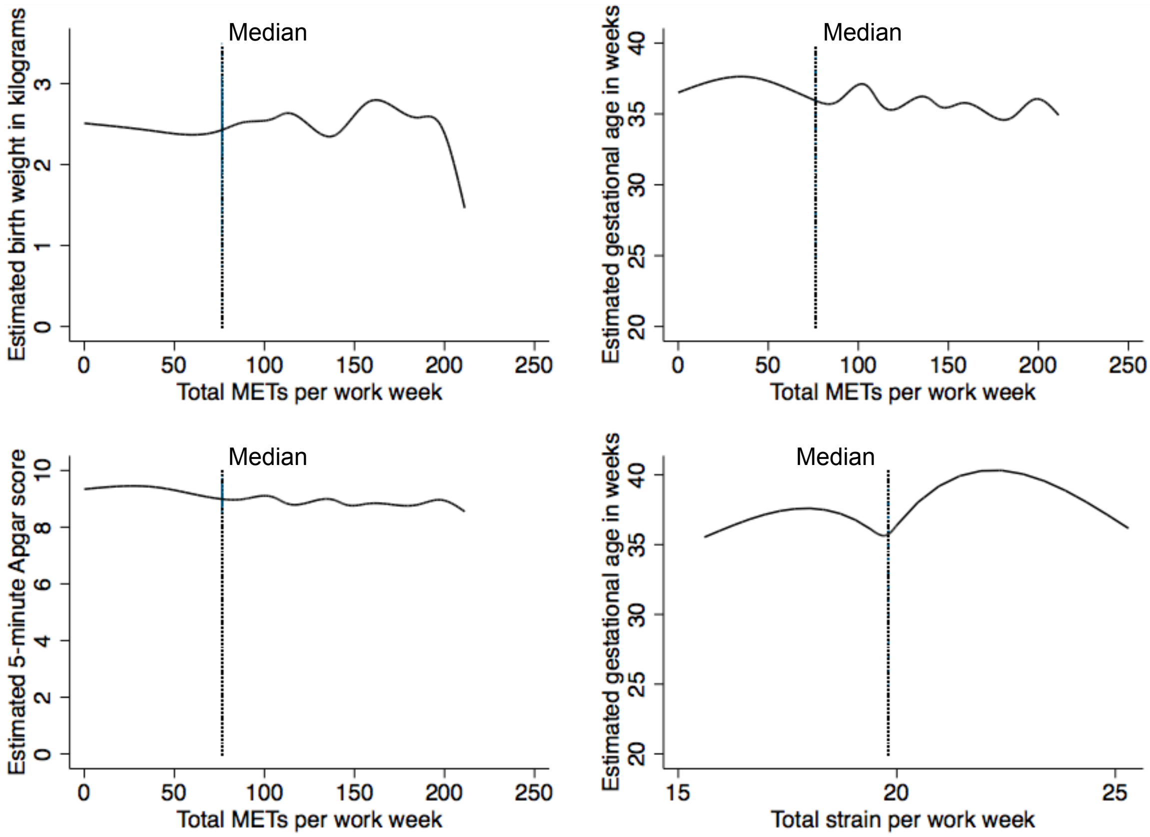


Figure 7.1 The effects of occupational physical activity (METs) on estimated birth weight, gestational age and Apgar score, and of psychological strain on gestational age.

Multilevel logistic spline regression analyses

Similar to birth weight, occupational physical activity, there was a trend to higher probability of delivery by caesarean section if occupational physical activity exceeded 175 METs/week (p=0.07; Figure 7.2). There were no other effects of physical activity or psychological strain. However, further investigation of psychological strain components showed that infants of mothers who had lower perceived control (below median) were more than twice as likely to be admitted to a neonatal ward compared with those of mothers, who felt they were more in control (p=0.02).

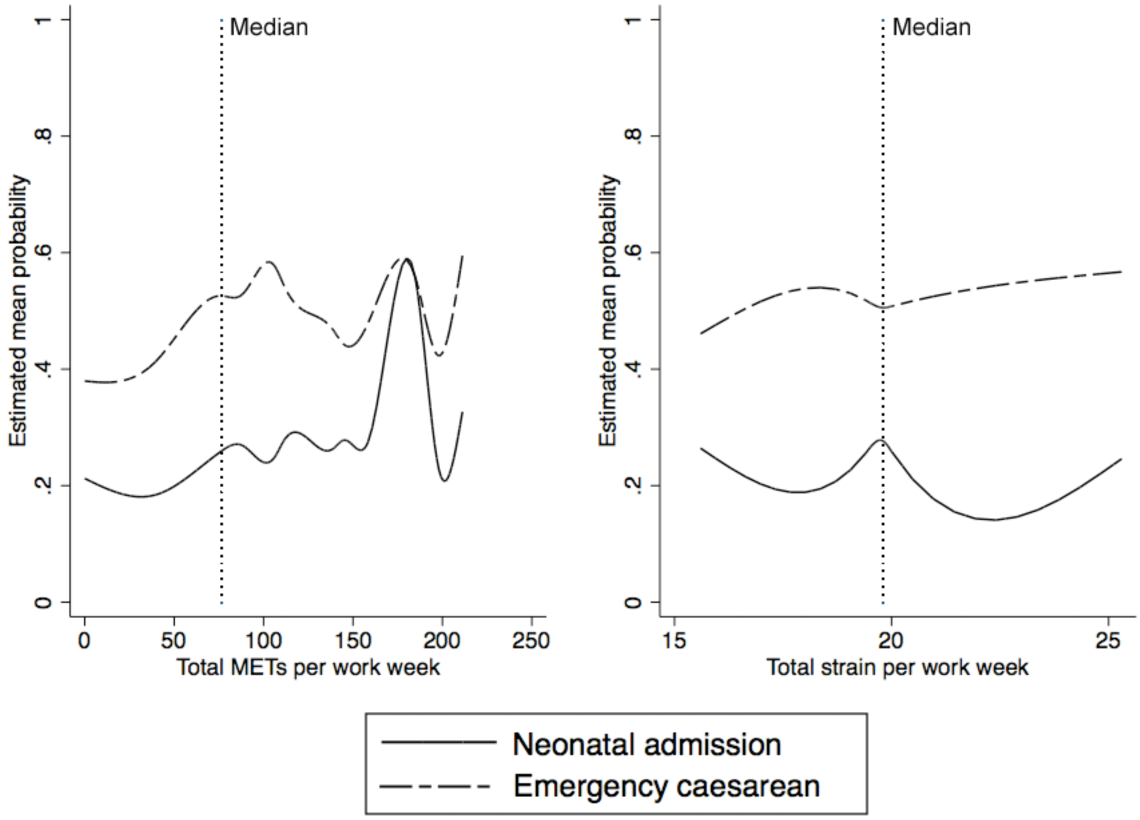


Figure 7.2 The effect of occupational physical activity, defined as metabolic equivalents (METs) per week, and psychological strain on estimated probabilities of neonatal hospital admission and delivery by emergency caesarean.

7.5 Discussion

The findings from this study suggest that maternal occupational physical activity does not affect perinatal outcomes in twin pregnancies, although birth weight showed a trend towards a decrease as 175 METs/week were exceeded (p=0.07). Work-related psychological strain, on the other hand, had a significantly positive effect on gestational length. Further examination of the components of strain showed that neonatal admissions were more likely to occur if mothers perceived lower levels of occupational control.

7.5.1 Occupational physical activity and perinatal outcomes

I did not replicate findings from previous studies of occupational physical activity and perinatal outcomes. Furthermore, the results did not support my original hypothesis, whereby light physical activity would affect perinatal outcomes in twin pregnancies. My findings suggest a negative effect on birth weight if 11.5 hours of light activity is exceeded, as opposed to a 7-hour threshold described in previous research[183]. Previous studies found that the effect of physical activity during pregnancy is dependent on maternal physical fitness[182-185]. According to the findings described in this chapter, there seems to be an optimal level of maternal physical fitness, which has negative effects on infant-related birth outcomes when not met or surpassed. Studies have also found that physical fitness levels are related to mode of delivery[197], as giving birth naturally is extremely strenuous on the human body and caesarean section is considered major surgery for which one needs to be physically fit. Mothers, who are more physically active at work, would be more physically fit compared with those who do mostly sedentary work, and are therefore more physically capable to withstand the major impact of childbirth[197]. A possible explanation for the lack of effect on any of these factors might be that the current sample did not consist of mothers who had extremely strenuous jobs. Therefore, occupational physical activity remained moderate at most. Perhaps higher physical activity levels would have resulted in significant effects on birth weight, gestational age, Apgar scores and delivery mode, as have been found in singleton research. Nevertheless, physical activity in this study could be considered representative of pregnant mothers, as it is unlikely that mothers participate in highly strenuous activities during pregnancy[198, 199].

7.5.2 Occupational psychological strain and perinatal outcomes

Occupational psychological strain has not been found to have any effect on perinatal outcomes in singleton pregnancies. However, findings from the current study suggest that higher occupational strain was significantly associated with longer gestations and higher occupational control was related to lower risks of neonatal admissions. Jobs with high demand and high control have low occupational strain, and are generally management or professional jobs. High strain jobs, on the other hand, are generally jobs with fewer responsibilities and for which no university degree is necessary[192]. It is possible that these mothers have the opportunity or feel more inclined to take maternity leave earlier than mothers in occupations with more responsibilities[200].

Little is known about the biological pathways that are involved in the association of stress and pregnancy outcomes. In their review, Hobel et al. (2010)[201] outlined that stress is indeed related with preterm birth and low birth weight. The authors also suggest that increased levels of corticotrophin-releasing hormone might prevent the placenta from optimum functioning, which may lead to an unfavourable intrauterine environment that precedes preterm birth and low birth weight. However, more research is needed to identify the extent to which stress-related hormones affect the intrauterine environment and how it can be prevented or treated.

7.5.3 Limitations

The use of job title as proxy for occupational physical activity and psychological strain did not provide details on leisure-time physical activity and maternal physical fitness levels prior to their pregnancy, which standardised questionnaires (such as the International Physical Activity Questionnaire[202]) may have provided. However, many physical activity questionnaires may not be appropriate to provide information about energy expenditure as they include different activities[203] or rely on self-report[204]. Similar arguments could be brought forward against the use of standardised psychological strain questionnaires, such as the Job Content Questionnaire[205]. Activity monitors and biological measurements[206] could be a costly, but potentially more objective measure of physical activity and psychological strain. Considering previous studies described in §7.1, in which too much physical or psychological strain were related to worse pregnancy outcomes, I do not believe my findings would be very different if I had used a measurement other than job title.

Finally, ethnicity was not included in the regression models, because it did not have an effect on perinatal outcomes or maternal occupation in the current sample. This could be due to the majority (81%) of the current sample being White. However, the choice to work during pregnancy could be dependent on ethnic origin and should be considered in future research.

7.5.4 Conclusion

According to the findings in this chapter, there is no evidence that moderate occupational physical activity in twin pregnancies is harmful. Furthermore, evidence from previous studies show that some physical fitness may be beneficial during childbirth[197]. Although occupational psychological strain needs to be studied in more detail, the current findings suggest that jobs with more responsibility may lead to shorter gestations and higher risks of neonatal admission. Due to the methodological limitations and incomplete data of the current study, it is difficult to provide clinically relevant advice. For now, advice on timing of maternity leave in twin pregnancies should remain up to the discretion of the clinician, and decisions should be made together with mothers when they have been fully informed about the inconclusive evidence for a relationship between working during pregnancy and pregnancy outcomes as described in the introduction and discussion sections of this chapter.

CHAPTER 8

THE HERITABILITY OF BODY MASS INDEX

"I wish I had your genes." – BiRTHS parent

8.1 Abstract

Background: Increased body mass index (BMI) is a worldwide health issue. Individual differences in the susceptibility to increased BMI could be related to genes or environment. I performed a systematic review of genetic studies on BMI in pre-adolescence, young adulthood and late adulthood.

Methods: I searched PubMed and EMBASE with heritability, body mass index, BMI, weight, height, anthropometry and twins as search terms. Studies reporting intra-pair correlations of healthy twin pairs that were raised together were included. This resulted in the inclusion of 8,179 monozygotic and 9,977 dizygotic twin pairs from twelve published studies in addition to individual participant data for 629 monozygotic and 594 dizygotic pairs from four twin registries.

Results: Structural equation modelling with intra-pair twin correlations showed that the heritability of BMI remained high over all age categories ranging from 61% (95% CI 54-64%) to 80% (95% CI 76-81%) for male and female subjects combined, while unique environmental influences increased from 14% (95% CI 13-15%) to 40% (95% CI 37-43%) with increasing age.

Conclusions: Heritability of BMI remains consistently high over different age categories. Environmental changes over time do not seem to have as big a relative impact on an individual's weight as previously reported, suggesting a mainly genetic influence on variation in BMI over the years.

8.2 Introduction

In the previous chapters, I have described my research based on the Birmingham Registry for Twin and Heritability Studies (BiRTHS)[78]. Besides this, I have investigated the heritability of body mass index (BMI) in a meta-analysis as part of my doctoral research[1]. Body composition is a worry for many people and known to be related to various health issues. The current obesity epidemic[207, 208] has caused an increased emphasis on healthy diets, exercise and research into possible causes and consequences of excess weight gain.

8.2.1 The obesity epidemic

Between 35 and 65% of the population in Europe and nearly 80% of the United States is overweight (body mass index; BMI>25 kg/m^2) or obese (BMI>30 kg/m^2) according to the latest estimates by the World Health Organisation. Furthermore, nearly 43 million children under five were overweight in 2010[209]. Increased body mass is related to many health problems, including cardiovascular disease and type 2 diabetes[208]. Causes of obesity and overweight have been linked to the imbalance between calorie intake and physical activity[210]. Over the past few decades, food has become more available, and cheaper. This, and the changes in marketing of food, has led to people feeling more inclined to go out for meals and buy more highly processed foods[211]. Jobs and modes of transport allow us to be sedentary for most of the day, causing more energy intake than expenditure[212].

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[1] This study was published in the European Journal of Epidemiology under the title 'Heritability of BMI in pre-adolescence, young and late adulthood' (Nan et al., 2012) and can be accessed via www.springerlink.com. A copy of the published paper can be found in Appendix X.

8.2.2 The heritability of BMI

In addition to these environmental explanations for excessive weight gain, there is evidence that BMI also has a strong genetic component. Results on the difference in the genetic influence at different developmental stages are inconclusive. It has been reported that the heritability may increase from childhood into adolescence[213, 214] and decrease[215] after that. This means that the relative importance of genetic and environmental effects on the variance of BMI change over time. Common environmental influences within a twin pair seem to have a larger influence in early life[71], than in adulthood where common environmental effects are negligible[214, 215]. Only a few studies have looked into gender differences in the heritability of BMI. There is room for debate whether the heritability of BMI in males and females is influenced by the same[72] or different[216] genes. Wisniewski et al.[217] concluded in their review that gender differences in relation to childhood obesity in particular have been understudied. They argue that body composition and hormonal influences are different for males and females, and thus gender differences should be investigated when looking at risk factors for obesity.

8.2.3 Rationale of this chapter

Following a cohort from early life to late adulthood in order to estimate the heritability of BMI is a lengthy process. It is much easier to collate data and results from various studies that have investigated the heritability of BMI at different ages for a cross-sectional analysis. In this systematic review I studied the heritability of BMI, using previously reported intra-pair twin correlations from published studies and unpublished individual participant dataset (IPDs) from four additional twin registries.

8.2.4 Aim of this chapter

This chapter aims to:

- investigate how the heritability of BMI changes in pre-adolescence, young adulthood and late adulthood, and as a function of sex.

8.3 Methods

8.3.1 Search methods

I conducted a structured literature search of MEDLINE and EMBASE databases at regular intervals until January 2012. Search terms that were used as free text and MeSH terms were heritability, body mass index, BMI, weight, height, anthropometry and twins. See Appendix IX for a diagram of the search results.

In addition to published articles, I obtained IPDs from the Netherlands Twin Registry (NTR)[218], Carolina African American Twin Study of Aging (CAATSA)[219], Murcia Twin Register (MTR)[220] and the Leuven Longitudinal Twin Study (LLTS)[221], which used subjects from the East Flanders Prospective Twin Study (EFPTS)[222], comprising a total of 1,223 twin pairs. The height and weight data in the IPDs were used to calculate BMI and intra-twin pair correlations in order for it to be in a comparable format to the published data.

8.3.2 Inclusion/exclusion criteria

Articles were included if the classic twin method was used on population-based samples, where monozygotic and dizygotic intra-twin pair correlations on a trait are compared. Finally, articles were selected if they reported intra-pair twin correlations on BMI of healthy monozygotic and dizygotic twins that were raised together or published sufficient information to calculate these correlations.

Articles were excluded from the review if birth weight of the subjects was less than 500g or gestational age at birth was less than 22 weeks, based on the minimum criteria for a viable live birth as described in the International Classification of Disease (10th ed.)[79]. If several articles were published on data from the same cohort, and it was unclear whether these were the same subjects or not, the largest study was selected for inclusion. IPD entries with missing zygosity, gender, age or BMI were excluded.

8.3.3 Statistical analyses

The studies were grouped into three age categories: pre-adolescence (9-11 years), young adulthood (18-22 years) and late adulthood (49-65 years). There were two longitudinal registries. The first one was the LLTS[221], for which IPD data was available. Average BMI was calculated for those subjects in the LLTS who had several measurements within one age category. Average BMI was log transformed to reduce skewness of the data, before intra-twin pair correlations were calculated.

The second longitudinal study was the Twins Early Development Study (TEDS). Data from this registry were used in three papers[223-225] within the 9-11 years category. Average correlations were calculated by applying a Fisher's z transformation on the extracted intra-twin pair correlations.

Heritability analyses

Structural equation modelling, using correlations derived from published articles and the IPDs, was done in Mx[226, 227] to estimate genetic (A) and common (C) and unique (E) environmental influences on the variance of BMI in each age category. Two models were fitted sequentially, as proposed by Sullivan et al.[228]. The first model allowed for the factor loadings on A, C and E to be freely estimated for each primary study[229]. These factor loadings were set to be equal across all studies in the second model. Heterogeneity was determined by calculating Chi-square differences between both models, and studies were considered heterogeneous if the Chi-square difference was statistically significant at p=0.05. Estimates from the second model are reported as pooled variance components[228]. I performed further sex-specific analysis, which only included articles that reported intra-twin pair correlations separately for men and women.

8.4 Results

8.4.1 Participants

Table 8.1 Characteristics of studies included in the meta-analysis.

Author(s), year of publication	Country	Method BMI measurement	Total MZ pairs	Total DZ pairs
Wardle et al., 2008[19]	United Kingdom	Self report and clinical examination	1813	3279
Haworth et al., 2008a[17]	United Kingdom	Self report	2209	2536
Haworth et al., 2008[18]	United Kingdom	Self report	1857	1669
Faith et al., 1999[29]	United States	Clinical examination	41	25
Silventoinen et al., 2008[26]	Sweden	Clinical examination	1582	1864
Harris et al., 1995[24]	Norway	Self report	866	751
Korkeila et al., 1991[25]	Finland	Self report	1173	2340
Carmichael & McGue, 1995[7]	United States	Self report	137	136
Cardon et al., 1994[28]	United States	Clinical examination	134	134
Wade et al., 2001[31]	United States	Clinical examination	527	248
Nelson et al., 2000[27]	Sweden	Clinical examination	27	36
Stunkard et al., 1986[30]	United States	Clinical examination	1974	2097
LLTS[15]	Belgium	Clinical examination	158	146
NTR[12]	Netherlands	Self report	341	277
CAATSA[13]	United States	Clinical examination	66	86
Murcia[14]	Spain	Self report	64	85

MZ = monozygotic, DZ = dizygotic, LLTS = Leuven Longitudinal Twin Study, NTR = Netherlands Twin Registry, CAATSA = Carolina African American Twin Study of Aging, Murcia = Murcia Twin Register

Sixteen studies were included in the final analysis, resulting in a total of 1,912 pre-adolescent, 5,367 young adult and 1,248 late adult monozygotic twin pairs, and 2,499 adolescent, 4,444 young adult and 1,375 late adult dizygotic twin pairs from population-based cohorts. Intra-pair correlations were obtained from twelve studies, of which seven were carried out in

Europe[223-225, 230-233] and five in the United States[215, 234-237]. I acquired IPDs for the remaining studies from three European twin cohorts[218, 220-222] and an American twin registry[219].

Table 8.2 Extracted sample sizes and intra-pair correlations by age category and study.

	Male				Female				Combined			
	Monozygotic		Dizygotic		Monozygotic		Dizygotic		Monozygotic		Dizygotic	
Article	N	r	N	r	N	r	N	r	N	r	N	r
Pre-adolescence												
Wardle et al., 2008[229]*	845	0.84	818	0.45	968	0.87	840	0.55	1813	0.86	3279	0.49
Haworth et al., 2008a[227]*	679	0.38	547	0.44	804	0.88	593	0.52	1483	0.86	2090	0.47
Haworth et al., 2008[228]*	-	-	-	-	-	-	-	-	1857	0.86	1669	0.50
Faith et al., 1999[239]	-	-	-	-	-	-	-	-	41	0.85	25	0.24
LLTS[225]	24	0.88	16	0.36	18	0.95	21	0.57	42	0.93	37	0.55
NTR[222]	49	0.87	49	0.61	59	0.88	39	0.72	111	0.88	91	0.65
Young adulthood												
Silventoinen et al., 2008[236]	1582	0.84	1864	0.42	-	-	-	-	-	-	-	-
Harris et al., 1995[234]	380	0.70	342	0.36	486	0.79	409	0.36	-	-	-	-
Korkeila et al., 1991[235]	379	0.77	817	0.41	468	0.72	830	0.33	-	-	-	-
Stunkard et al., 1986[240]	1974	0.81	2097	0.42	-	-	-	-	-	-	-	-
LLTS[225]	18	0.86	19	0.28	19	0.90	17	0.39	37	0.88	36	0.36
NTR[222]	28	0.81	19	0.61	33	0.84	30	0.31	61	0.82	49	0.47
Late adulthood												
Carmichael & McGue, 1995[219]	-	-	-	-	-	-	-	-	137	0.69	136	0.33
Cardon et al., 1994[238]	134	0.67	134	0.24	-	-	-	-	-	-	-	-
Wade et al., 2001[241]	131	0.53	68	0.35	396	0.59	180	0.37	-	-	-	-
Nelson et al., 2000[237]	27	0.67	36	0.33	-	-	-	-	-	-	-	-
CAATSA[223]	11	0.89	19	0.38	22	0.67	24	0.38	33	0.75	43	0.37
Murcia[224]	-	-	-	-	64	0.73	85	0.31	-	-	-	-

*Average sample size and intra-pair correlations of these studies used in meta-analysis (r_{mz} = 0.86 (N=1718), r_{dz} = 0.48 (N=2346). These studies are combined under TEDS in further analyses

MZ = monozygotic, DZ = dizygotic, LLTS = Leuven Longitudinal Twin Study, NTR = Netherlands Twin Registry, CAATSA = Carolina African American Twin Study of Aging, Murcia = Murcia Twin Register

General study characteristics and intra-pair correlations for monozygotic and dizygotic twins of all included studies can be found in Table 8.1 and Table 8.2 respectively. The sex-specific analyses of the IPDs suggested no significant difference in variance components for male and female subjects. Therefore, I reported results on the combined male–female meta-analyses only. Forest plots of heritability estimates from each study are provided in Figure 8.1.

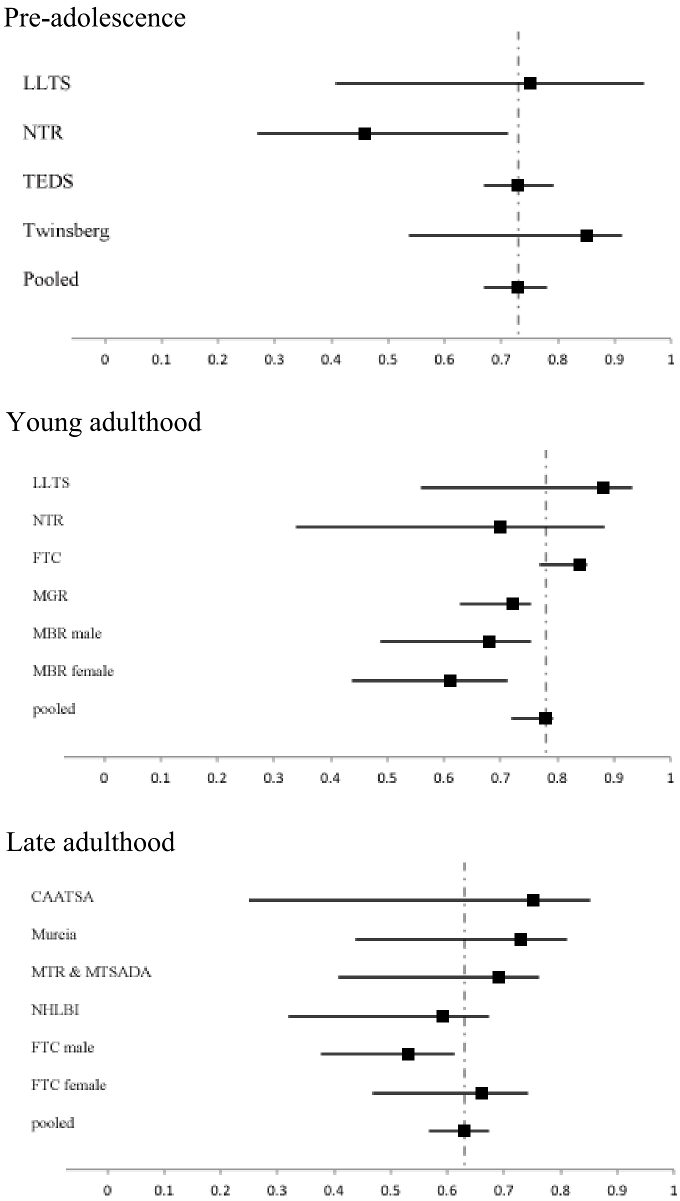

Figure 8.1 Forrest plots of individual and pooled heritability estimates of body mass index in pre-adolescence (9-11 years), young adulthood (18-22 years) and late adulthood (49-65 years).

LLTS=Leuven Longitudinal Twin Study, NTR=Netherlands Twin Registry, TEDS=Twins Early Development Study, FTC=Finnish Twin Cohort, MGR=Multi-Generation Register, MBR=Medical Birth Registry, CAATSA=Carolina African American Twin Study of Aging, Murcia=Murcia Twin Register, MTR & MTSADA=Minnesota Twin Registry & Minnesota Twin Study of Adult Development and Aging, NHLBI=National Heart, Lung and Blood Institute Twin Study. Pooled heritability estimates for pre-adolescence, young adulthood and late adulthood are 0.75, 0.80 and 0.60 respectively.

8.4.2 Heritability analyses

Figure 8.2 shows the variance components estimates for all age categories. Heritability of BMI remains high over the age categories, with estimates of 0.75 (95% confidence interval

(CI) 0.70-0.80) in pre-adolescence, 0.80 (95% CI 0.76-0.81) in young adulthood, and somewhat lower in late adulthood at 0.61 (95% CI 0.54-0.64).

Shared and unique environmental factors seem to follow opposite patterns from each other. As age increases, the proportion of shared environmental influences decreased from small to negligible. Overall, the proportion of variance explained by unique environmental factors seemed to generally increase from 0.14 (95% CI 0.13-0.15) in preadolescence to 0.39 (95% CI 0.36-0.43) in late adulthood.

A model in which the proportion of variance explained by A, C and E were equated over all studies for pre-adolescence and late adulthood fitted as well as the model with free estimates for each individual study, suggesting there was no significant heterogeneity between the studies. However, this was not the case in the young adulthood category, where significant heterogeneity between the studies was present (χ^2= 21.98, df=9, p=0.01).

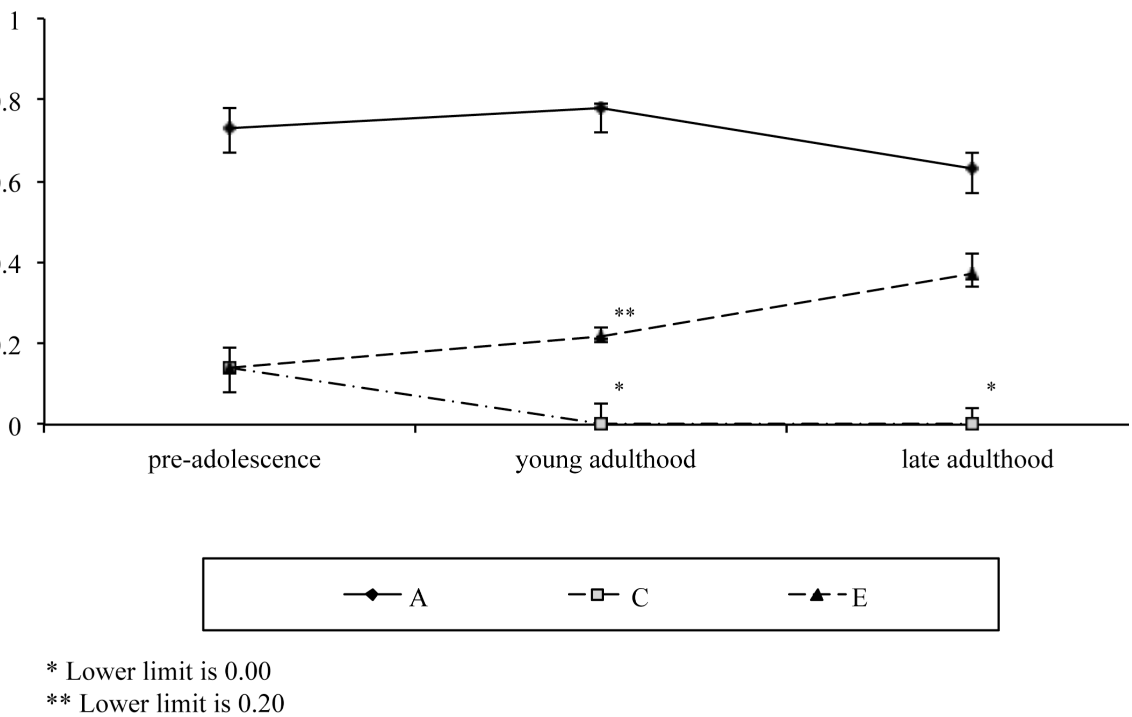

Figure 8.2 Pooled estimates of additive genetic (A), common environmental (C) and unique environmental (E) variance components of body mass index and their 95% confidence intervals for males and females combined in pre-adolescence, young adulthood and late adulthood.

8.5 Discussion

The findings show that heritability of BMI remains high over several age categories. Unique environmental influences on BMI increase with increasing age group, while common environmental factors decrease to being negligible at an older age.

Many studies, including the current, have shown that BMI is highly heritable[72, 225, 231]. So far, about 20 susceptibility genes have been associated with obesity[238, 239]. However, these identified candidate genes only account for about 1% of the total genetic variation of obesity and leave much to be discovered[240]. More specifically, matters are complicated by the fact that different obesity related genes come into play at different stages in life; some play a role in either childhood or adult obesity, whereas others are associated with both[223, 239], emphasizing the importance of studying heritability of BMI at difference ages. No studies, to my knowledge, have reported on the change in heritability over a lifespan. I investigated the heritability of BMI in several age categories in an attempt to study this change.

8.5.1 Heritability of BMI

I found that the heritability of BMI remains high throughout the three age categories and generally increases from pre-adolescence into young adulthood, in accordance with previous studies[223, 241], while it decreases after young adulthood. However, a recent study by Ortega-Alonso et al.[242] found that the heritability of BMI does continue to increase up to old age. Ortega-Alonso et al.[242] suggest that narrower age ranges may be able to show this increase later in life, as the rate of change seems to slow down with age. Contrary to this study, which only included women in young to late adulthood, my analyses included both male and female subjects from pre-adolescence to late adulthood. The inclusion of male subjects in my analyses could be an alternative explanation for why I have found a decrease of heritability in late adulthood, where Ortega-Alonso et al.[242] did not. More data on BMI of older twins would be needed to verify or refute this.

I have shown that common environmental factors have less influence on individual difference in BMI in late adulthood, whereas unique environmental influences increase steadily from pre-adolescence to late adulthood. This pattern is not surprising as we expect their interests and lifestyles to diverge as twins separate from their shared household[243]. My findings of high heritability, while common environmental factors become negligent after young adulthood add confidence to the growing evidence within the field[244].

8.5.2 Limitations

To my knowledge, this is the largest meta-analysis on the heritability of BMI at several ages that combines published correlations and raw data in the same analysis. A large review of published estimates by Maes et al.[245] showed similar results. Additionally, Maes et al.[245] performed a more comprehensive raw data analysis which included data on relative body weight of parents, siblings, spouses and offspring of twins. This method allows for the investigation of any kind of special twin environment that could influence heritability estimations, which is not possible with the classical twin design that I have used in the current meta-analysis. Not only do familiarity, genes and environment separately play a role in determining BMI, interactions between food intake, physical activity and genetic predispositions to excessive weight gain are also possible explanations for obesity[246, 247]. My data did not allow for the investigation of possible gene-environment interactions. Another limitation to the current study is the choice of three age categories. The timing of pubertal growth spurts depends on genetics[1, 248], diet and physical activity[249]. The conclusion might have been different if I had been able to include more categories in the analyses. However, due to lack of raw data for subjects in puberty, I decided to not include this in the analyses.

More data would be needed to adequately investigate this. Due to the nature of our data I was also not able to adequately investigate possible sex differences in heritability of BMI. Some studies have shown that there is indeed a sex difference, such that heritability estimates for women are generally higher than for men[72, 250]. Information on dizygotic opposite sex twin pairs would be needed in order to perform these analyses.

8.5.3 Clinical relevance

Many studies are investigating possible causes and remedies for obesity and its health consequences, with physical activity and inactivity[251-253] amongst the most common environmental factors that are studied. However, only few studies have looked into the genetic influences on obesity and how these change over a lifetime[254].

My findings suggest that there are differences related to either gene expression or gene-environment interaction at different ages across the age categories. The lack of significant heterogeneity in our pre-adolescent and late adulthood data from several countries and over a number of years suggests that genetic influences are still strong, despite the increased relative environmental influence on individual differences in BMI. This in turn suggests that the effectiveness of any environmental intervention would be largely dependent on an individual's genetic predisposition to weight gain and weight loss. Therefore, interventions would probably be most effective if aimed at certain risk groups as opposed to the entire population. This would, however, not be the most efficient method of treating and preventing obesity.

8.5.4 Conclusion

The results in this chapter give reason to do more research into this in order to improve current interventions for overweight and obesity. Moreover, the results confirm that the known candidate genes for obesity only make up for about 1% of the heritability of BMI. Therefore, more obesity-related genes are yet to be identified. My findings also suggest that findings from longitudinal studies would be plausible, as the relative influences of the variance components seem to stay stable over time. In any of these instances, variance components estimates in young adulthood should be verified first. As longitudinal studies over an entire lifespan are not the most time-efficient when looking at changes in heritability of BMI, a cross-sectional study would be the best approximation. A cross-sectional or a combination of cross-sectional and longitudinal studies with raw data could shed more light on the relationship between genes, environment, age and BMI.

CHAPTER 9

GENERAL DISCUSSION

"Coming together is a beginning.
Keeping together is progress.
Working together is success." – Henry Ford

In the introduction of this thesis, I outlined twin-singleton differences in growth and psychological development. There was reason to believe that the effects of ante- and perinatal risk factors on postnatal development (found in singleton research) may be different in twins. Little is known about the effect of risk factors, such as poor antenatal growth, low birth weight and shorter gestations, on the development of healthy twins in the infancy period. I therefore set out to investigate these effects in this thesis. To demonstrate the use of the twin design in epidemiology and to illustrate the changes in genetic and environmental influences on BMI, I included the heritability of BMI as a sub-study in my doctoral research.

9.1 Summary of findings

The most important findings in this thesis are that:

- twins perform below the normal range in the first year of life when assessed with a singleton-based questionnaire, even when low birth weight and shorter gestations have been taken into account. Particularly gross motor skills seem to take the longest time to catch up to singleton standards. Furthermore, in contrast to previous singleton studies, older maternal age does not seem to have a protective influence on early developmental skills and very little association was found with low birth weight. This could indicate that the influence of antenatal factors could be more important than postnatal factors in infancy (Chapter 4);
- larger antenatal head circumference in late pregnancy (approximately >33 weeks) seems an important indicator of decreased gross motor skills in infancy. This could be related to antenatal brain development and insufficient antenatal synaptic pruning

(Chapter 5) or the postnatal changes in communicative and problem-solving skills might not be large enough compared with gross motor skills to detect any effects;

- there is indeed a twin-singleton difference in postnatal growth. Increased weight gain and head circumference growth were associated with worse developmental skills at 24 months, implying that an early focus on physical growth, or apparent catch-up growth, may impair later developmental performance (Chapter 6);
- according to findings in this thesis, there is no evidence that moderate occupational physical activity in twin pregnancy is related to adverse outcomes. However, jobs with more responsibility may lead to shorter gestations (Chapter 7);
- heritability of BMI remains consistently high over different age categories, which has consequences for the search for obesity-related genes and interventions (Chapter 8).

As the results and limitations have been discussed in detail in each individual results chapter, I will focus more on putting my findings in the context of neurodevelopment. I will also discuss the implications for clinical practice and future research, and the dissemination of my findings.

9.2 BiRTHS in context

9.2.1 Theoretical model

In Chapter 1, I proposed a diagram that described the relationship between antenatal risk factors and developmental delays (Figure 1.3). I investigated the relationships that were based on previous literature (solid lines) and did not find the expected relationships between birth outcomes and developmental skills. My next step was to investigate potential other paths to developmental delays (dashed lines). The results from Chapter 5 suggested that worse infant developmental skills might be related to impaired antenatal development of the brain, for which antenatal head circumference could be a proxy. Hüppi et al. (1996)[255] provide some evidence for this theory, as they found that preterm infants had structural brain development delays and behavioural delays at corrected age 40 weeks, compared with term-born infants. It is more likely that delays in brain development occurred due to unfavourable antenatal conditions than due to preterm birth[255]. Therefore, it is likely that delayed brain development could be observed in utero and should be included in the theoretical model as an additional pathway between unfavourable antenatal conditions and developmental delays. Furthermore, there is some evidence that early postnatal catch-up growth is associated with worse developmental skills[55] as outlined and investigated in Chapter 6, but it remains unclear whether there is a direct association between delayed postnatal growth and developmental delays and requires more research.

Myelinated white matter, which is important for communication between different brain areas, has been reported to be decreased in low-risk preterm infants without brain injury[255]. Furthermore, decreased myelination has been suggested to occur specifically in frontal and pre-central brain areas[256], which are important for executive and motor functions[257, 258]. Preterm infants were also found to have decreased cortical volumes in certain brain areas, including sensorimotor, which were related to decreased cognitive skills at 8 years[259]. Furthermore, cortical brain volume in twins born after 33 weeks gestation was decreased compared with singletons of the same gestational age[260], which could be an indication of placental insufficiency during the last trimester in twin pregnancies[19] and suggests that healthy twins would still be at higher risk of developmental delays compared with same-aged singletons. The specific brain areas that seem to be affected by preterm birth could explain findings of worse development in motor skills in previous studies[55, 118] as well as in this thesis. Currently, potential postnatal supplements to improve growth and brain development (and consequently cognitive performance) in preterm infants are caffeine[261], glutamine[262] and fatty acids[263]. However, in light of preventing developmental delays, it would be useful if we could identify a relationship between antenatal delays in brain or physical development and adverse antenatal conditions due to maternal smoking, stress, insufficient nutrition (placental insufficiency) or obstetric complications among others. Although unfavourable antenatal conditions have been associated with adverse pregnancy outcomes that are related to *postnatal* growth and developmental delays[54], the association of maternal and obstetric factors with *antenatal* brain development remains unknown. Technological advances make it possible to perform magnetic resonance imaging of the foetal brain[264, 265], which is currently useful in clinical practice for the diagnosis of abnormalities. Additionally, this technique could have the potential to provide a clearer picture of antenatal brain development and how it is influenced by environmental factors.

9.2.2 BiRTHS in numbers

The original plan for BiRTHS was to recruit 220 families (440 twins), who would complete the 2-year follow-up. In reality, I only recruited 170 families for the 2-year follow-up study, of which about half could be included in my analyses due to incomplete follow-up data. The main reason for this was that recruitment was slower than anticipated and therefore, those who were recruited in the last year of the study period would never be eligible for the 18- or 24-month follow-up. Also, parents were often too busy or forgot to complete the questionnaires on time and were often very reluctant to come back to the hospital for anthropometric measurements of the twins. This resulted in disappointingly small amounts of useable data and occasionally 'sub-optimal' statistical methods to compensate (e.g. Chapter 6). Furthermore, the fact that I had data for less than half of the follow-up measurements for most families, forced me to estimate longitudinal trajectories with the use of cross-sectional methods (Chapters 4 and 6). The small amount of useable data was also due to the questionnaires that were used. For example, we did not ask any questions that were related to parental socioeconomic status and did not use more comprehensive questionnaires for occupation and physical activity. I might have been able to perform more detailed analyses in this thesis if I had had more data on these variables.

Based on the original power calculations, 170 families would be too few to perform any heritability analyses. Therefore, I did post-hoc power calculations to determine whether I had sufficient power to detect mean differences, which was the basis of most of my analyses. A sample size of 150 was of sufficient size to detect differences of at least 0.23SD on a standardised scale if power=0.80 and α=0.05, or 0.28SD if α=0.01. Minimal detectable mean differences were 0.27SD (α=0.05) and 0.32SD (α=0.01) if power=0.90. Despite having sufficient power to detect mean differences presented in this thesis, some results were based on very little data, particularly in the second year. All of these issues combined may have lessened the strength of my study and prevented me from providing more conclusive answers. Instead, the results I present provide a more general overview of infant twin development and could provide guidance for more detailed investigations of potential risk factors in future research.

9.2.3 Representing the United Kingdom

Considering that BiRTHS was meant to be a population cohort, it is important that the cohort is representative of the UK population. Ethnicity has been previously found to moderate the effect of other factors that influence child development. For example, women in ethnic minority groups, particularly Pakistani and Bangladeshi women who make up most of the minority groups in the West Midlands[266], tend to be unemployed more frequently than women of other ethnicities and generally choose parenthood over employment[267]. On the one hand, this could be related to socioeconomic disadvantages, which have been linked to more strict parenting styles[5] that might lead to worse developmental outcomes in children[268]. On the other hand, children might receive more undivided attention from unemployed parents, which may positively influence cognitive development[123].

According to data from the Office of National Statistics (ONS)[269] closest to the peak recruitment period for BiRTHS (mid-2009), about 88% of the population in England and Wales were White and 86% in Birmingham[266]. The ethnic diversity in BiRTHS was very similar, with the majority (81%) being White. The large proportion of White participants could explain why the addition of ethnicity did not improve the regression models in this thesis. Additionally, average maternal age in BiRTHS was similar to that reported from other twin studies within[71, 270] and outside of the UK[271], and within the range reported on the UK population by ONS[272] (25-35 years for singletons and multiples combined).

9.2.4 Potential bias

Although similar to English and Welsh proportions, ethnicity could have been related to sampling bias in BiRTHS, whereby non-English speaking parents were not approached as translated study materials were not available and follow-up sessions would be very difficult to arrange. I did not record any details of families that were not approached or declined consent. It is therefore not possible to estimate how recruitment from three specific hospitals, of which two were located in very ethnically (and socioeconomically) diverse areas, could have influenced selection bias. However, it is possible that we may have excluded a disproportionately high number of non-English speaking parents than if we had included a wider range of hospitals in our study.

As described in section 9.2.3, ethnicity has been related to parental[268] and socioeconomic[5, 273] factors that might influence child development. Furthermore, there are differences between ante- and postnatal growth of Caucasian children and children of other ethnic backgrounds[274-

[276]. The exclusion of non-English speaking parents may therefore have led to an overestimation of developmental trajectories and ante- and postnatal growth patterns. Also, it is possible that the effect of potential risk factors for developmental delay may not be representative for the general population, as the relationship between ante- and perinatal risk factors and postnatal development may be mediated by ethnicity or socioeconomic status. Although there is no data to quantify the proportion of non-English speaking parents in the UK population, it is unlikely that this group would be sufficiently large to have significantly changed my results.

Participation bias is another common issue in cohort studies, which often include a disproportionately high number of participants who are well educated and in paid employment, because they are more likely to participate in research studies[277]. A similar trend for participation bias can be found in the BiRTHS cohort where 85% of parents were in paid employment, compared with an overall 71% in the UK during the study recruitment period[266]. In Chapter 7, I described the current literature on maternal employment and pregnancy outcomes. As various studies have reported different findings, ranging from negative to positive associations, it is not possible to determine whether having fewer unemployed parents or more white-collar employees in the study sample could have caused an over- or underestimation of the results and how large this over- or underestimation would have been. Taking these biases into account, results that are presented in this thesis can only be applied to the general population with confidence when the external validity can be confirmed. Other types of bias are unlikely to occur in cohort studies and are therefore not discussed.

9.2.5 Twin registries around the globe

In Chapter 8, I have already mentioned several twin studies and registries in other countries. Van Dongen et al. (2012)[278] provide a comprehensive overview of many twin registries within and outside of Europe. The variation in primary research interests for these twin registries is large. They include the Barker hypothesis[222], ageing[279], cognitive development[280], and cardiovascular disease[281]. The BiRTHS research theme fits in well with these health-related themes and adds the advantage of antenatal participant recruitment, which seems to be unique at the moment. This has been particularly valuable in that social support, depression and physical activity data in pregnancy were collected prospectively. Also, the prospective nature of the study and regular follow-up of participating families has great potential to provide important information about the development of twins and the wellbeing of their parents from a very early age onwards. Contrary to surveillance studies or studies that mainly rely on medical databases[282], BiRTHS makes use of hypothesis-driven data collection, which means that the collected data is sufficiently detailed to adequately answer specific questions that are raised in previous research or clinical practice (in this case longitudinal follow-up of growth and development). Finally, some established registries extracted anthropometric data from medical records[283, 284]. However, this could increase measurement error as hospital staff turnover is quite frequent and measuring equipment could vary between clinics. Having only a few trained researchers to take measurements with the same equipment has likely minimised measurement error in the BiRTHS cohort.

Data collection (other than anthropometric data) in other studies ranges from postal questionnaires (e.g. in the Australian Twin Registry[285]) to in-depth interviews in the Chinese[286] and German[287] twin registries. The choice to use postal questionnaires to assess developmental skills in BiRTHS was a practical one. The Ages and Stages Questionnaire (ASQ-3; psychometric values described in Chapter 4) was a time-efficient alternative for larger test batteries that can take up to several hours to complete, such as the Griffiths Mental Development Scales[95]. Not only would it have been unfeasible to provide face-to-face assessments at each follow-up, parents would very likely be overwhelmed if their twins were assessed in person each time[288]. Furthermore, face-to-face assessments would only be a reflection of a specific moment of contact with the twins. Parents, on the other hand, can see their children develop every day and could answer questions about their children's development better than a researcher could during a home or clinic visit. One could argue that the ASQ-3 is limited, as children cannot score higher than 'normal development' on each subscale. However, this is unlikely to have been an issue in the current study, because most twins performed below the mean and would probably not have scored 'above average' on more comprehensive assessments that include 'better than normal' categories.

9.3 Clinical implications

9.3.1 Twin-specific standards

Several previous studies[23, 48] have already stated that twin-specific growth charts should be developed due to ante- and postnatal twin-singleton differences. The unsuitability of singleton growth standards and definitions can be exemplified by cases of stunting. Stunting is defined as length below the 5th percentile on an age-appropriate growth chart[289]. It has been previously associated with poor cognitive outcomes[66, 162]. However, I found no associations of length with developmental skills although the twins in BiRTHS were below the 3rd percentile in the first 3 months. There are several possible explanations for these different findings. The first is that previous studies on stunting reported results of developing and low-income countries[66, 162], and may not be comparable to twins in the UK. It is also possible that there is a twin-singleton difference between the association of length and developmental skills, although there is no evidence to support this. Finally, the most likely reason is that the definition of stunting cannot be correctly applied in infant twins, because it is more likely for twins to fall below the 3rd centile at this early age due to different twin growth trajectories in infancy[48]. Similarly, antenatal growth patterns differ significantly from that of singletons[141]. This, together with findings presented in this thesis, strengthens the argument for twin-specific growth standards. Although some postnatal growth charts already exists[48, 49], only a few antenatal growth charts are available for twins, but based on birth weight[23, 290]. In their 2011 report, the National Institute for Health and Clinical Excellence recommended to use prospective ultrasound data in order to create more accurate twin-specific growth charts[291]. Growth measurements from BiRTHS combined with available ultrasound data from other twin cohorts could be used to achieve this.

Considering that infant twins show developmental delays compared with singletons and higher multiples follow similar or even worse developmental trajectories[292], one could argue for the creation of twin-specific norm scores in developmental assessments. However, only 1-2% of deliveries are twin births[11, 293] and higher multiples are even rarer[293]. As development is dependent on many country- or culture-specific factors, such as ethnicity and socioeconomic status[276], this would mean that country-specific twin standards would need to be created. Furthermore, developmental assessments should be based on one norm group to be meaningful. Therefore, it is questionable whether the creation of twin-specific norms for developmental assessments would facilitate useful interpretations of the results. Regardless of whether twin-specific growth and developmental standards are available or not, clinicians and researchers should be aware of these differences to interpret assessment outcomes appropriately.

9.3.2 Pregnancy management guidelines and maternal occupation

To provide twins with the best possible start to life and development, it is vital to investigate potential risk factors during pregnancy. Because many mothers are in paid employment and continue to work throughout their pregnancy[176], I studied the association of occupational physical activity and psychological strain with perinatal outcomes in Chapter 7. Most guidelines by the Royal College of Obstetricians and Gynaecologists[294] regarding physical activity are subjective to the woman's own health and the health of her pregnancy. However, increased body temperature following physical activity in pregnancy has been related to detrimental effects on foetal development[295], regardless of maternal physical fitness. Therefore, low-impact physical activity that does not increase maternal body temperature is recommended[296]. Supplementary to leisure-time physical activity in previous research, I found no evidence to discourage light to moderate occupational physical activity in twin pregnancy.

It has already been established that mothers of twins experience excessive amounts of stress compared with singletons[271, 297]. Although work-related psychological strain has been taken into account in the current occupational health and safety guidelines in the United Kingdom, this additional psychological burden of a twin or higher multiple pregnancy is not acknowledged. I found that jobs with more responsibility (higher psychological strain) might lead to shorter gestations and higher risks of neonatal admission (Chapter 7). It should be noted that my study mostly included jobs that involved moderate physical activity and psychological strain. Further research in a wider range of jobs is needed before more definitive guidelines can be made. Nonetheless, employers should be aware that psychological strain may be related to adverse outcomes in twin pregnancies and appropriate measures should be taken, such as regular evaluations and offering shorter working days or longer maternity leave to relieve work stress.

9.4 Future research

Findings described in this thesis suggest that more research is needed, particularly in the area of antenatal growth in relation to developmental skills. As explained in Chapter 5, studies of antenatal head circumference and developmental skills did not provide consistent results. Although further research is needed to clarify this relationship, it is also important to determine whether any interventions are possible to optimise antenatal head circumference. For example, maternal smoking and the use of folic acid supplements have been related to foetal growth[152] and could provide interventions that would be fairly straightforward to implement. I do recommend that any future research should be conducted in collaboration with clinicians and potentially researchers from other disciplines, so that results can be as comprehensive as possible. In the following paragraphs, I will describe the various possibilities for collaboration and how these can fit in an ideal study design.

9.4.1 Research questions arising from clinical practice

In the 2011 Health and Lifestyle survey undertaken by the Twin and Multiple Births Association (Tamba), parents were asked to review their experiences with UK health care services in the first 18 months of their children's lives[298]. The report that followed this survey stated that postnatal depression rates had declined since 2008, following improved support from health care professionals and Tamba. However, only 36% of parents were offered parent education classes specifically aimed at multiple births, and 21% of mothers were unprepared for the possibility of their children to be premature and admitted to special care wards. Furthermore, advice about breastfeeding was considered inadequate and contradictory. Recommendations were made following the report. In summary, both health care professionals and parents need to be provided with better training and support in order to prepare for the birth and infancy period of multiples. Also, other surveys have resulted in proposals for better education of hospital staff and expectant mothers[198, 299], as they seem to believe physical activity would be harmful and therefore avoid it unnecessarily. These are examples of how surveys of current clinical practice could give direction to translational research.

9.4.2 Cross-disciplinary collaborations

The model I presented in Figure 1.3 can only be verified and expanded by collaborating cross-disciplinarily. The components in the model are likely to be mediated by maternal mental state[146, 300], socioeconomic[5, 268] and genetic factors[68-70], and interactions between parents and children[123, 129]. In Chapters 1 and 8, I described how twins could be used to study the heritability of traits. However, (epi)geneticists could use the collected DNA in BiRTHS to investigate heritability in more detail by identifying specific genes involved in certain traits for example. Similarly, there is more data on maternal and infant health and social factors in BiRTHS that can be used by colleagues in other fields. Particularly social scientists and developmental psychologist might be interested in these data.

The first of such cross-disciplinary collaborations was with the Department of Psychology at The University of Birmingham for which parents in BiRTHS were offered the option to participate in an infant electroencephalogram (EEG) study at 3 months at our clinic at Birmingham Children's Hospital. The EEG study aimed to identify the relationship between infant brain activity and maternal depressive state, in which the twins in BiRTHS served as a control group.

9.4.3 Database linkage

Collaborations on a larger scale have resulted in linkage of various databases, making studies even more interesting and powerful. Some established twin registries have set up collaborations with military service records[283] or even government and medical records using personal identification numbers[279, 282, 301]. Although data collection is limited to the available data in these records, it is an efficient method of identifying eligible participants (twins and higher multiples in this case) and to reduce loss to follow-up due to address changes. There have also been initiatives to link various research databases across borders and disciplines. Linkage of twin studies across borders has led to the GenomEUtwin project[302], in which 12 studies pooled their data for genetic analyses. The general aim of such a project is to enhance datasets in order to identify genes that may not be identified in individual datasets. The Biobanking and Biomolecular Resources Research Infrastructure[303] consists of 59 members, including research institutions, research councils and government bodies. It serves as a platform to facilitate and collaborate on biomedical research. Including twin studies (GenomEUtwin for example) could create new possibilities for genetic and heritability research.

Linking various databases for more than just recruitment is a powerful tool to enhance the currently available data in individual studies. Some studies make use of indirect patient identifiers (probabilistic matching) to match patients included in each database[304]. This avoids the ethical issues of confidentiality and patient privacy, but it is not a full-proof method, as details may change or incorrectly recorded in different clinics. Dokholyan et al. (2009)[305] outline the regulatory and ethical issues involved in linking clinical databases. They suggest that any database set up for research purposes should be reviewed by an independent review board and that patient consent should be asked before collecting personal identifiers. However, there are currently no widespread guidelines for the linkage of databases with personal identifiers. This is particularly an issue if researchers had unlimited access to patients' personal details. It is therefore important to outline the extent to which databases will be linked before the start of a study. Furthermore, there is a need for transparency towards potential research participants, particularly in the informed consent stage. More research into database linkage is needed in order to determine the best method to conduct large-scale, collaborative studies, while preserving the rights of participants.

9.4.4 The role of multimedia in research

Bringing research to the attention of the general public to recruit participants can be done through modern media as demonstrated in the German Observational Study of Adult Twins[287], where the study was published in the newspaper. One mother was recruited into BiRTHS after she had seen the study referenced in a self-help book[306]. It shows potential for references in the media as a recruitment tool and may have been more beneficial for BiRTHS recruitment if we had known about the author's intent to refer to the study.

Not only can the media be used in the recruitment process, it is also an efficient way to bring research findings to the attention of the general public. In the case of twin research, established associations like Tamba are an important source of information for interested individuals. Many twin registries even maintain websites to update the public about the latest findings and events[278]. Companies have already found the development of online social networks, such as Twitter and Facebook, a successful method of marketing their products[307]. Using the profitable business as an analogy, researchers could market their research via these social networks and ensure that anyone who is interested can have access to the available information. The use of non-scientific media to convey research findings to the lay public comes with a major issue of misinformation. Researchers should ensure they provide sufficient context for the study and outline important limitations in press releases and interviews[308, 309]. It is therefore important that researchers, clinicians and journalists work closely together to provide the public with the best possible information.

9.4.5 An integrated study design – the future of twin registries

There are currently initiatives to combine the various collaborations described in §9.4.1-9.4.4 into one large research design, in which there are links between researchers, clinical professionals, the media and the general public. The EPOS consortium[310] is working on such a design in the field of geoscience, for example. However, although the various collaborations occur individually in twin research, there has not yet been a move towards an integrated design. Figure 9.1 is a simplified diagram of the study design that I propose for future twin research, based on the EPOS consortium design and existing collaborations in twin research: Clinicians, who see twin families, raise potential research questions with researchers. Ideally, researchers should make use of cross-disciplinary collaborations and database linkage to investigate the proposed research questions. Research findings are fed back to clinicians via journal publications and suggestions for policy or guideline updates. Researchers outside of the consortium can also be reached via journal publications. Finally, clinicians and researchers inform the media together, so that twin families and the general public can receive relevant information. The main condition in order for this model to work (aside from ethical issues) is communication between the various parties involved, particularly between clinicians and researchers. Efforts should be made to investigate the feasibility of this model and closer collaboration between clinicians and researchers should be encouraged.

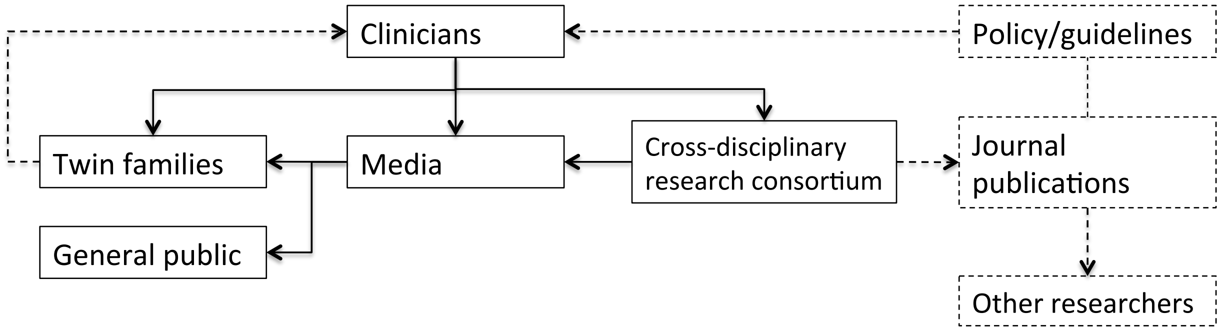

Figure 9.1 Proposed integrated study design for twin research.
Dashed lines (---) represent pathways for feedback, solid lines represent pathways for direct communication. Cross disciplinary research consortium includes database linkage where possible.

9.5 Conclusion

The goal that I had in mind for this thesis was to provide additional information to clinicians, researchers and parents of twins so that they are better prepared for what to expect and understand why twins may seem to differ from singletons in terms of physical and cognitive development. I was unable to provide conclusive answers to the questions I had set in Chapter 1, because I did not achieve the intended size and completeness for the dataset. However, I do believe that the information provided in this thesis is of value, because only very limited information is currently available on the development of twins in infancy.

On one hand, clinicians and researchers should be aware of twin-singleton differences and should take these into account when assessing twin growth and development. This is particularly important when providing feedback to parents, as it can be quite distressing to hear that their child is underperforming according to current standards. On the other hand, the research presented in this thesis should be an additional source of information to parents, who have frequently told me that the available information about twins is insufficient or irrelevant to their situation (e.g. development of extremely premature twins or twins with severe health issues, while their twins are healthy). Until the first results from BiRTHS have been published, I have endeavoured to provide as many ways as possible for parents to contact me for information and for me to keep them informed about recent analyses and presentations. Once studies have been published, parents should be able to access further details via Tamba.

The findings presented in this thesis will need to be replicated in other populations and a more complete dataset. More research is needed, particularly with regard to ethnicity and potential biological explanations of developmental delay. Further follow-up of the twins is needed to study any environmental influences as children grow up. Nonetheless, the current study provides promising results and should be considered a stepping-stone for further research into the influences of ante-, peri- and postnatal factors on early child development. The BiRTHS cohort provides a good platform to continue this research. However, as population-based cohort studies rely heavily on sufficient participants, the future potentially lies in the collaboration between researchers, clinicians and the media to generate more public awareness and enthusiasm for research participation.

CHAPTER 10

REFERENCES

1. Beunen G, Thomis M, Maes HH, et al. Genetic variance of adolescent growth in stature. *Annals of Human Biology* 2000; **27**(2): 173 - 86.
2. Griffiths LJ, Dezateux C, Cole TJ. Differential parental weight and height contributions to offspring birthweight and weight gain in infancy. *Int J Epidemiol* 2007; **36**(1): 104-7.
3. Singhal A, Kennedy K, Lanigan J, et al. Nutrition in infancy and long-term risk of obesity: evidence from 2 randomized controlled trials. *Am J Clin Nutr* 2010; **92**(5): 1133-44.
4. Karmiloff-Smith A. Development itself is the key to understanding developmental disorders. *Trends Cogn Sci* 1998; **2**(10): 389-98.
5. Bradley RH, Corwyn RF. Socioeconomic status and child development. *Annu Rev Psychol* 2002; **53**: 371-99.
6. Homer CJ, James SA, Siegel E. Work-related psychosocial stress and risk of preterm, low birthweight delivery. *Am J Public Health* 1990; **80**(2): 173-7.
7. Nordentoft M, Lou HC, Hansen D, et al. Intrauterine growth retardation and premature delivery: the influence of maternal smoking and psychosocial factors. *Am J Public Health* 1996; **86**(3): 347-54.
8. Papiernik E, Zeitlin J, Delmas D, et al. Differences in outcome between twins and singletons born very preterm: results from a population-based European cohort. *Hum Reprod* 2010; **25**(4): 1035-43.
9. Smith GC, Gutovich J, Smyser C, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. *Ann Neurol* 2011.
10. Devoe LD. Antenatal fetal assessment: multifetal gestation--an overview. *Semin Perinatol* 2008; **32**(4): 281-7.
11. Hall JG. Twinning. *Lancet* 2003; **362**(9385): 735-43.
12. Luu TM, Vohr B. Twinning on the brain: the effect on neurodevelopmental outcomes. *Am J Med Genet C Semin Med Genet* 2009; **151C**(2): 142-7.
13. Black M, Bhattacharya S. Epidemiology of multiple pregnancy and the effect of assisted conception. *Seminars in Fetal & Neonatal Medicine* 2010; **15**(6): 306-12.
14. Price TS, Freeman B, Craig I, Petrill SA, Ebersole L, Plomin R. Infant zygosity can be assigned by parental report questionnaire data. *Twin Res* 2000; **3**(3): 129-33.
15. Joseph KS, Fahey J, Platt RW, et al. An outcome-based approach for the creation of fetal growth standards: do singletons and twins need separate standards? *Am J Epidemiol* 2009; **169**(5): 616-24.
16. Hack KE, Derks JB, Elias SG, et al. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. *BJOG* 2008; **115**(1): 58-67.
17. Acosta-Rojas R, Becker J, Munoz-Abellana B, et al. Twin chorionicity and the risk of adverse perinatal outcome. *Int J Gynaecol Obstet* 2007; **96**(2): 98-102.
18. Lee YM, Wylie BJ, Simpson LL, D'Alton ME. Twin chorionicity and the risk of stillbirth. *Obstet Gynecol* 2008; **111**(2 Pt 1): 301-8.
19. Cleary-Goldman J, D'Alton ME. Growth abnormalities and multiple gestations. *Semin Perinatol* 2008; **32**(3): 206-12.
20. Knox E, Martin W. Multiples clinic: a model for antenatal care. *Semin Fetal Neonatal Med* 2010; **15**(6): 357-61.
21. Bakr AF, Karkour T. What is the optimal gestational age for twin delivery. *BMC Pregnancy Childbirth* 2006; **6**: 3.
22. Udom-Rice I, Inglis SR, Skupski D, Adams D, Chervenak FA. Optimal gestational age for twin delivery. *J Perinatol* 2000; **20**(4): 231-4.
23. Gielen M, Lindsey PJ, Derom C, et al. Twin-specific intrauterine 'growth' charts based on cross-sectional birthweight data. *Twin Res Hum Genet* 2008; **11**(2): 224-35.

24. Shinwell ES, Haklai T, Eventov-Friedman S. Outcomes of multiplets. *Neonatology* 2009; **95**(1): 6-14.
25. Villalbi JR, Salvador J, Cano-Serral G, Rodriguez-Sanz MC, Borrell C. Maternal smoking, social class and outcomes of pregnancy. *Paediatr Perinat Epidemiol* 2007; **21**(5): 441-7.
26. Luciano A, Bolognani M, Biondani P, Ghizzi C, Zoppi G, Signori E. The influence of maternal passive and light active smoking on intrauterine growth and body composition of the newborn. *Eur J Clin Nutr* 1998; **52**(10): 760-3.
27. Horta BL, Victora CG, Menezes AM, Halpern R, Barros FC. Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking. *Paediatr Perinat Epidemiol* 1997; **11**(2): 140-51.
28. Pringle PJ, Geary MP, Rodeck CH, Kingdom JC, Kayamba-Kay's S, Hindmarsh PC. The influence of cigarette smoking on antenatal growth, birth size, and the insulin-like growth factor axis. *J Clin Endocrinol Metab* 2005; **90**(5): 2556-62.
29. Power C, Jefferis BJ. Fetal environment and subsequent obesity: a study of maternal smoking. *Int J Epidemiol* 2002; **31**(2): 413-9.
30. Jones KL. The effects of alcohol on fetal development. *Birth Defects Res C Embryo Today* 2011; **93**(1): 3-11.
31. Pinto SM, Dodd S, Walkinshaw SA, Siney C, Kakkar P, Mousa HA. Substance abuse during pregnancy: effect on pregnancy outcomes. *Eur J Obstet Gynecol Reprod Biol* 2010; **150**(2): 137-41.
32. Snijder CA, te Velde E, Roeleveld N, Burdorf A. Occupational exposure to chemical substances and time to pregnancy: a systematic review. *Hum Reprod Update* 2012; **18**(3): 284-300.
33. Rogers I, Euro-Blcs Study Group. The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life. *Int J Obes Relat Metab Disord* 2003; **27**(7): 755-77.
34. Poulsen P, Vaag A. The intrauterine environment as reflected by birth size and twin and zygosity status influences insulin action and intracellular glucose metabolism in an age- or time-dependent manner. *Diabetes* 2006; **55**(6): 1819-25.
35. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet* 2003; **361**(9363): 1089-97.
36. Odd DE, Rasmussen F, Gunnell D, Lewis G, Whitelaw A. A cohort study of low Apgar scores and cognitive outcomes. *Archives of Disease in Childhood - Fetal Neonatal Edition* 2008; **93**(2): F115-20.
37. Merenstein DJ, Gatti ME, Mays DM. The Association of Mode of Delivery and Common Childhood Illnesses. *Clin Pediatr (Phila)* 2011.
38. Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. *BMJ* 1999; **319**(7206): 339-43.
39. Wadhwa PD. Psychoneuroendocrine processes in human pregnancy influence fetal development and health. *Psychoneuroendocrinology* 2005; **30**(8): 724-43.
40. Rondo PH, Ferreira RF, Nogueira F, Ribeiro MC, Lobert H, Artes R. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur J Clin Nutr* 2003; **57**(2): 266-72.
41. Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev* 2010; **81**(1): 131-48.
42. Gabbe SG, Turner LP. Reproductive hazards of the American lifestyle: work during pregnancy. *Am J Obstet Gynecol* 1997; **176**(4): 826-32.

43. Bonzini M, Coggon D, Godfrey K, Inskip H, Crozier S, Palmer KT. Occupational physical activies, working hours and outcome of pregnancy: findings from the Southampton Women's Survey. *Occupational and Environmental Medicine* 2009; **66**(10): 685-90.
44. Abeysena C, Jayawardana P, Seneviratne Rde A. Effect of psychosocial stress and physical activity on low birthweight: a cohort study. *J Obstet Gynaecol Res* 2010; **36**(2): 296-303.
45. Leonard LG. Depression and anxiety disorders during multiple pregnancy and parenthood. *J Obstet Gynecol Neonatal Nurs* 1998; **27**(3): 329-37.
46. Sorensen HT, Sabroe S, Rothman KJ, et al. Birth weight and length as predictors for adult height. *Am J Epidemiol* 1999; **149**(8): 726-9.
47. Estourgie-van Burk G, Bartels M, Boomsma D, Delemare-van de Wall H. Body size of twins compared with siblings and the general population: From birth to late adolescence. *The Journal of Pediatrics* 2010; **156**(4): 586-91.
48. van Dommelen P, de Gunst M, van der Vaart A, van Buuren S, Boomsma D. Growth references for height, weight and body mass index of twins aged 0-2.5 years. *Acta Paediatr* 2008; **97**(8): 1099-104.
49. Ooki S, Yokoyama Y. Physical growth charts from birth to six years of age in Japanese twins. *J Epidemiol* 2004; **14**(5): 151-60.
50. Griffin IJ, Cooke RJ. Development of whole body adiposity in preterm infants. *Early Hum Dev* 2012; **88 Suppl 1**: S19-24.
51. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992; **35**(7): 595-601.
52. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 2000; **320**(7240): 967-71.
53. Dulloo AG. Thrifty energy metabolism in catch-up growth trajectories to insulin and leptin resistance. *Best Pract Res Clin Endocrinol Metab* 2008; **22**(1): 155-71.
54. Datar A, Jacknowitz A. Birth weight effects on children's mental, motor, and physical development: evidence from twins data. *Maternal and Child Health Journal* 2009; **13**(6): 780-94.
55. Hediger ML, Overpeck MD, Ruan WJ, Troendle JF. Birthweight and gestational age effects on motor and social development. *Paediatric and Perinatal Epidemiology* 2002; **16**(1): 33-46.
56. Laucht M, Esser G, Schmidt MH. Developmental outcome of infants born with biological and psychosocial risks. *J Child Psychol Psychiatry* 1997; **38**(7): 843-53.
57. Gasson N, Piek JP. A longitudinal investigation of the development of fullterm, preterm and 'at-risk' infants from birth to four years using a parent report measure. In: Katsikitis M, editor. 38th Annual APS Conference; 2003 October 2 - 5; Perth; 2003. p. 78 - 82.
58. Siegel LS, Saigal S, Rosenbaum P, et al. Predictors of development in preterm and full-term infants: a model for detecting the at risk child. *Journal of Pediatric Psychology* 1982; **7**(2): 135-48.
59. Piek JP, Dawson L, Smith LM, Gasson N. The role of early fine and gross motor development on later motor and cognitive ability. *Human Movement Science* 2008; **27**: 668 - 81.
60. van Haastert IC, de Vries LS, Helders PJ, Jongmans MJ. Early gross motor development of preterm infants according to the Alberta Infant Motor Scale. *Journal of Pediatrics* 2006; **149**(5): 617-22.
61. Goyen TA, Lui K. Longitudinal motor development of "apparently normal" high-risk infants at 18 months, 3 and 5 years. *Early Human Development* 2002; **70**(1-2): 103-15.

62. Pike KC, Crozier SR, Lucas JS, et al. Patterns of fetal and infant growth are related to atopy and wheezing disorders at age 3 years. *Thorax* 2010; **65**(12): 1099-106.
63. Fancourt R, Campbell S, Harvey D, Norman AP. Follow-up study of small-for-dates babies. *Br Med J* 1976; **1**(6023): 1435-7.
64. Desch LW, Anderson SK, Snow JH. Relationship of head circumference to measures of school performance. *Clin Pediatr (Phila)* 1990; **29**(7): 389-92.
65. Deutsch CK, Joseph RM. Brief report: cognitive correlates of enlarged head circumference in children with autism. *J Autism Dev Disord* 2003; **33**(2): 209-15.
66. Crookston BT, Dearden KA, Alder SC, et al. Impact of early and concurrent stunting on cognition. *Matern Child Nutr* 2011; **7**(4): 397-409.
67. Silva A, Metha Z, O'Callaghan F J. The relative effect of size at birth, postnatal growth and social factors on cognitive function in late childhood. *Ann Epidemiol* 2006; **16**(6): 469-76.
68. Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. *Am J Epidemiol* 2007; **165**(7): 734-41.
69. Beardsall K, Ong KK, Murphy N, et al. Heritability of childhood weight gain from birth and risk markers for adult metabolic disease in prepubertal twins. *J Clin Endocrinol Metab* 2009; **94**(10): 3708-13.
70. Fabsitz RR, Sholinsky P, Carmelli D. Genetic influences on adult weight gain and maximum body mass index in male twins. *Am J Epidemiol* 1994; **140**(8): 711-20.
71. Koeppen-Schomerus G, Wardle J, Plomin R. A genetic analysis of weight and overweight in 4-year-old twin pairs. *International Journal of Obesity* 2001; **25**: 838 - 44.
72. Schousboe K, Willemsen G, Kyvik K, et al. Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. *Twin Research* 2003; **6**(5): 409 - 21.
73. MacGregor AJ, Snieder H, Schork NJ, Spector TD. Twins. Novel uses to study complex traits and genetic diseases. *Trends Genet* 2000; **16**(3): 131-4.
74. Purcell S. Variance components models for gene-environment interaction in twin analysis. *Twin Res* 2002; **5**(6): 554-71.
75. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet* 2002; **3**(11): 872-82.
76. Plomin R, DeFries JC, McClearn GE, McGuffin P. Nature, nurture, and behavior. New York: Worth Publishers and W.H.Freeman and Company; 2001.
77. Rice TK. Familial resemblance and heritability. *Adv Genet* 2008; **60**: 35-49.
78. Krone RE, Ewer AK, Barrett TG, et al. The Birmingham Registry for Twin and Heritability Studies (BiRTHS). *Twin Res Hum Genet* 2006; **9**(6): 907-12.
79. WHO. International Classification of Diseases (ICD). 10th ed; 2010.
80. Broadhead WE, Gehlbach SH, de Gruy FV, Kaplan BH. The Duke-UNC Functional Social Support Questionnaire. Measurement of social support in family medicine patients. *Med Care* 1988; **26**(7): 709-23.
81. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol* 2003; **56**(12): 1163-9.
82. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 1987; **150**: 782-6.
83. Squires JT, Bricker D, Potter L. Excerpts from ASQ-3 User's Guide: Brookes Publishing Co. Inc.; 2009.
84. Oragene DNA (OG-250). http://www.dnagenotek.com/ROW/products/OG250.html (accessed 29 August 2012).

85. StataCorp. Stata Statistical Software: Release 11. In: College Station T, editor.: StataCorp LP; 2009.
86. Berk L. Intelligence. Child development. 6th ed. Boston, MA: Pearson Education, Inc; 2003.
87. Berk L. Language development. Child development. 6th ed. Boston, MA: Pearson Education, Inc; 2003.
88. Berk L. Personality and social development. Child development. 6th ed. Boston, MA: Pearson Education, Inc; 2003.
89. Berk L. Infancy: Early learning, motor skills and perceptual capacities. Child development. 6th ed. Boston, MA: Pearson Education, Inc; 2003.
90. Anderson V, Northam E, Hendy J, Wrennal J. Cognitive development. Developmental Neuropsychology. New York, NY: Psychology Press Ltd.; 2005.
91. Klamer A, Lando A, Pinborg A, Greisen G. Ages and Stages Questionnaire used to measure cognitive deficit in children born extremely preterm. *Acta Paediatrica* 2005; **94**(9): 1327-9.
92. Limbos MM, Joyce DP. Comparison of the ASQ and PEDS in screening for developmental delay in children presenting for primary care. *Journal of Developmental & Behavioral Pediatrics* 2011; **32**(7): 499-511.
93. Page FP. Parents Evaluation of Developmental Status (PEDS). *Indian Pediatrics* 2003; **40**(5): 439-40; author reply 41-2.
94. Bayley N. Bayley scales of infant and toddler development. 3rd ed. San Antonia, TX: Harcourt Assessment, Inc; 2006.
95. McLean M, McCormick K, Baird S. Concurrent validity of the Griffiths' Mental Develpment Scales with a population of children under 24 months. *Journal of Early Intervention* 1991; **15**(4): 338 - 44.
96. Sutcliffe AG, Derom C. Follow-up of twins: health, behaviour, speech, language outcomes and implications for parents. *Early Human Development* 2006; **82**(6): 379-86.
97. Ronalds GA, De Stavola BL, Leon DA. The cognitive cost of being a twin: evidence from comparisons within families in the Aberdeen children of the 1950s cohort study. *British Medical Journal* 2005; **331**(7528): 1306.
98. Drillien CM. School disposal and performance for children of different birthweight born 1953-1960. *Archives of Disease in Childhood* 1969; **44**(237): 562-70.
99. Cooke R. Does neonatal and infant neurodevelpmental morbidity of multiples and singletons differ? *Seminars in Fetal & Neonatal Medicine* 2010; **15**: 362 - 6.
100. Posthuma D, De Geus EJ, Bleichrodt N, Boomsma DI. Twin-singleton differences in intelligence? *Twin Res* 2000; **3**(2): 83-7.
101. Pike A, Iervolino AC, Eley TC, Price T, Plomin R. Environmental risk and young children's cognitive and behavioral development. *International Journal of Behavioral Development* 2006; **30**(1): 55 - 66.
102. de Moura DR, Costa JC, Santos IS, et al. Risk factors for suspected developmental delay at age 2 years in a Brazilian birth cohort. *Paediatric and Perinatal Epidemiology* 2010; **24**(3): 211-21.
103. Rose SA, Feldman JF, Jankowski JJ, Van Rossem R. A Cognitive Cascade in Infancy: Pathways from Prematurity to Later Mental Development. *Intelligence* 2008; **36**(4): 367-78.
104. Berglund E, Eriksson M, Westerlund M. Communicative skills in relation to gender, birth order, childcare and socioeconomic status in 18-month-old children. *Scandivian Journal of Psychology* 2005; **46**(6): 485-91.
105. Rutter M, Thorpe K, Greenwood R, Northstone K, Golding J. Twins as a natural experiment to study the causes of mild language delay: I: Design; twin-singleton differences

in language, and obstetric risks. *Journal of Child Psychology & Psychiatry* 2003; **44**(3): 326-41.
106. Thorpe K. Twin children's language development. *Early Human Development* 2006; **82**(6): 387-95.
107. Petrill SA, Pike A, Price T, Plomin R. Chaos in the home and socioeconomic status are associated with cognitive development in early childhood: Environmental mediators identified in a genetic design. *Intelligence* 2004; **32**: 445 - 60.
108. Lung FW, Shu BC, Chiang TL, Lin SJ. Twin-singleton influence on infant development: a national birth cohort study. *Child: Care, Health and Development* 2009; **35**(3): 409-18.
109. Mundy P, Kasari C, Sigman M, Ruskin E. Nonverbal communication and early language acquisition in children with Down syndrome and in normally developing children. *J Speech Hear Res* 1995; **38**(1): 157-67.
110. Laakso M-L, Poikkeus AM, Katajamäki J, Lyytinen P. Early intentional communication as a predictor of language development in young toddlers. *First Language* 1999; **19**: 207 - 31.
111. Rietveld MJ, Hudziak JJ, Bartels M, van Beijsterveldt CE, Boomsma DI. Heritability of attention problems in children: longitudinal results from a study of twins, age 3 to 12. *Journal of Child Psychology & Psychiatry* 2004; **45**(3): 577-88.
112. Leung GP, Chan CC, Chung RC, Pang MY. Determinants of activity and participation in preschoolers with developmental delay. *Research in Developmental Disabilities* 2010; **32**(1): 289-96.
113. Iverson JM. Developing language in a developing body: the relationship between motor development and language development. *Journal of Child Language* 2010; **37**(2): 229-61.
114. Yokoyama Y, Wada S, Sugimoto M, Saito M, Matsubara M, Sono J. Comparison of motor development between twins and singletons in Japan: a population-based study. *Twin Res Hum Genet* 2007; **10**(2): 379-84.
115. McPhillips M, Jordan-Black JA. The effect of social disadvantage on motor development in young children: a comparative study. *J Child Psychol Psychiatry* 2007; **48**(12): 1214-22.
116. Jeng SF, Yau KI, Liao HF, Chen LC, Chen PS. Prognostic factors for walking attainment in very low-birthweight preterm infants. *Early Human Development* 2000; **59**(3): 159-73.
117. Sullivan MC, Margaret MM. Perinatal morbidity, mild motor delay, and later school outcomes. *Developmental Medicine & Child Neurology* 2003; **45**(2): 104-12.
118. Thun-Hohenstein L, Largo RH, Molinari L, Kundu S, Duc G. Early fine motor and adaptive development in high-risk appropriate for gestational age preterm and healthy term children. *European Journal of Pediatrics* 1991; **150**(8): 562-9.
119. Murray GK, Veijola J, Moilanen K, et al. Infant motor development is associated with adult cognitive categorisation in a longitudinal birth cohort study. *Journal of Child Psychology & Psychiatry* 2006; **47**(1): 25-9.
120. Krone RE, Ewer AK, Barrett TG, et al. The Birmingham Registry for Twin and Heritiability Studies (BiRTHS). *Twin Research and Human Genetics* 2006; **9**(6): 907-12.
121. Gutierrez RG. Tricks of the trade: Getting the most out of xtmixed. North American Stata Users Group Meeting; 2008; 2008.
122. Asbury K, Almeida D, Hibel J, Harlaar N, Plomin R. Clones in the classroom: a daily diary study of the nonshared environmental relationship between monozygotic twin differences in school experience and achievement. *Twin Research and Human Genetics* 2008; **11**(6): 586-95.

123. Rutter M, Redshaw J. Annotation: growing up as a twin: twin-singleton differences in psychological development. *Journal of Child Psychology & Psychiatry* 1991; **32**(6): 885-95.
124. Roze E, Meijer L, Van Braeckel KN, Ruiter SA, Bruggink JL, Bos AF. Developmental trajectories from birth to school age in healthy term-born children. *Pediatrics* 2010; **126**(5): e1134-42.
125. Janssen AJ, Akkermans RP, Steiner K, et al. Unstable longitudinal motor performance in preterm infants from 6 to 24 months on the Bayley Scales of Infant Development--Second edition. *Research in Developmental Disabilities* 2011; **32**(5): 1902-9.
126. Sherlock RL, Synnes AR, Koehoorn M. Working mothers and early childhood outcomes: lessons from the Canadian National Longitudinal Study on Children and Youth. *Early Human Development* 2008; **84**(4): 237-42.
127. Waldfogel J, Han WJ, Brooks-Gunn J. The effects of early maternal employment on child cognitive development. *Demography* 2002; **39**(2): 369-92.
128. Harvey E. Short-term and long-term effects of early parental employment on children of the National Longitudinal Survey of Youth. *Developmental Psychology* 1999; **35**(2): 445-59.
129. Fergusson E, Maughan B, Golding J. Which children receive grandparental care and what effect does it have? *Journal of Child Psychology & Psychiatry* 2008; **49**(2): 161-9.
130. Erdemoglu E, Mungan T, Tapisiz OL, Ustunyurt E, Caglar E. Effect of inter-twin delivery time on Apgar scores of the second twin. *Australian and New Zealand Journal of Obstetrics and Gynaecology* 2003; **43**(3): 203-6.
131. Rossi A, Mullin P, Chmait R. Neonatal outcomes of twins according to birth order, presentation and mode of delivery: a systematic review and meta-analysis*. *Bjog* 2011; **118**(5): 523-32.
132. Tideman E. Longitudinal follow-up of children born preterm: cognitive development at age 19. *Early Human Development* 2000; **58**(2): 81-90.
133. McLoyd VC. Socioeconomic disadvantage and child development. *Am Psychol* 1998; **53**(2): 185-204.
134. Blickstein I. Growth aberration in multiple pregnancy. *Obstet Gynecol Clin North Am* 2005; **32**(1): 39-54, viii.
135. Glinianaia SV, Skjaerven R, Magnus P. Birthweight percentiles by gestational age in multiple births. A population-based study of Norwegian twins and triplets. *Acta Obstet Gynecol Scand* 2000; **79**(6): 450-8.
136. Smith AP, Ong S, Smith NC, Campbell D. A prospective longitudinal study of growth velocity in twin pregnancy. *Ultrasound Obstet Gynecol* 2001; **18**(5): 485-7.
137. Loos RJ, Derom C, Derom R, Vlietinck R. Determinants of birthweight and intrauterine growth in liveborn twins. *Paediatr Perinat Epidemiol* 2005; **19 Suppl 1**: 15-22.
138. Ong S, Lim MN, Fitzmaurice A, Campbell D, Smith AP, Smith N. The creation of twin centile curves for size. *Bjog* 2002; **109**(7): 753-8.
139. Dias T, Arcangeli T, Bhide A, Mahsud-Dornan S, Papageorghiou A, Thilaganathan B. Second-trimester assessment of gestational age in twins: validation of singleton biometry charts. *Ultrasound Obstet Gynecol* 2011; **37**(1): 34-7.
140. Devoe LD, Ware DJ. Antenatal assessment of twin gestation. *Semin Perinatol* 1995; **19**(5): 413-23.
141. Blickstein I. Normal and abnormal growth of multiples. *Semin Neonatol* 2002; **7**(3): 177-85.
142. Ananth CV, Vintzileos AM, Shen-Schwarz S, Smulian JC, Lai YL. Standards of birth weight in twin gestations stratified by placental chorionicity. *Obstet Gynecol* 1998; **91**(6): 917-24.

143. Mattsson N, Rosendahl H, Luukkaala T. Good accuracy of ultrasound estimations of fetal weight performed by midwives. *Acta Obstet Gynecol Scand* 2007; **86**(6): 688-92.
144. Walker SP, Thame MM, Chang SM, Bennett F, Forrester TE. Association of growth in utero with cognitive function at age 6-8 years. *Early Hum Dev* 2007; **83**(6): 355-60.
145. Harvey D, Prince J, Bunton J, Parkinson C, Campbell S. Abilities of children who were small-for-gestational-age babies. *Pediatrics* 1982; **69**(3): 296-300.
146. Henrichs J, Schenk JJ, Barendregt CS, et al. Fetal growth from mid- to late pregnancy is associated with infant development: the Generation R Study. *Dev Med Child Neurol* 2010; **52**(7): 644-51.
147. Gale CR, O'Callaghan FJ, Godfrey KM, Law CM, Martyn CN. Critical periods of brain growth and cognitive function in children. *Brain* 2004; **127**(Pt 2): 321-9.
148. van Batenburg-Eddes T, de Groot L, Steegers EA, et al. Fetal programming of infant neuromotor development: the generation R study. *Pediatr Res* 2010; **67**(2): 132-7.
149. Vanderploeg R. Clinician's guid to neuropsychological assessment. 2nd Ed. ed: Psychology Press; 1999.
150. Cleary-Goldman J, Malone FD, Vidaver J, et al. Impact of maternal age on obstetric outcome. *Obstet Gynecol* 2005; **105**(5 Pt 1): 983-90.
151. Lumley J. Defining the problem: the epidemiology of preterm birth. *Bjog* 2003; **110 Suppl 20**: 3-7.
152. Mook-Kanamori DO, Steegers EA, Eilers PH, Raat H, Hofman A, Jaddoe VW. Risk factors and outcomes associated with first-trimester fetal growth restriction. *JAMA* 2010; **303**(6): 527-34.
153. Law J, Lee M, Hülse M, Tomassetti A. The infant development timeline and its application to robot shaping. *Adaptive Behavior* 2011; **19**(5): 335-58.
154. Peterson J, Taylor HG, Minich N, Klein N, Hack M. Subnormal head circumference in very low birth weight children: neonatal correlates and school-age consequences. *Early Hum Dev* 2006; **82**(5): 325-34.
155. Horemuzova E, Soder O, Hagenas L. Growth charts for monitoring postnatal growth at NICU of extreme preterm-born infants. *Acta Paediatr* 2012; **101**(3): 292-9.
156. Cole TJ. Growth monitoring with the British 1990 growth reference. *Arch Dis Child* 1997; **76**(1): 47-9.
157. Bertino E, Di Nicola P, Giuliani F, et al. Evaluation of postnatal growth of preterm infants. *J Matern Fetal Neonatal Med* 2011; **24 Suppl 2**: 9-11.
158. Patel AL, Engstrom JL, Meier PP, Jegier BJ, Kimura RE. Calculating postnatal growth velocity in very low birth weight (VLBW) premature infants. *J Perinatol* 2009; **29**(9): 618-22.
159. Buckler JM, Green M. A comparison of the early growth of twins and singletons. *Ann Hum Biol* 2004; **31**(3): 311-32.
160. Howe TH, Sheu CF, Wang TN, Hsu YW, Wang LW. Neuromotor outcomes in children with very low birth weight at 5 yrs of age. *Am J Phys Med Rehabil* 2011; **90**(8): 667-80.
161. Taki Y, Hashizume H, Sassa Y, et al. Correlation among body height, intelligence, and brain gray matter volume in healthy children. *Neuroimage* 2012; **59**(2): 1023-7.
162. Dewey KG, Begum K. Long-term consequences of stunting in early life. *Matern Child Nutr* 2011; **7 Suppl 3**: 5-18.
163. Belfort MB, Rifas-Shiman SL, Sullivan T, et al. Infant growth before and after term: effects on neurodevelopment in preterm infants. *Pediatrics* 2011; **128**(4): e899-906.
164. Reolon RK, Rotta NT, Agranonik M, Silva AA, Goldani MZ. Influence of intrauterine and extrauterine growth on neurodevelopmental outcome of monozygotic twins. *Braz J Med Biol Res* 2008; **41**(8): 694-9.

165. LMSgrowth. Medical Research Council; 1998.
166. Cooke RJ. Catch-up growth: implications for the preterm and term infant. *Eur J Clin Nutr* 2010; **64 Suppl 1**: S8-S10.
167. Knickmeyer RC, Kang C, Woolson S, et al. Twin-singleton differences in neonatal brain structure. *Twin Res Hum Genet* 2011; **14**(3): 268-76.
168. Ordaz SJ, Lenroot RK, Wallace GL, et al. Are there differences in brain morphometry between twins and unrelated singletons? A pediatric MRI study. *Genes Brain Behav* 2010; **9**(3): 288-95.
169. Penhune VB. Sensitive periods in human development: evidence from musical training. *Cortex* 2011; **47**(9): 1126-37.
170. Cheong JL, Hunt RW, Anderson PJ, et al. Head growth in preterm infants: correlation with magnetic resonance imaging and neurodevelopmental outcome. *Pediatrics* 2008; **121**(6): e1534-40.
171. Cole TJ, Donaldson MD, Ben-Shlomo Y. SITAR--a useful instrument for growth curve analysis. *Int J Epidemiol* 2010; **39**(6): 1558-66.
172. Johnson L, Llewellyn CH, van Jaarsveld CH, Cole TJ, Wardle J. Genetic and environmental influences on infant growth: prospective analysis of the Gemini twin birth cohort. *PLoS One* 2011; **6**(5): e19918.
173. Methods and Development. WHO Child Growth Standards: Growth velocity based on weight, length and head circumference: World Health Organisation; 2009.
174. Touwslager RN, Gielen M, Derom C, et al. Determinants of infant growth in four age windows: a twin study. *J Pediatr* 2011; **158**(4): 566-72 e2.
175. Duffield M. Trends in female employment. *UK National Statistics: Labour Market Trends* 2002.
176. UK National Statistics: Labour Market. 2011. http://www.statistics.gov.uk/hub/labour-market/index.html (accessed 29/06/2011.
177. Halliday-Bell JA. Laboratory work and adverse pregnancy outcomes. *Occupational medicine* 2010; **60**(4): 310.
178. Farrow A, Shea KM, Little RE. Birthweight of term infants and maternal occupation in a prospective cohort of pregnant women. The ALSPAC Study Team. *Occup Environ Med* 1998; **55**(1): 18-23.
179. Domingues MR, Matijasevich A, Barros AJD. Physical activity and preterm birth. A literature review. *Sports Medicine* 2009; **39**(1): 961-75.
180. Vrijkotte TGM. First-Trimester Working Conditions and Birthweight: A Prospective Cohort Study. *American Journal of Public Health* 2009; **99**(8): 1409.
181. Bell JF, Zimmerman FJ, Diehr PK. Maternal work and birth outcome disparities. *Matern Child Health J* 2008; **12**(4): 415-26.
182. Hatch MC, Shu X, McLean DE, et al. Maternal exercise during pregnancy, physical fitness, and fetal growth. *American Journal of Epidemiology* 1993; **137**(10).
183. Takito MY, Benito MH. Physical activity during pregnancy and fetal outcomes: a case-control study. *Revista de saúde pública* 2010; **44**(1): 90-101.
184. Brown W. The benefits of physical activity during pregnancy. *Journal of Science and Medicine in Sport* 2002; **5**(1): 37-45.
185. Lumbers ER. Exercise in pregnancy: Physiological basis of exercise prescription for the pregnant woman. *Journal of Science and Medicine in Sport* 2002; **5**(1): 20-31.
186. Escriba-Aguir V, Perez-Hoyos S, Saurel-Cubizolles MJ. Physical load and psychological demand at work during pregnancy and preterm birth. *Int Arch Occup Environ Health* 2001; **74**(8): 583-8.
187. Bungum TJ, Peaslee DL, Jackson AW, Perez MA. Exercise during pregnancy and type of delivery in nulliparae. *J Obstet Gynecol Neonatal Nurs* 2000; **29**(3): 258-64.

188. Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ* 1999; **318**(7181): 427-31.
189. Jiang ZD, Zang Z, Wilkinson AR. Cochlear function in 1-year-old term infants born with hypoxia-ischaemia or low Apgar scores. *J Paediatr Child Health* 2012; **48**(2): 160-5.
190. Friedman SA, Schiff E, Kao L, Kuint J, Sibai BM. Do twins mature earlier than singletons? Results from a matched cohort study. *Am J Obstet Gynecol* 1997; **176**(6): 1193-6; discussion 6-9.
191. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and Science in Sports and Exercise* 2000; **32**(9 Suppl): S498-504.
192. de Smet P, Sans S, Dramaix M, et al. Gender and regional differences in perceived job stress across Europe. *Eur J Public Health* 2005; **15**(5): 536-45.
193. Bryan E. The impact of multiple preterm births on the family. *Hosp Med* 2003; **64**(11): 648-50.
194. Helder OK, Brug J, Looman CW, van Goudoever JB, Kornelisse RF. The impact of an education program on hand hygiene compliance and nosocomial infection incidence in an urban neonatal intensive care unit: an intervention study with before and after comparison. *Int J Nurs Stud* 2010; **47**(10): 1245-52.
195. Ganzeboom KS, Mairuhu G, Reitsma JB, Linzer M, Wieling W, van Dijk N. Lifetime cumulative incidence of syncope in the general population: a study of 549 Dutch subjects aged 35-60 years. *J Cardiovasc Electrophysiol* 2006; **17**(11): 1172-6.
196. Pate RR, Pratt M, Blair SN, et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA* 1995; **273**(5): 402-7.
197. Melzer K, Schutz Y, Soehnchen N, et al. Effects of recommended levels of physical activity on pregnancy outcomes. *Am J Obstet Gynecol* 2010; **202**(3): 266 e1-6.
198. Clarke PE, Gross H. Women's behaviour, beliefs and information sources about physical exercise in pregnancy. *Midwifery* 2004; **20**(2): 133-41.
199. Borodulin K, Evenson KR, Herring AH. Physical activity patterns during pregnancy through postpartum. *BMC Womens Health* 2009; **9**: 32.
200. Lyness KS, Thompson CA, Francesco AM, Judiesch MK. Work and pregnancy: individual and organizational factors influencing organizational commitment, timing of maternity leave, and return to work. *Sex Roles* 1999; **41**(7-8): 485-508.
201. Hobel CJ, Goldstein A, Barrett ES. Psychosocial stress and pregnancy outcome. *Clin Obstet Gynecol* 2008; **51**(2): 333-48.
202. Hagstromer M, Oja P, Sjostrom M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutr* 2006; **9**(6): 755-62.
203. Neilson HK, Robson PJ, Friedenreich CM, Csizmadi I. Estimating activity energy expenditure: how valid are physical activity questionnaires? *Am J Clin Nutr* 2008; **87**(2): 279-91.
204. Prince SA, Adamo KB, Hamel ME, Hardt J, Gorber SC, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. *Int J Behav Nutr Phys Act* 2008; **5**: 56.
205. Karasek R, Brisson C, Kawakami N, Houtman I, Bongers P, Amick B. The Job Content Questionnaire (JCQ): an instrument for internationally comparative assessments of psychosocial job characteristics. *J Occup Health Psychol* 1998; **3**(4): 322-55.
206. Rachele JN, McPhail SM, Washington TL, Cuddihy TF. Practical physical activity measurement in youth: a review of contemporary approaches. *World J Pediatr* 2012; **8**(3): 207-16.

207. James PT, Leach R, Kalamara E, Shayeghi M. The worldwide obesity epidemic. *Obes Res* 2001; **9 Suppl 4**: 228S-33S.
208. WHO. The World Health Organization warns of the rising threat of heart disease and stroke as overweight and obesity rapidly increase. WHO news release. 2005.
209. WHO. March 2011 2011. http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed January 24 2012).
210. Dietz WH, Gortmaker SL. Do we fatten our children at the television set? Obesity and television viewing in children and adolescents. *Pediatrics* 1985; **75**: 807 - 12.
211. Cohen D. Obesity and the built environment: changes in environmental cues cause energy imbalances. *International Journal of Obesity* 2008; **32**: S137 - S42.
212. Hu F, Li T, Colditz G. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *Journal of the American Medical Association* 2003; **289**(14): 1785 - 91.
213. Haworth CM, Carnell S, Meaburn EL, Davis OS, Plomin R, Wardle J. Increasing heritability of BMI and stronger associations with the FTO gene over childhood. *Obesity (Silver Spring)* 2008; **16**(12): 2663-8.
214. Lajunen H, Kaprio J, Keski-Rahkonen A, et al. Genetic and environmental effects on body mass index during adolescence: a prospective study among Finnish twins. *International Journal of Obesity* 2009; **33**: 559 - 67.
215. Carmichael C, McGue M. A cross-sectional examination of height, weight, and body mass index in adult twins. *Journal of Gerontology: Biological Sciences* 1995; **50A**(4): B237 - B44.
216. Zillikens M, Yazdanpanah M, Pardo L, et al. Sex-specific genetic effects influence variation in body composition. *Diabetologia* 2008; **51**: 2233 - 41.
217. Wisniewski A, Chernausek S. Gender in childhood obesity: family environment, hormones, and genes. *Gender Medicine* 2009; **6**: 76 - 85.
218. Boomsma D, de Geus E, Vink J, et al. Netherlands Twin Register: From twins to twin families. *Twin Research and Human Genetics* 2006; **9**(6): 849 - 57.
219. Whitfield K, Brandon D, Wiggins S, Vogler G, McClearn G. Does intact pair status matter in the study of African American Twins? The Carolina African American Twin Study of Aging. *Experimental Aging Research* 2003; **29**(4): 407 - 23.
220. Ordoñana J, Pérez-Riquelme F, González-Javier F, Carrillo E, Goméz-Amor J, Martínez-Selva J. An initiative in Spain for the study of women's health: The Murcia Twin Registry. *Twin Research and Human Genetics* 2006; **9**(6): 865 - 7.
221. Beunen G, Maes H, Vlietinck R, et al. Univariate and multivariate genetic analysis of subcutaneous fatness and fat distribution in early adolescence. *Behavior Genetics* 1998; **28**(4): 179 - 288.
222. Derom C, Vlietinck R, Thiery E, Leroy F, Fryns J, Derom R. The East Flanders Prospective Twin Survey (EFPTS). *Twin Research and Human Genetics* 2006; **9**(6): 733 - 8.
223. Haworth C, Carnell S, Meaburn E, Davis O, Plomin R, Wardle J. Increasing heritability of BMI and stronger associations with the FTO gene over childhood. *Obesity* 2008a; **16**(12): 2663 - 8.
224. Haworth C, Plomin R, Carnell S, Wardle J. Childhood obesity: genetic and environmental overlap with normal-range BMI. *Obesity* 2008; **16**(7): 1585 - 90.
225. Wardle J, Carnell S, Haworth C, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *American Journal of Clinical Nutrition* 2008; **87**: 398 - 404.
226. Neale M. Mx: Statistical Modeling 5th ed: Department of Psychiatry, Box 126 MCV, Richmond, VA 23298; 1999.

227. Neale M, Cardon L. Methodology for genetic studies of twins and families. Dordrecht, the Netherlands: Kluwer Academic Publishers; 1992.
228. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003; **60**(12): 1187-92.
229. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* 2000; **157**(10): 1552-62.
230. Harris J, Tambs K, Magnus P. Sex-specific effects for body mass index in the new Norwegian Twin Panel. *Genetic Epidemiology* 1995; **12**: 251 - 65.
231. Korkeila M, Kaprio J, Rissanen A, Koskenvuo M. Effects of gender and age on the heritability of body mass index. *International Journal of Obesity* 1991; **15**: 647 - 54.
232. Silventoinen K, Magnusseon P, Tynelius P, Kaprio J, Rasmussen F. Heritability of body size and muscle strength in young adulthood: A study of one million Swedish men. *Genetic Epidemiology* 2008; **32**(4): 341 - 9.
233. Nelson T, Vogler G, Pedersen N, Hong Y, Miles T. Genetic and environmental influences on body fat distribution, fasting insulin levels and CVD: are the influences shared? *Twin Research and Human Genetics* 2000; **3**(1): 43-50.
234. Cardon L, Carmelli D, Fabsitz R, Reed T. Genetic and environmental correlations between obesity and body fat distribution in adult male twins. *Human Biology* 1994; **66**(3): 465 - 79.
235. Faith M, Pietrobelli A, Nuñez C, Heo M, Heymsfield S, Allison D. Evidence for independent genetic influences of fat mass and body mass index in a pediatric twin sample. *Pediatrics* 1999; **104**: 61 - 7.
236. Stunkard A, Foch T, Hrubec Z. A twin study of human obesity. *Journal of the American Medical Association* 1986; **256**(1): 51-4.
237. Wade T, Bulik C, Heath A, Martin N, Eaves L. The influence of genetic and environmental factors in estimations of current body size, desired body size, and body dissatisfaction. *Twin Research and Human Genetics* 2001; **4**(4): 260-5.
238. Berulava T, Horsthemke B. The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels. *Eur J Hum Genet* 2010; **18**(9): 1054-6.
239. Böttcher Y, Körner A, Kovacs P, Kiess W. Obesity genes: implication in childhood obesity. *Occasional Review* 2012; **22**(1): 31-6.
240. Hofker M, Wijmenga C. A supersized list of obesity genes. *Nat Genet* 2009; **41**(2): 139-40.
241. Pietiläinen K, Kaprio J, Räsänen M, Rissanen A, Rose R. Genetic and environmental influences on the tracking of body size from birth to early adulthood. *Obesity Research* 2002; **10**(9): 875 - 84.
242. Ortega-Alonso A, Sipilä S, Kujala U, Kaprio J, Rantanen T. Genetic influences on change in BMI from middle to old age: A 29-year follow-up of twin sisters. *Behavior Genetics* 2009; **39**: 154 - 64.
243. Nelson M, Gordon-Larsen P, North K, Adair L. Body mass index gain, fast food, and physical activity: Effects of shared environments over time. *Obesity* 2006; **14**(4): 701 - 9.
244. Silventoinen K, Rokholm B, Kaprio J, Sørensen T. The genetic and environmental influences on childhood obesity: a systematic review of twin and adoption studies. *International Journal of Obesity* 2009.
245. Maes H, Neale M, Eaves L. Genetic and environmental factors in relative body weight and human adiposity. *Behavior Genetics* 1997; **27**(4): 325 - 51.
246. Faith M, Berkowitz R, Stallings V, Kerns J, Storey M, Stunkard A. Parental feeding attitudes and styles and child body mass index: Prospective analysis of a gene-environment interaction. *Pediatrics* 2004; **114**: e429 - e36.

247. Martínez J, Corbalán M, Sánchez-Villegas A, Forga L, Marti A, Martínez-González M. Obesity risk is associated with carbohydrate intake in women carrying the Gln27Glu β2-adrenoceptor polymorphism. *The Journal of Nutrition* 2003; **133**: 2549 - 54.
248. Silventoinen K, Haukka J, Dunkel L, Tynelius P, Rasmussen F. Genetics of pubertal timing and its associations with relative weight in childhood and adult height: The Swedish Young Male Twins Study. *Pediatrics* 2008; **121**(4): e885 - e91.
249. Rogol A, Clark P, Roemnich J. Growth and pubertal development in children and adolescents: effects of diet and physical activity. *American Journal of Clinical Nutrition* 2000; **72**(supplement): 521S - 8S.
250. Hur Y-M. Sex difference in heritability of BMI in South Korean adolescent twins. *Obesity* 2007; (15): 2908 - 11.
251. Harrington D, Elliott S. Weighing the importance of neighbourhood: A multilevel exploration of the determinants of overweight and obesity. *Social Science & Medicine* 2009; **68**: 593 - 600.
252. Kremers S, de Bruijn G-J, Visscher T, van Mechelen W, de Vries N, Brug J. Environmental influences on energy balance-related behaviors: A dual-process view. *International Journal of Behavioral Nutrition and Physical Activity* 2006; **3**: 9.
253. Pietiläinen K, Kaprio J, Borg P, et al. Physical inactivity and obesity: A vicious circle. *Obesity* 2008; **16**(2): 409 - 14.
254. Mustelin L, Silventoinen K, Pietiläinen K, Rissanen A, Kaprio J. Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. *International Journal of Obesity* 2009; **33**: 29 - 36.
255. Huppi PS, Schuknecht B, Boesch C, et al. Structural and neurobehavioral delay in postnatal brain development of preterm infants. *Pediatr Res* 1996; **39**(5): 895-901.
256. Mewes AU, Huppi PS, Als H, et al. Regional brain development in serial magnetic resonance imaging of low-risk preterm infants. *Pediatrics* 2006; **118**(1): 23-33.
257. Blakemore SJ, Choudhury S. Development of the adolescent brain: implications for executive function and social cognition. *J Child Psychol Psychiatry* 2006; **47**(3-4): 296-312.
258. Hanakawa T, Immisch I, Toma K, Dimyan MA, Van Gelderen P, Hallett M. Functional properties of brain areas associated with motor execution and imagery. *J Neurophysiol* 2003; **89**(2): 989-1002.
259. Peterson BS, Vohr B, Staib LH, et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. *JAMA* 2000; **284**(15): 1939-47.
260. Huppi PS, Maier SE, Peled S, et al. Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatr Res* 1998; **44**(4): 584-90.
261. Doyle LW, Cheong J, Hunt RW, et al. Caffeine and brain development in very preterm infants. *Ann Neurol* 2010; **68**(5): 734-42.
262. de Kieviet JF, Oosterlaan J, Vermeulen RJ, Pouwels PJ, Lafeber HN, van Elburg RM. Effects of Glutamine on Brain Development in Very Preterm Children at School Age. *Pediatrics* 2012.
263. O'Connor DL, Hall R, Adamkin D, et al. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. *Pediatrics* 2001; **108**(2): 359-71.
264. Glenn OA, Barkovich J. Magnetic resonance imaging of the fetal brain and spine: an increasingly important tool in prenatal diagnosis: part 2. *AJNR Am J Neuroradiol* 2006; **27**(9): 1807-14.
265. Glenn OA, Barkovich AJ. Magnetic resonance imaging of the fetal brain and spine: an increasingly important tool in prenatal diagnosis, part 1. *AJNR Am J Neuroradiol* 2006; **27**(8): 1604-11.

266. Medland A. Portrait of the West Midlands: Office for National Statistics, 2011.
267. Dale A, Lindley J, Dex S. A life-stage perspective on ethnic differences in women's economic activity in Britain. *European Sociological Review* 2006; **22**(4): 459 - 76.
268. Mesman J, van Ijzendoorn MH, Bakermans-Kranenburg MJ. Unequal in opportunity, equal in process: Parental sensitivity promotes positive child development in ethnic minority families. *Child Development Perspectives* 2012; **6**(3): 239-50.
269. Office for National Statistics. Population estimates by ethnic group 2002-2009, 2011.
270. van Jaarsveld CH, Johnson L, Llewellyn C, Wardle J. Gemini: a UK twin birth cohort with a focus on early childhood weight trajectories, appetite and the family environment. *Twin Res Hum Genet* 2010; **13**(1): 72-8.
271. Choi Y, Bishai D, Minkovitz CS. Multiple births are a risk factor for postpartum maternal depressive symptoms. *Pediatrics* 2009; **123**(4): 1147-54.
272. Zumpe J, Dormon O, Jefferies J. Childbearing among UK born and non-UK born women living in the UK, 2011.
273. Shields MA, Wheatley Price S. Language fluency and immigrant employment prospects: evidence from Britain's ethnic minorities. *Applied Economics Letters* 2001; **8**: 741-5.
274. de Hoog ML, van Eijsden M, Stronks K, Gemke RJ, Vrijkotte TG. The role of infant feeding practices in the explanation for ethnic differences in infant growth: the Amsterdam Born Children and their Development study. *Br J Nutr* 2011; **106**(10): 1592-601.
275. Sacker A, Kelly YJ. Ethnic differences in growth in early childhood: an investigation of two potential mechanisms. *Eur J Public Health* 2012; **22**(2): 197-203.
276. Drooger JC, Troe JW, Borsboom GJ, et al. Ethnic differences in prenatal growth and the association with maternal and fetal characteristics. *Ultrasound Obstet Gynecol* 2005; **26**(2): 115-22.
277. Galea S, Tracy M. Participation rates in epidemiologic studies. *Ann Epidemiol* 2007; **17**(9): 643-53.
278. van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nat Rev Genet* 2012; **13**(9): 640-53.
279. Stazi MA, Cotichini R, Patriarca V, et al. The Italian Twin Project: from the personal identification number to a national twin registry. *Twin Res* 2002; **5**(5): 382-6.
280. Trouton A, Spinath FM, Plomin R. Twins early development study (TEDS): a multivariate, longitudinal genetic investigation of language, cognition and behavior problems in childhood. *Twin Res* 2002; **5**(5): 444-8.
281. Snieder H, Treiber FA. The Georgia Cardiovascular Twin Study. *Twin Res* 2002; **5**(5): 497-8.
282. Skytthe A, Kyvik K, Holm NV, Vaupel JW, Christensen K. The Danish Twin Registry: 127 birth cohorts of twins. *Twin Res* 2002; **5**(5): 352-7.
283. Goldberg J, Curran B, Vitek ME, Henderson WG, Boyko EJ. The Vietnam Era Twin Registry. *Twin Res* 2002; **5**(5): 476-81.
284. Sung J, Cho SH, Cho SI, et al. The Korean Twin Registry--methods, current stage, and interim results. *Twin Res* 2002; **5**(5): 394-400.
285. Hopper JL. The Australian Twin Registry. *Twin Res* 2002; **5**(5): 329-36.
286. Yang H, Li X, Cao W, et al. Chinese National Twin Registry as a resource for genetic epidemiologic studies of common and complex diseases in China. *Twin Res* 2002; **5**(5): 347-51.
287. Spinath FM, Angleitner A, Borkenau P, Riemann R, Wolf H. German Observational Study of Adult Twins (GOSAT): a multimodal investigation of personality, temperament and cognitive ability. *Twin Res* 2002; **5**(5): 372-5.

288. Rocheleau CM, Romitti PA, Hockett Sherlock S, Sanderson WT, Bell EM, Druschel C. Effect of survey instrument on participation in a follow-up study: a randomization study of a mailed questionnaire versus a computer-assisted telephone interview. *BMC Public Health* 2012; **12**(1): 579.
289. Vesel L, Bahl R, Martines J, et al. Use of new World Health Organization child growth standards to assess how infant malnutrition relates to breastfeeding and mortality. *Bulletin of the World Health Organization* 2010; **88**: 39-48.
290. Roberts CL, Lancaster PA. National birthweight percentiles by gestational age for twins born in Australia. *J Paediatr Child Health* 1999; **35**(3): 278-82.
291. Multiple pregnancy: the management of twin and triplet pregnancies in the antenatal period, 2011.
292. Feldman R, Eidelman AI. Does a triplet birth pose a special risk for infant development? Assessing cognitive development in relation to intrauterine growth and mother-infant interaction across the first 2 years. *Pediatrics* 2005; **115**(2): 443-52.
293. McKeever TM, Lewis SA, Smith C, et al. Siblings, multiple births, and the incidence of allergic disease: a birth cohort study using the West Midlands general practice research database. *Thorax* 2001; **56**(10): 758-62.
294. Exercise in pregnancy (RCOG Statement 4): Royal College of Obstetricians and Gynaecologists, 2006.
295. Wang TW, Apgar BS. Exercise during pregnancy. *Am Fam Physician* 1998; **57**(8): 1846-52, 57.
296. Larsson L, Lindqvist PG. Low-impact exercise during pregnancy--a study of safety. *Acta Obstet Gynecol Scand* 2005; **84**(1): 34-8.
297. Baor L, Soskolne V. Mothers of IVF Twins: The Mediating Role of Employment and Social Coping Resources in Maternal Stress. *Women Health* 2012; **52**(3): 252-64.
298. Tamba. Tamba Health and Lifestyle Survey, 2011.
299. Luke B, Brown MB, Misiunas R, et al. Specialized prenatal care and maternal and infant outcomes in twin pregnancy. *Am J Obstet Gynecol* 2003; **189**(4): 934-8.
300. Van Dijk AE, Van Eijsden M, Stronks K, Gemke RJ, Vrijkotte TG. Maternal depressive symptoms, serum folate status, and pregnancy outcome: results of the Amsterdam Born Children and their Development study. *Am J Obstet Gynecol* 2010; **203**(6): 563 e1-7.
301. Kaprio J, Pulkkinen L, Rose RJ. Genetic and environmental factors in health-related behaviors: studies on Finnish twins and twin families. *Twin Res* 2002; **5**(5): 366-71.
302. Twin Research: Australian Academic Press; 2003.
303. Yuille M, van Ommen GJ, Brechot C, et al. Biobanking for Europe. *Brief Bioinform* 2008; **9**(1): 14-24.
304. Lone NI, Simpson C, Kavanagh K, et al. Seasonal Influenza Vaccine Effectiveness in the community (SIVE): protocol for a cohort study exploiting a unique national linked data set. *BMJ Open* 2012; **2**(2): e001019.
305. Dokholyan RS, Muhlbaier LH, Falletta JM, et al. Regulatory and ethical considerations for linking clinical and administrative databases. *Am Heart J* 2009; **157**(6): 971-82.
306. Cooke K. The Rough Guide to pregnancy and birth: Rough Guides; 2010.
307. Jansen BJ, Zhang M, Sobel K, Chowdury A. Twitter power: Tweets as electronic word of mouth. *Journal of the American Society for Information Science and Technology* 2009; **60**: 2169-88.
308. Dentzer S. Communicating medical news--pitfalls of health care journalism. *N Engl J Med* 2009; **360**(1): 1-3.
309. Woloshin S, Schwartz LM, Casella SL, Kennedy AT, Larson RJ. Press releases by academic medical centers: not so academic? *Ann Intern Med* 2009; **150**(9): 613-8.

310. European Plate Observing System. http://www.epos-eu.org (accessed 27 October 2012.
311. Doull IJ, McCaughey ES, Bailey BJ, Betts PR. Reliability of infant length measurement. *Arch Dis Child* 1995; **72**(6): 520-1.
312. Bartram JL, Rigby AS, Baxter PS. The "Lasso-o" tape: stretchability and observer variability in head circumference measurement. *Arch Dis Child* 2005; **90**(8): 820-1.
313. Stomfai S, Ahrens W, Bammann K, et al. Intra- and inter-observer reliability in anthropometric measurements in children. *Int J Obes (Lond)* 2011; **35 Suppl 1**: S45-51.

APPENDIX I:

RECRUITMENT DOCUMENTS

A-I.i Patient information sheet

The Birmingham Registry for Twin and Heritability Studies (BiRTHS)

Chief Investigator: Prof Maurice Zeegers
The University of Birmingham

Name of your doctor:

Name of your hospital:

PATIENT INFORMATION SHEET

We would like to invite you to take part in a research study and registry of multiples and their family members. We are currently recruiting expectant parents of twins or more who have been referred to a special multiples clinic.

The aim of this study is to provide insights into the way differences between individuals are determined by hereditary and environmental influences. We would also like to investigate how life of parents of multiples changes from a social point of view. In order to take this research forward we are asking parents expecting multiples to join the Birmingham Registry for Twin and Heritability Studies (BiRTHS). With the help of registered multiples and their families, the BiRTHS will examine the influence of genes and environment on the growth and development of children. We would be grateful if you would join the BiRTHS and fill in a few questionnaires when you come to the hospital for your antenatal appointments and after delivery. We would like your permission to take cord blood from your babies to genetically determine whether they are identical or non-identical. We will feedback this information to you. We do not take blood from the babies themselves. Once registered, we will invite you and your babies to a special multiples clinic for regular follow-ups not only of growth and weight, but also to monitor your babies' development in detail. After each visit you will receive detailed feedback on the developmental status of your children. In addition, we will look through your and your babies' medical records and will ask you to complete short and easy to fill in questionnaires. You also have the option to participate in an electroencephalogram (EEG) study, in which your babies' brain waves will be measured. This is a safe procedure that is not invasive or painful. You will find more detailed information about this included in your information pack.

Taking part is entirely voluntary. Before you decide whether you would like to join, it is important for you to understand why the research is being done and what it exactly will involve. Please take time to read the following detailed information carefully and discuss it with others if you wish. Please do not hesitate to contact me if anything is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

Thank you for taking the time to read this information sheet. A BiRTHS team member will speak to you again at your next clinic appointment to discuss whether you wish to participate in the study or not.

Yours truly

Professor Maurice Zeegers

Detailed study information

1. What is the purpose of the study?

In this study we investigate the influence of genes and environment on the development of children. We will also investigate how life of parents of multiples changes from a social point of view. This will include research on the relation between maternal depression and child development. This has not been investigated in mums of multiples so far.

2. Why have we been chosen?

You have been referred to a special multiples clinic as you are expecting more than one baby. We are asking prospective parents of multiples to join the Birmingham Registry for Twin and Heritability Studies. We intend to recruit at least 200 twins over the next two years.

3. Do we have to take part?

It is up to you to decide whether or not to join. You do not have to decide straight away. When you come to antenatal multiples clinic, you will be asked if you would like to join the registry and participate in the study. If you agree to take part you will be asked to sign a consent form. If you do decide to take part you are free to withdraw at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you and your babies receive or your or your babies' relationship with your doctor.

4. What will happen to us/our babies if we take part?

Once you have agreed to take part and have signed a consent form, a scientific study will run alongside your and your babies' standard care. When you come to antenatal clinic and to the follow-up appointments for your babies we will ask you to fill in a questionnaire. The questions will be on your background, medical history and lifestyle. We would need you to give us about 30 minutes of your time to fill in the questionnaires. We will also ask you to let the midwife take blood from the placental end of your babies' umbilical cords at birth. We do not take blood of your babies. In addition, we will ask you to give us permission to look through your and your babies' medical records. After discharge, we will invite you to regular follow-up clinics at our clinics at Birmingham Women's Hospital or Birmingham Children's Hospital. These clinics will be held every 3 to 6 months. At each visit your babies' growth and weight will be measured and a detailed developmental screen of your children will be carried out. After each visit you will receive detailed feedback on the developmental status of your children. We will also ask you to fill in a questionnaire at each visit. The questionnaire will contain questions about changes in your lifestyle after the birth of your babies. For your convenience and to avoid any double investigations we will ask for your permission to look through your babies' personal child health records ("red booklets"). We can document our findings in the babies' personal health record. Each follow-up appointment will take about 45 minutes.

At the moment we have funding to run the study over a 2-year period. However, as we will collect data on growth and development there will be a much longer period of interest. This study could be considered a first approach to future studies, so we might come back to you and your babies later and ask for further follow-up appointments. Of course any subsequent study will have separate ethical approval and we will ask you and your children for consent.

5. What do we have to do?

Other than your normal care we would need you to complete our questionnaires and to donate samples of your babies' cord blood at birth. In addition, we would need you to visit the special postnatal clinics for further follow-up of your babies.

6. What will happen to the blood samples taken as part of this study?

The blood samples will be stored centrally at a laboratory at The University of Birmingham. These blood samples will be used to genetically determine whether your babies are identical or non-identical. We will feedback this information to you. In addition, we would later use the samples donated as part of this study for future research. Such research projects have not yet been planned and could occur many years in the future. These future research projects may involve studies of your babies' genes and DNA. By giving your consent for your babies' blood to be stored you will be offering your babies' samples as a gift.

The blood and DNA samples will be stored under strict confidentiality and security and are coded, so that researchers receiving the samples do not know your or your babies' names or any other personal details. Researchers who wish to use the samples that are stored will only be given access to the samples after their research has been approved by an independent Research Ethics Committee who makes sure that the research is in the interest of patients and is carried out safely.

7. What are the possible disadvantages and risks of taking part?

There are no risks of taking part. This study will run alongside your and your babies' routine care and follow-up; it will not influence this process.

8. What are the possible benefits of taking part?

There is no intended immediate clinical benefit from taking part in this study. However, your children's growth and development will be closely monitored for the first two years of their life. After each visit you will receive an extensive report about your babies' development. If we are concerned, we will inform you and your GP, and a specialist appointment will be arranged. Furthermore, we will be able to tell you whether your children are identical ("monozygotic") or non-identical ("dizygotic"). You will receive this information. The information obtained from this study may result in changes in the future care and follow-up of expecting mothers during multiple pregnancy and care and follow-up of their babies.

9. What if new information becomes available?

This study does not influence your routine care and follow-up. However, any new discoveries or information relating to this will automatically be incorporated into the standard treatment provided by your doctors.

10. What happens when the research study stops?

When the study stops your and your children's routine care and follow-up will continue in the normal way, although it may incorporate new discoveries or information generated by this study.

11. What if something goes wrong?
As this study does not influence your and your babies' routine care and follow-up, the normal National Health Service complaints mechanisms should be followed.

12. Will my/our taking part in this study be kept confidential?
All information that is collected about you and your babies during the course of the research will be kept strictly confidential. If you agree to take part in this study we will need you to sign a consent form. You will be given a copy of the consent form and this information sheet to keep.

Upon registration in the BIRTHS we would collect some contact details from you including your current address and telephone number. These details will be used to ask you again for future studies after the first research study is closed. You can of course reject this request. By registration in the BIRTHS you are not obliged to do anything! Your contact details will be kept strictly confidential and only members of the BiRTHS research team would be allowed access to them.

Information on all patients entered into this study will be sent to the BiRTHS Study Office. This is located at The University of Birmingham where it will be retained in secure storage and handled according to the 1998 Data Protection Act. No personally identifiable information will be released from the BiRTHS study office. Limited clinical information may be passed on to the researchers within the UK. It would not be possible to identify any patient from this information and any information provided will be handled according to the normal standard of medical confidentiality and data protection.

13. What will happen to the results of the research study?
Important results from the study will be published as they come available, which may be during the course of the study or after the study has finished, and this could possibly take several years. We intend that any results will be published in scientific journals or will be presented at meetings involved with this field of twin research. These publications will be available upon request from your GP. You and your children will not be identified in any report or publication.

14. Who is organizing and funding the research?

The research is being organised by the Unit of genetic Epidemiology at the Department of Public Health and Epidemiology at The University of Birmingham in collaboration with the Neonatal Unit at the Birmingham Women's Hospital, Birmingham. The Birmingham Children's Hospital Research Foundation funds the research. The doctors conducting this study are not being paid for including and looking after you within this study.

15. Who has reviewed the study?

This study has been positively reviewed as a multi-centre study by the Solihull Research Ethics Committee and by scientific experts affiliated with The Birmingham Children's Hospital Research Foundation and The University of Birmingham. The two major UK twin associations: The "Multiple Births Foundation" (MBF) and the "Twins and Multiple Birth Association" (TAMBA) have give written declarations to support the BiRTHS.

16. What if I have other concerns or would like further information?

If you have any concerns or other questions about this study or the way it has been carried out, you should contact the number listed below.

Contact details:

Name: Cassandra Nan

Telephone: [redacted]

A-I.ii Patient consent form

BiRTHS
The Birmingham Registry for Twin and Heritability Studies

Date:	
Interviewer:	
Study Number	
Date of Birth (DD/MM/YYYY)	

PATIENT CONSENT FORM

Title of Project: The Birmingham Registry for Twin and Heritability Studies (BiRTHS)

Please initial inside the box

- I/we agree to take part in the **registry** of the Birmingham Registry for Twin and Heritability Studies but not the study. ☐
- I/we agree to take part in the **follow-up study** of the Birmingham Registry for Twin and Heritability Studies. ☐
- I/we agree to take part in the **EEG study** at the Birmingham Children's Hospital when the babies are 3 months old. ☐

1. I/we confirm that I/we have read and understand the information sheets for the above study and have had the opportunity to ask questions. ☐

2. I/we understand that my/our participation is voluntary and that I am/we are free to withdraw at any time, without giving a reason, without our or our babies' medical care or legal rights being affected. ☐

3. I/we understand that the mother's pregnancy notes and my/our babies' medical records (hospital notes, Red Booklet) may be looked at by members of the BiRTHS research team and regulatory authority representatives and that photocopies of these medical records may be taken. I/we understand that strict confidentiality will be maintained at all times. ☐

4. I/we agree for our babies' cord blood to be stored and used for determination of zygosity and extracting DNA and for future biological research projects which have received appropriate scientific and ethical approval. ☐

5. I/we agree for my contact details to be stored and used to contact me/us about aspects of the study, either by telephone or by post. ☐

6. I/We agree that our General Practitioner may be contacted. ☐

7. I/We agree to become part of the Birmingham's Twin Register and that our and our babies' data may be used for follow-up and future research. ☐

8. I/we understand that we will not get paid for participating in the above Birmingham Registry for Twin and Heritability Studies. ☐

Name of Mother	Date	Signature
Name of Father	Date	Signature
Name of person taking consent	Date	Signature

(1 for parents; 1 for researcher; 1 to be kept with hospital notes)

A-I.iii Contact details sheet

BiRTHS
The Birmingham Registry for Twin and Heritability Studies

CONFIDENTIAL DATA - MOTHER (CONTACT DETAILS)

-Highly confidential-

Hospital		
(tick✓ one only)	Women’s	☐
	Heartlands	☐
	City	☐

Mothers details	
Hospital number	
NHS number	
Study number	
Name (family, first)	
DOB (dd/mm/yy)	
Contact address	
E-mail address	
Telephone number	
Name and address of GP	
Name of partner	
Informed consent taken (dd/mm/yy)	
Estimated date of delivery	

APPENDIX II

QUESTIONNAIRES

A-II.i Antenatal maternal questionnaire

BiRTWS
The Birmingham Registry for Twin and Heritability Studies

Parent Specific Antenatal Questionnaire

MOTHER

Could you please complete this questionnaire? The information will help us understand your twins' growth and development. Please be aware that

- **No social values will be given to any of your answers**
- **Confidentiality will be maintained at all times**
- **You are free not to answer any questions.**

If you have questions or need help in completing this questionnaire, please let us know.

Thank you for your cooperation!

Study number ………………

Date completed (*dd/mm/yyyy*) ………………

Hospital (Please tick✓ 1 only)
☐ Birmingham Women's Hospital
☐ Birmingham Heartlands Hospital
☐ Birmingham City Hospital

Do you need an interpreter?
☐ No
☐ Yes, for the following language: …………………. I make use of the interpreting services of ☐ the NHS ☐ a family member ☐ a friend

Date of birth (*dd/mm/yyyy*) …………… **Age**………… *years*

Height ……………………….*cm*

……….*ft* ………*inches*

Weight in early pregnancy (approx. 12 weeks) ……………………….*kg* **Date** …………………

……………………….*lbs*

Weight today ……………………….*kg*

……………………….*lbs*

How far along are you currently in your pregnancy? …………. *weeks*, calculated according to ☐ last menstrual period ☐ ultra sound scan

Country of birth ………………………..

Preferred language spoken ………………………..

<u>Ethnic group</u> (Please tick✓ 1 only)

Asia
- ☐ India
- ☐ Pakistan
- ☐ Bangladesh
- ☐ China
- ☐ Far East Asia (Japan, Korea)
- ☐ South East Asia (Malaysia, Thailand, Phillipines)

Europe
- ☐ Britain (England, Scotland, Wales)
- ☐ Ireland (incl. Northern Ireland, Eire)
- ☐ Northern Europe (Denmark, Norway, Sweden)
- ☐ Western Europe (France, Germany, Netherlands)
- ☐ Eastern Europe (Balkans, Poland, Russia)
- ☐ Southern Europe (Cyprus, Greece, Italy, Spain, Turkey)

Caribbean
- ☐ Barbados, Jamaica, Trinidad and Tobago

Middle East
- ☐ Egypt, Israel, Syria, Yemen

Africa
- ☐ North African (Morocco, Algeria)
- ☐ Sub-Sahara (Somalia, Kenya, Nigeria)

Other
- ☐ ……………………………..

Decline of information
- ☐ Please tick✓ this box if you do not wish to answer this question

---

Employment

Job title ……………………………….

☐ Full-time ☐ Part-time, ………….. hours per week (please specify here)

If you don't work, please tick✓ one of the following

I am ☐ a housewife ☐ a student ☐ unemployed

Twins

Are there twins in the family?

☐ I/We don't know

☐ No

☐ Yes, ☐ We already have twins

☐ Twins in the family of the MOTHER, they are our …..…...............................

* Please include all the twins in mother's family you know of and how they are related to you. Use the empty space below to continue

☐ Twins in the family of the FATHER, they are our…………………………………..

* Please include all the twins in mother's family you know of and how they are related to you. Use the empty space below to continue

Do you think your twins will be identical ("monozygotic")?

☐ No ☐ Yes ☐ I/We don't know

Have you smoked before pregnancy and/or are you currently smoking?

Before pregnancy During pregnancy

☐ No ☐ Yes, …….. (number per day) ☐ No ☐ Yes, ……….. (number per day)

If stopped smoking, when (*dd/mm/yyyy*) …………………..

Have you consumed alcohol and/or do you currently consume any alcohol?

Before pregnancy During pregnancy

☐ No ☐ Yes, ………(units per week) ☐ No ☐ Yes, ………. (units per week)

Have you taken and/or are you currently taking medical drugs?

Before pregnancy
☐ No ☐ Yes

During pregnancy
☐ No ☐ Yes

Have you taken and/or are you currently taking non-medical drugs?

Before pregnancy
☐ No ☐ Yes

During pregnancy
☐ No ☐ Yes

Exercise

Here is a list of activities and space for you to list any other sports you participate(d) in. Please fill in **how many hours per week** you used to spend on each activity at different ages and now that you are pregnant.

	12-19 yrs	19-29 yrs	>30 yrs	Now
Walking (including to school, work, shopping or as a leisure activity)				
Cycling (including to school, work, shopping or as a leisure activity)				
Gardening				
Housework (excluding childcare)				
……………………. (type of sport)				
……………………. (type of sport)				
……………………. (type of sport)				
……………………. (type of sport)				

How many hours per week did you spend on sports and physical activity in the *last year before you were pregnant* and *now that you are pregnant*?

	Hours per week	
Type of sport (*please list below*)	in the ***LAST YEAR BEFORE*** pregnancy	now ***DURING*** pregnancy

<u>Social support</u>

Here is a list of some things that other people do for us or give us that may be helpful or supportive. Please read each statement carefully and put a tick✓ in the box that is closest to your sitation.

------------------------**Example**--------------------------**Example**------------------------------

	As much as I would like	←------→			Much less than I would like
I get enough vacation time	☐	☑	☐	☐	☐

If you put a tick where we have, it means that you get <u>almost</u> *as much vacation time as you would like, but not quite as much as you would like*

------------------------**Example**--------------------------**Example**------------------------------

	As much as I would like	←------→		Much less than I would like		Not applicable
I have people who care what happens to me	☐	☐	☐	☐	☐	☐
I get love and affection	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about problems at work or with my housework	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about my personal and family problems	☐	☐	☐	☐	☐	☐
I get to talk to someone about money matters	☐	☐	☐	☐	☐	☐
I get invitations to go out and do things with other people	☐	☐	☐	☐	☐	☐
I get useful advice about important things in life	☐	☐	☐	☐	☐	☐
I get help when I am sick in bed	☐	☐	☐	☐	☐	☐

A-II.ii Antenatal paternal questionnaire

BiRTHS
The Birmingham Registry for Twin and Heritability Studies

Parent Specific Antenatal Questionnaire

PARTNER

Could you please complete this questionnaire? The information will help us understand your twins' growth and development. Please be aware that

- **No social values will be given to any of your answers**
- **Confidentiality will be maintained at all times**
- **You are free not to answer any questions.**

If you have questions or need help in completing this questionnaire, please let us know.

Thank you for your cooperation!

Study number ………………

Date completed (*dd/mm/yyyy*) ………………

Hospital (Please tick✓ 1 only)
☐ Birmingham Women's Hospital
☐ Birmingham Heartlands Hospital
☐ Birmingham City Hospital

Do you need an interpreter?
☐ No
☐ Yes, for the following language: …………………. I make use of the interpreting services of ☐ the NHS ☐ a family member ☐ a friend

Date of birth (*dd/mm/yyyy*) …………… **Age**………… *years*

Height ………………………*cm*

……….*ft* ………*inches*

Weight ………………………*kg*

………………………*lbs*

Are you a blood relation to the mother?

☐ No ☐ Yes, I am her ……………………. (please specify relation here)

Country of birth ………………………..

Preferred language spoken ………………………..

<u>Ethnic group</u> (Please tick✓ 1 only)

Asia

☐ India
☐ Pakistan
☐ Bangladesh
☐ China
☐ Far East Asia (Japan, Korea)
☐ South East Asia (Malaysia, Thailand, Phillipines)

Europe

☐ Britain (England, Scotland, Wales)
☐ Ireland (incl. Northern Ireland, Eire)
☐ Northern Europe (Denmark, Norway, Sweden)
☐ Western Europe (France, Germany, Netherlands)
☐ Eastern Europe (Balkans, Poland, Russia)
☐ Southern Europe (Cyprus, Greece, Italy, Spain, Turkey)

Caribbean

☐ Barbados, Jamaica, Trinidad and Tobago

Middle East

☐ Egypt, Israel, Syria, Yemen

Africa

☐ North African (Morocco, Algeria)
☐ Sub-Sahara (Somalia, Kenya, Nigeria)

Other

☐ ……………………………..

Decline of information

☐ Please tick✓ this box if you do not wish to answer this question

---

<u>Employment</u>

Job title ………………………………

☐ Full-time ☐ Part-time, ………….. *hours per week* (please specify here)

If you don't work, please tick✓ one of the following

I am ☐ a housewife/husband ☐ a student ☐ unemployed

Do you smoke?

☐ No ☐ Yes, …….. (number per day)

If stopped smoking, when (*dd/mm/yyyy*) …………………..

Do you consume any alcohol?

☐ No ☐ Yes, ………(units per week)

Do you take any medical drugs?

☐ No ☐ Yes

Do you take any non-medical drugs?

☐ No ☐ Yes

Social support

Here is a list of some things that other people do for us or give us that may be helpful or supportive. Please read each statement carefully and put a tick✓ in the box that is closest to your situation.

------------------------**Example**--------------------------**Example**------------------------------

	As much as I would like	←------→			Much less than I would like
I get enough vacation time	☐	☑	☐	☐	☐

If you put a tick where we have, it means that you get almost *as much vacation time as you would like, but not quite as much as you would like*

------------------------**Example**--------------------------**Example**------------------------------

	As much as I would like	←------→		Much less than I would like		Not applicable
I have people who care what happens to me	☐	☐	☐	☐	☐	☐
I get love and affection	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about problems at work or with my housework	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about my personal and family problems	☐	☐	☐	☐	☐	☐
I get to talk to someone about money matters	☐	☐	☐	☐	☐	☐
I get invitations to go out and do things with other people	☐	☐	☐	☐	☐	☐
I get useful advice about important things in life	☐	☐	☐	☐	☐	☐
I get help when I am sick in bed	☐	☐	☐	☐	☐	☐

A-II.iii Edinburgh Postnatal Depression Scale

[Source: Cox, JL., Holden, JM., Sagovsky, R. Detection of postnatal depression: development of the 10-item Edinburgh Postnatal Depression Scale. *British Journal of Psychiatry*. 1987;**150**:782-786]

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The Birmingham Registry for Twin and Heritability Studies

EPDS (Mothers only) - Antenatal

Date:	
Interviewer:	
Study Number	
Date of Birth (DD/MM/YYYY)	

<u>Edinburgh Postnatal Depression Scale (EPDS[62])</u>

As you are pregnant with twins, we would like to know how you are feeling. Please TICK THE BOX of the answer which comes closest to how you have felt IN THE PAST 7 DAYS, not just how you feel today.

Here is an example, already completed.

I have felt happy:

Yes, all the time	
Yes, most of the time	✓
No, not very often	
Not at all	

This would mean: "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

In the past 7 days:

1. I have been able to laugh and see the funny side of things

As much as I always could	
Not quite so much	
Definitely not so much	
Not at all	

2. I have looked forward with enjoyment to things

As much as I ever did	
Rather less than I used to	
Definitely less than I used to	
Hardly at all	

3. I have blamed myself unnecessarily when things went wrong

Yes, most of the time	
Yes, some of the time	
Not very often	
No, never	

4. I have been anxious or worried for no good reason

No, not at all	
Hardly ever	
Yes, sometimes	
Yes, very often	

5. I have felt scared or panicky for no very good reason

Yes, quite a lot	
Yes, sometimes	
No, not much	
No, not at all	

6. Things have been getting on top of me

Yes, most of the time I haven't been able to cope at all	
Yes, sometimes I haven't been coping as well as usual	
No, most of the time I have coped quite well	
No, I have been coping as well as ever	

7. I have been so unhappy that I have had difficulty sleeping

Yes, most of the time	
Yes, sometimes	
Not very often	
No, not at all	

8. I have felt sad or miserable

Yes, most of the time	
Yes, quite often	
Not very often	
No, not at all	

9. I have been so unhappy that I have been crying

Yes, most of the time	
Yes, quite often	
Only occasionally	
No, never	

10. The thought of harming myself has occurred to me

Yes, quite often	
Sometimes	
Hardly ever	
Never	

A-II.iv Postnatal parental questionnaire

BiRTHS

The Birmingham Registry for Twin and Heritability Studies

PARENT SPECIFIC QUESTIONNAIRE - POSTNATAL

Study number

Time point

Date completed (*dd/mm/yyyy*) ………………

Do you need an interpreter?

☐ No

☐ Yes, for the following language: …………………. I make use of the interpreting services of ☐ the NHS ☐ a family member ☐ a friend

<u>Mother</u>

Date of birth (*dd/mm/yyyy*) **Age**………… *years*

Height ……………………….*cm*

……….*ft* ………*inches*

Weight today ……………………….*kg*

……………………….*lbs*

<u>Employment</u> *MOTHER*

Job title ………………………………

☐ Full-time ☐ Part-time, ………….. hours per week (please specify here)

If you don't work, please tick✓ one of the following

I am ☐ a housewife ☐ a student ☐ unemployed

<u>Employment</u> *PARTNER*

Job title ………………………………

☐ Full-time ☐ Part-time, ………….. hours per week (please specify here)

If you don't work, please tick✓ one of the following

I am ☐ a housewife/husband ☐ a student ☐ unemployed

Twins

Date of birth (*dd/mm/yyyy*) **Age**………… *months*

Gestational age at birth ………………. *weeks* and ……….. *days*

Do you think your twins will be identical ("monozygotic")?
☐ No ☐ Yes ☐ I/We don't know

Do your twins look alike?
☐ Not at all ☐ Not too much ☐ Yes, slightly ☐ Yes, you can hardly distinguish between them

Do you (as parents) **ever confuse your twins?**
☐ Never ☐ Sometimes ☐ Often

Do family members ever confuse your twins?
☐ Never ☐ Sometimes ☐ Often

Do strangers ever confuse your twins?
☐ Never ☐ Sometimes ☐ Often

Which hand is the main one for writing or working with?

Mother
☐ Left ☐ Right ☐ Both ☐ Unknown

Father
☐ Left ☐ Right ☐ Both ☐ Unknown

Twin 1
☐ Left ☐ Right ☐ Both ☐ Unknown

Twin 2
☐ Left ☐ Right ☐ Both ☐ Unknown

The following questions are about the general wellbeing of your twins please tick✓ as appropriate and specify where needed.

How are you feeding your twins?

Twin 1	Twin 2
☐ Breastfeeding	☐ Breastfeeding
☐ Formula	☐ Formula
☐ Both	☐ Both

How much time does your baby sleep compared to other babies you know?

Twin 1	Twin 2
☐ I don't know	☐ I don't know
☐ Very little	☐ Very little
☐ Normal	☐ Normal
☐ A lot	☐ A lot
☐ Very much	☐ Very much

Has one of your twins been sick?

Twin 1	Twin 2
☐ No	☐ No
☐ Yes, …………………… (please specify, e.g. flu or cold)	☐ Yes, …………………………………….

Has one of your twins been seen by a doctor regarding illness?

Twin 1	Twin 2
☐ No	☐ No
☐ Yes, ………………. (please specify what illness and doctor)	☐ Yes, ……………………….……...

Has one of your twins been admitted to hospital?

Twin 1	Twin 2
☐ No	☐ No
☐ Yes, ……………………….. (please specify why and when)	☐ Yes, ……………….………..

Does one of your twins need regular medication?

Twin 1	Twin 2
☐ No	☐ No
☐ Yes, ………………………..…(please name medication)	☐ Yes, ………………………..

Exercise *MOTHER*

Here is a list of activities and space for you to list any other sports you participate(d) in. Please fill in **how many hours per week** you spend on each activity now that you have your babies.

	Hours per week
Walking (including to school, work, shopping or as a leisure activity)	
Cycling (including to school, work, shopping or as a leisure activity)	
Gardening	
Housework (excluding childcare)	
……………………. (type of sport)	
……………………. (type of sport)	
……………………. (type of sport)	
……………………. (type of sport)	

Drugs *MOTHER*

Do you currently smoke?

☐ No ☐ Yes, …………….. (number per day)

If stopped smoking, when (*dd/mm/yyyy*) …………………..

Do you consume any alcohol?

☐ No ☐ Yes, …………….. (units per week)

Do you use any medical drugs?

☐ No ☐ Yes

Do you use any non-medical drugs?

☐ No ☐ Yes

<u>Social support</u> *MOTHER*

Here is a list of some things that other people do for us or give us that may be helpful or supportive. Please read each statement carefully and put a tick✓ in the box that is closest to your sitation.

------------------------**Example**--------------------------**Example**-------------------------------

	As much as I would like	←---→			Much less than I would like
I get enough vacation time	☐	☑	☐	☐	☐

If you put a tick where we have, it means that you get <u>almost</u> *as much vacation time as you would like, but not quite as much as you would like*

------------------------**Example**--------------------------**Example**-------------------------------

	As much as I would like	←---→		Much less than I would like		Not applicable
I have people who care what happens to me	☐	☐	☐	☐	☐	☐
I get love and affection	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about problems at work or with my housework	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about my personal and family problems	☐	☐	☐	☐	☐	☐
I get to talk to someone about money matters	☐	☐	☐	☐	☐	☐
I get invitations to go out and do things with other people	☐	☐	☐	☐	☐	☐
I get useful advice about important things in life	☐	☐	☐	☐	☐	☐
I get help when I am sick in bed	☐	☐	☐	☐	☐	☐

<u>Social support</u> *PARTNER*

Here is a list of some things that other people do for us or give us that may be helpful or supportive. Please read each statement carefully and put a tick✓ in the box that is closest to your sitation.

------------------------**Example**--------------------------**Example**------------------------------

	As much as I would like	←→			Much less than I would like
I get enough vacation time	☐	☑	☐	☐	☐

If you put a tick where we have, it means that you get <u>almost</u> *as much vacation time as you would like, but not quite as much as you would like*

------------------------**Example**--------------------------**Example**------------------------------

	As much as I would like	←→			Much less than I would like	Not applicable
I have people who care what happens to me	☐	☐	☐	☐	☐	☐
I get love and affection	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about problems at work or with my housework	☐	☐	☐	☐	☐	☐
I get to talk to someone I trust about my personal and family problems	☐	☐	☐	☐	☐	☐
I get to talk to someone about money matters	☐	☐	☐	☐	☐	☐
I get invitations to go out and do things with other people	☐	☐	☐	☐	☐	☐
I get useful advice about important things in life	☐	☐	☐	☐	☐	☐
I get help when I am sick in bed	☐	☐	☐	☐	☐	☐

APPENDIX III

ANTHROPOMETRIC MEASUREMENTS

A-III.i Weight

Weight, without clothes or nappies, was measured in kilograms (kg) with digital baby scales. We used the portable SECA 336 at Birmingham Children's Hospital. The Shekel electronic scale T-15-S was used at Birmingham Women's Hospital. Both scales have a capacity of 15kg, measuring at an accuracy of .005kg, and are designed to allow children to sit or lie down in them.

Hospital equipment, including scales, is checked every 12 months. If equipment is found out of calibration, it is sent to the manufacturer for calibration. Therefore, I did not need to calibrate the scales myself. I did check whether there were differences between the scales that we used with a 1kg weight and found a maximum difference of 1%, which I found acceptable.

A-III.ii Length

Length was measured in centimetres, using the Rollameter 100 by Harlow Healthcare. This is a portable measuring mat, which is appropriate for measuring infants. The Rollameter allows measurements up to 100 centimetres (cm) and is accurate to 1 millimetre (mm). Measurements were done with the infants lying on their backs and the top of their heads against the board on the top. Mothers were asked to hold the head of their child so that the eyes were facing straight towards the ceiling (Figure III.i). It was important to ensure that the hips were aligned with the measuring mat, legs were stretched and together, and ankles were at a 90-degree angle (Figure III.ii). The measuring tape that was incorporated in the bottom of the mat could then be pulled up to the bottom of the feet and length could be read off the tape.

Reliability of length measurements in infancy seems to be effected more by a child's posture and not so much by the measuring instrument or the observer[311]. Therefore, ensuring that the child was lying on the measuring mat properly should have facilitated maintaining reliable length measurements in our study.

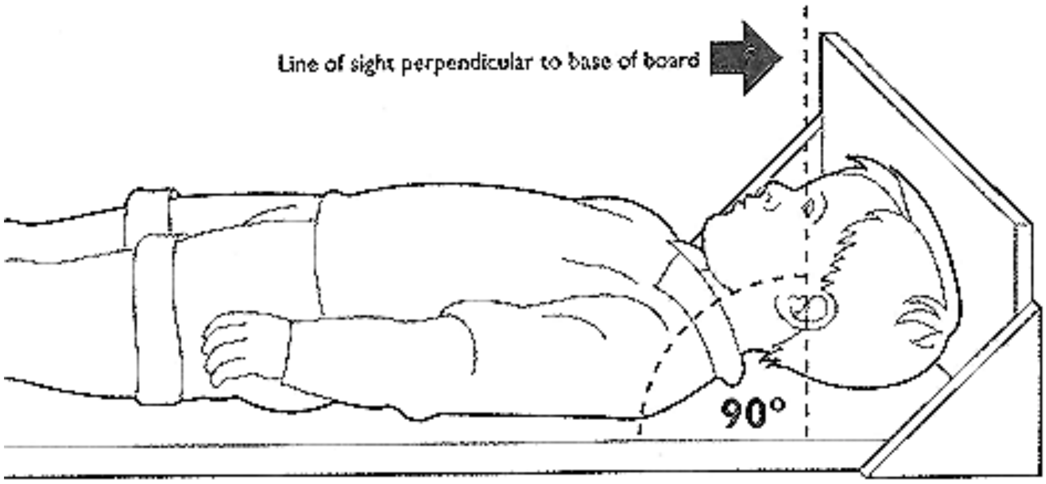

Figure III.i Line of sight should be straight towards the ceiling when measuring a child's length.
[Source: www.motherchildnutrition.org]

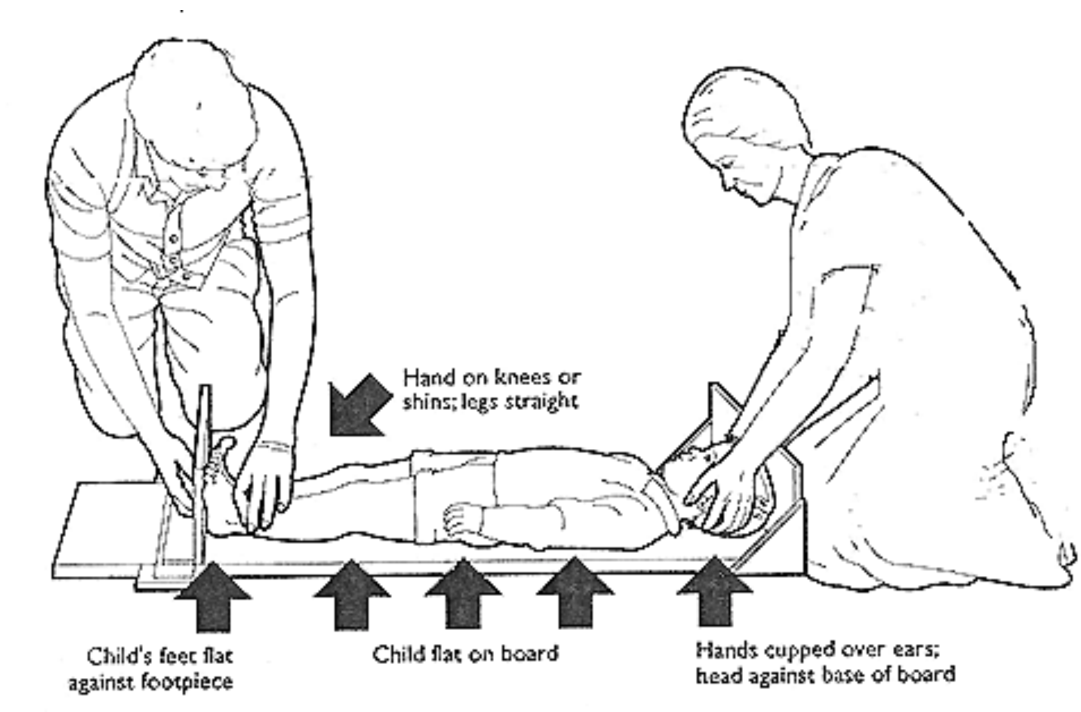

Figure III.ii Measuring a child's length.
[Source: www.motherchildnutrition.org]

A-III.iii Head circumference

I used re-usable Lasso-o measuring tapes from Harlow Healthcare to measure head circumference. They are made from paper-thin, non-stretch, non-shrink plastic 'syntape' and are accurate to the nearest millimetre. The Lasso-o was looped and placed over the infant's head around the largest possible circumference. This is normally midway between the eyebrows and hairline to the occipital prominence at the back of the head as illustrated in Figure III.iii. The Lasso-o was then pulled tight and the measurement was read from the marked place on the Lasso-o.

Although the measurements with Lasso-o tapes are highly reliable, Bartram et al. (2005)[312] describe how the stretchability of these tapes could produce variability in measurements, which could be clinically, but not statistically significant. They recommend that tapes be replaced at regular intervals. They also state that those using the tapes should be trained to use the same amount of pressure and take several measurements to ensure an accurate estimate. I replaced the tapes every 3 months, always took three measurements at each follow-up, and our training sessions ensured that all researchers used the same method.

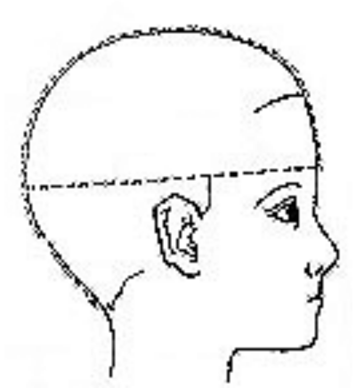

Figure III.iii Head circumference should be measured at the largest possible circumference. [Source: www.southwestmedical.com]

A-III.iv Mid-upper arm circumference

Mid-upper arm circumference was measured with the same Lasso-o measuring tape, on either arm. The midpoint of the upper arm was found in the middle between the olecranon in the elbow and the acromion in the shoulder. The midpoint was then marked with a pen or washable marker (Figure III.iv). After this, the Lasso-o was placed over the mark and measurements were read.

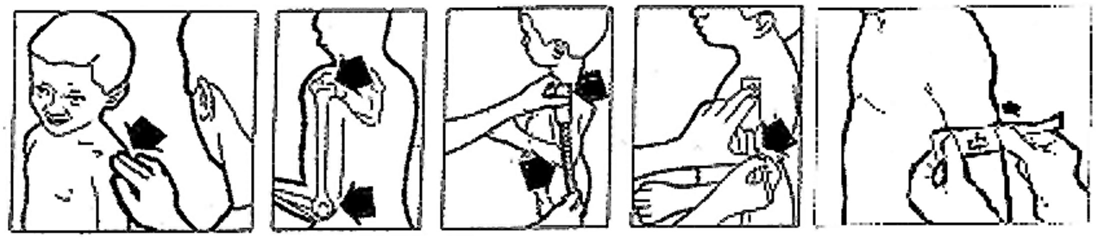

Figure III.iv Mid-upper arm circumference was measured on the midpoint of the upper arm between the olecranon and the acromion.
[Source: www.motherchildnutrition.org]

A-III.v Skinfold thickness (triceps)

Subcutaneous fat was measured with Holtain Skinfold Calipers, which has a capacity of measuring up to 48mm of subcutaneous tissue and has a dial graduation of 0.2mm. Measurements at the triceps were taken at the same midpoint of the upper arm, with the arm relaxed and stretched. The tissue was pinched between the thumb and middle finger and the calliper was placed underneath (Figure III.v). The calliper was then slowly released until the needle stopped moving, and a measurement could be read.

A multicentre study into the reliability of skinfold measurement in children concluded that a standardized method of measurement and trained staff ensured the realisation of acceptable inter- and intra-observer agreement. There are no indications of unreliable measurements due to the instrument or child.[313]

Figure III.v Measuring skinfold thickness on the same midpoint of the upper arm.
[Source: www.healthgoods.com]

A-III.vi Skinfold thickness (subscapular)

Subscapular skinfolds were measured with the same callipers and technique as triceps skinfolds. The location of these measurements was at the bottom point of the shoulder blade (scapula), roughly at the same level as the midpoint of the upper arm (Figure III.vi).

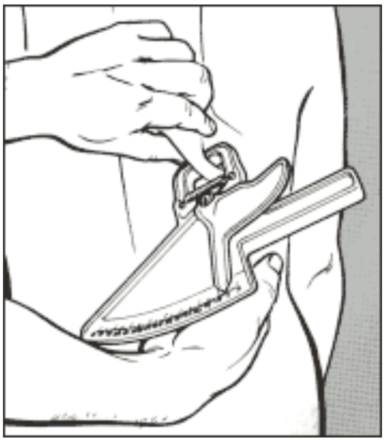

Figure III.vi Measuring subscapular skinfold thickness on roughly the same level as the midpoint of the upper arm.
[Source: www.healthgoods.com]

APPENDIX IV

CLINICAL RECORD FORMS

A-IV.i Maternal medical records

BiRTWS
The Birmingham Registry for Twin and Heritability Studies

CLINICAL RECORD FORM - MOTHER

Study number ………………

Date completed (*dd/mm/yyyy*) ……………… **Completed by** ………………….

Does MOTHER **need an interpreter?**
□ No
□ Yes, for the following language: ………………….
□ Use the interpreting services of □ the NHS □ a family member □ a friend

---

Does FATHER **need an interpreter?**
□ No
□ Yes, for the following language: ………………….
□ Use the interpreting services of □ the NHS □ a family member □ a friend

---

Date of birth (*dd/mm/yyyy*) …………… **Age**……….. *years*

Height ……………………….*cm*

……….*ft* ………*inches*

Weight in early pregnancy (approx. 12 weeks) ……………………….*kg* **Date** …………………

……………………….*lbs*

Weight at delivery ……………………….*kg*

……………………….*lbs*

BMI early pregnancy (*kg/m*^{2}) …………….. **BMI at birth** (*kg/m*^{2})……………..

Ethnic group (Please tick✓ 1 only)

Asia
☐ India
☐ Pakistan
☐ Bangladesh
☐ China
☐ Far East Asia (Japan, Korea)
☐ South East Asia (Malaysia, Thailand, Phillipines)

Europe
☐ Britain (England, Scotland, Wales)
☐ Ireland (incl. Northern Ireland, Eire)
☐ Northern Europe (Denmark, Norway, Sweden)
☐ Western Europe (France, Germany, Netherlands)
☐ Eastern Europe (Balkans, Poland, Russia)
☐ Southern Europe (Cyprus, Greece, Italy, Spain, Turkey)

Caribbean
☐ Barbados, Jamaica, Trinidad and Tobago

Middle East
☐ Egypt, Israel, Syria, Yemen

Africa
☐ North African (Morocco, Algeria)
☐ Sub-Sahara (Somalia, Kenya, Nigeria)

Other
☐ ……………………………..

Decline of information
☐ Please tick✓ this box if patient did not wish to answer

Employment *MOTHER*

Job title ………………………………

☐ Full-time ☐ Part-time, ………….. hours per week (please specify here)

If mother doesn't work, please tick✓ one of the following
Mother is ☐ a housewife/husband ☐ a student ☐ unemployed

Any previous pregnancies?
☐ No ☐ Yes, ……… (number), ………… (children born alive)

Any previous multiple pregnancies?
☐ No ☐ Yes, ……… (number)

Child 1

Date of birth (*dd/mm/yyyy*) ……………… **Gestational age at birth** ………..*weeks*

Birth weight (*grams*) …………………….

Condition now
☐ Alive and well
☐ Minor deficiencies (normal integration with some educational help)
☐ Moderate deficiencies (integration with additional help)
☐ Major disabilities (little or no integration despite intensive supervision/education)
☐ Dead

Child 2

Date of birth (*dd/mm/yyyy*) ……………… **Gestational age at birth** ………..*weeks*

Birth weight (*grams*) …………………….

Condition now
☐ Alive and well
☐ Minor deficiencies (normal integration with some educational help)
☐ Moderate deficiencies (integration with additional help)
☐ Major disabilities (little or no integration despite intensive supervision/education)
☐ Dead

Child 3

Date of birth (*dd/mm/yyyy*) ……………… **Gestational age at birth** ………..*weeks*

Birth weight (*grams*) …………………….

Condition now
☐ Alive and well
☐ Minor deficiencies (normal integration with some educational help)
☐ Moderate deficiencies (integration with additional help)
☐ Major disabilities (little or no integration despite intensive supervision/education)
☐ Dead

Current Pregnancy

Last menstrual period (*dd/mm/yyyy*) ....

Expected date of delivery (EDD) ....
As determined by ☐ last menstrual period ☐ ultra sound scan

Mode of conception

☐ Spontaneous
☐ Medical fertility treatment
☐ Assisted reproductive technique: ☐ ICSI
☐ IVF
☐ Eggs frozen
☐ Ova Stimulation, ……..………… (what stimulation)

Has the patient been admitted to hospital during pregnancy?

☐ No ☐ Yes (please specify below)

If the patient was admitted to hospital, specify why and when

☐ Premature labour ……………………….. (gestational age in weeks or date of admission)

☐ PROM ………………………..

☐ Hypertension ………………………..

☐ Pre-eclampsia ………………………..

☐ Diabetes Type 1 ………………………..

☐ Diabetes Type 2 ………………………..

☐ Growth retardation twin 1 ………………………..

☐ Growth retardation twin 2 ………………………..

☐ Absent end diastolic flow ………………………..

☐ Bleeding ………………………..

☐ HELLP syndrome ………………………..

☐ Other, ………………….. ………………………..

Smoking

Before pregnancy
☐ No ☐ Yes, ………. (number per day)

During pregnancy
☐ No ☐ Yes, …….. (number per day)

If stopped smoking, when (*dd/mm/yyyy*) …………………..

Alcohol

Before pregnancy
☐ No ☐ Yes, …….. (units per week)

During pregnancy
☐ No ☐ Yes, …….. (units per week)

Medical drugs

Before pregnancy
☐ No ☐ Yes

During pregnancy
☐ No ☐ Yes

Non-medical drugs

Before pregnancy
☐ No ☐ Yes

During pregnancy
☐ No v Yes

Any family history of the following:

Type 2 diabetes	☐ Mother	☐ Father
Cardiovascular disease	☐ Mother	☐ Father
Endocrine disorders	☐ Mother	☐ Father
Still births	☐ Mother	☐ Father
Multiple miscarriages	☐ Mother	☐ Father
Sudden infant death	☐ Mother	☐ Father
Genetic pathology	☐ Mother	☐ Father
Other, ………………………..	☐ Mother	☐ Father

<u>Father's details</u>
Date of birth (*dd/mm/yyyy*) …………… **Age**………… *years*

Height ……………………….*cm*

……….*ft* ……….*inches*

Weight ……………………….*kg*

……………………….*lbs*

Is father blood related to the mother?
☐ No ☐ Yes, he is her ………………………….. (specify relation)

<u>Ethnic group</u> (Please tick✓ 1 only)

Asia
☐ India
☐ Pakistan
☐ Bangladesh
☐ China
☐ Far East Asia (Japan, Korea)
☐ South East Asia (Malaysia, Thailand, Phillipines)

Europe
☐ Britain (England, Scotland, Wales)
☐ Ireland (incl. Northern Ireland, Eire)
☐ Northern Europe (Denmark, Norway, Sweden)
☐ Western Europe (France, Germany, Netherlands)
☐ Eastern Europe (Balkans, Poland, Russia)
☐ Southern Europe (Cyprus, Greece, Italy, Spain, Turkey)

Caribbean
☐ Barbados, Jamaica, Trinidad and Tobago

Middle East
☐ Egypt, Israel, Syria, Yemen

Africa
☐ North African (Morocco, Algeria)
☐ Sub-Sahara (Somalia, Kenya, Nigeria)

Other
☐ ……………………………..

Decline of information
☐ Please tick✓ this box if patient did not wish to answer

<u>Employment</u> *FATHER*

Job title ………………………………

☐ Full-time ☐ Part-time, ………….. hours per week (please specify here)

If father doesn't work, please tick✓ one of the following
Father is ☐ a housewife/husband ☐ a student ☐ unemployed

A-IV.ii Delivery details

BiRTHS
The Birmingham Registry for Twin and Heritability Studies

CLINICAL RECORD FORM – NEWBORN

BABY …. [insert number: 1, 2, 3… etc]

Study number ………………....

Date completed (*dd/mm/yyyy*) ……………… **Completed by** ………………….

Interpreter needed for parent?
□ No
□ Yes, for the following language: ………………….
□ Use the interpreting services of □ the NHS □ a family member □ a friend

Date of birth mother …………………….

Expected date of delivery (EDD) (*dd/mm/yyyy*) …………………….
As determined by □ last menstrual period □ ultra sound scan

Ethnic group (Please tick✓ 1 only) *MOTHER*

Asia
□ India
□ Pakistan
□ Bangladesh
□ China
□ Far East Asia (Japan, Korea)
□ South East Asia (Malaysia, Thailand, Phillipines)

Europe
□ Britain (England, Scotland, Wales)
□ Ireland (incl. Northern Ireland, Eire)
□ Northern Europe (Denmark, Norway, Sweden)
□ Western Europe (France, Germany, Netherlands)
□ Eastern Europe (Balkans, Poland, Russia)
□ Southern Europe (Cyprus, Greece, Italy, Spain, Turkey)

Caribbean
□ Barbados, Jamaica, Trinidad and Tobago

Middle East
□ Egypt, Israel, Syria, Yemen

Africa
□ North African (Morocco, Algeria)
□ Sub-Sahara (Somalia, Kenya, Nigeria)

Other □ ……………………………..

Decline of information
□ Please tick✓ this box if patient did not wish to answer this question

---

<u>Baby's details</u>

Date of birth (*dd/mm/yyyy*) …………….. **Gestational age** … *weeks* and ….. *days*

Time of birth (*hh/mm*) ………………………

Gender ………………………

Mode of delivery

☐ Spontaneous ☐ Induced ☐ Normal vaginal ☐ Instrumental

☐ Planned Caesarian ☐ Emergency Caesarian: ………………………..… (indication for Caesarian)

Presentation at birth

☐ Cephalic ☐ Breech ☐ Other, …………………..…….

Resuscitation needed?

☐ No ☐ Yes (specify below)

Details of resuscitation given

☐ Suction ☐ Oxygen ☐ Bag and mask

☐ Intubation ☐ Cardiac compression ☐ Drugs

APGAR

1 minute …………………….

5 minutes …………………….

10 minutes …………………….

Anthropometric data

Birth weight (*grams*) ……………………………….

Crown heel length (*cm*) ……………………………….

Occipito-frontal head circumference (*cm*)……………………………….

Location after birth

☐ Stayed with mother (transferred to ward) ☐ Transitional care ☐ Neonatal unit

---

Any postnatal complications?

☐ No ☐ Yes (specify below)

Postnatal complications details

☐ Ventilation: Oxygen ☐ Ventilation: CPAP ☐ Ventilation: Intubation
☐ Respiratory Distress Syndrome
☐ Infection
☐ Hypoglycaemia
☐ Hyperbilirubinaemia
☐ Intolerance of enteral feeding
☐ IUGR
☐ Organic abnormality
☐ Other, ……………………………

Mode of feeding

☐ Breastfeeding ☐ Formula ☐ Both

Tube needed?

☐ No ☐ Yes, ……………..……. *days*

……….…………… (total parenteral nutrition required)

<u>**Discharge details**</u>

Gestational age …………………………….. *weeks*

Weight (*grams*) ……………………………..

Crown heel length (*cm*)……………………………….

Occipito-frontal head circumference (*cm*)……………………………….

Days hospitalised ………………………………..

Days spent in neonatal unit ………………………..

Days spent on transitional care ward …………………………….

A-IV.iii Ultrasound scans

Date (dd/mm/yyyy):	**Gestational weeks:**	
	Baby I	Baby II
Biparietal diameter (mm)		
Biparietal diameter (percentile)		
Head circumference (mm)		
Head circumference (percentile)		
Femur length (mm)		
Femur length (percentile)		
Abdominal circumference (mm)		
Abdominal circumference (percentile)		
Estimated fetal weight (g)		
Estimated fetal weight (percentile)		
Anomaly detected		
If yes, specify		

	Baby I	Baby II
Heart Actvity		
Placenta		
Fetal Movement		
Kidney/Stomach/Bladder		
Heart 4 Chamber		
Skull		
Ventricles		
Cerebellum		
Spine		
EDF		
Amniotic Fluid		
Normal		
Oligohydramnios		
Polyhydramanios		
Presentation		
Cephalic		
Breech		
Other		
Fetal Well Being		
Umbilical Artery EDV		
Mid-Cerebral EDV		
Left Uterine Artery		
Right Uterine Artery		
Other EDV		

APPENDIX V

ADDITIONAL RESULTS FOR CHAPTER 4

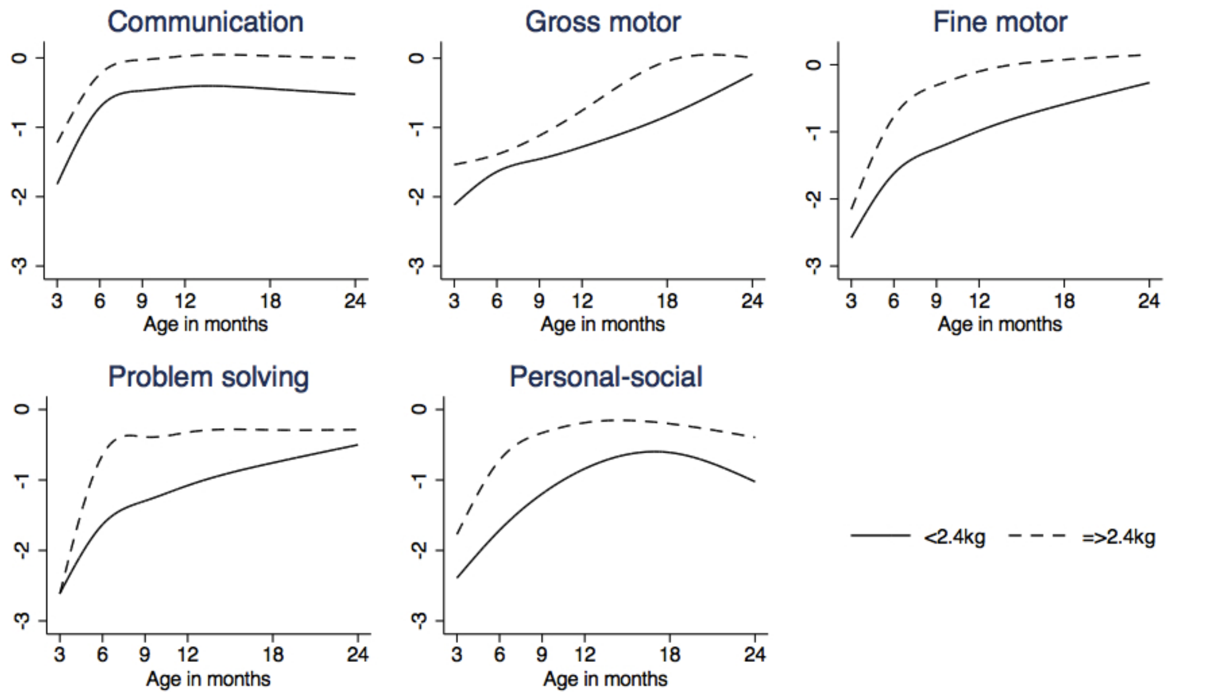

Figure V.i Twins' developmental trajectories assessed with the Ages and Stages Questionnaires, stratified by birth weight (<2.4 kilograms [kg] and =>2.4kg).

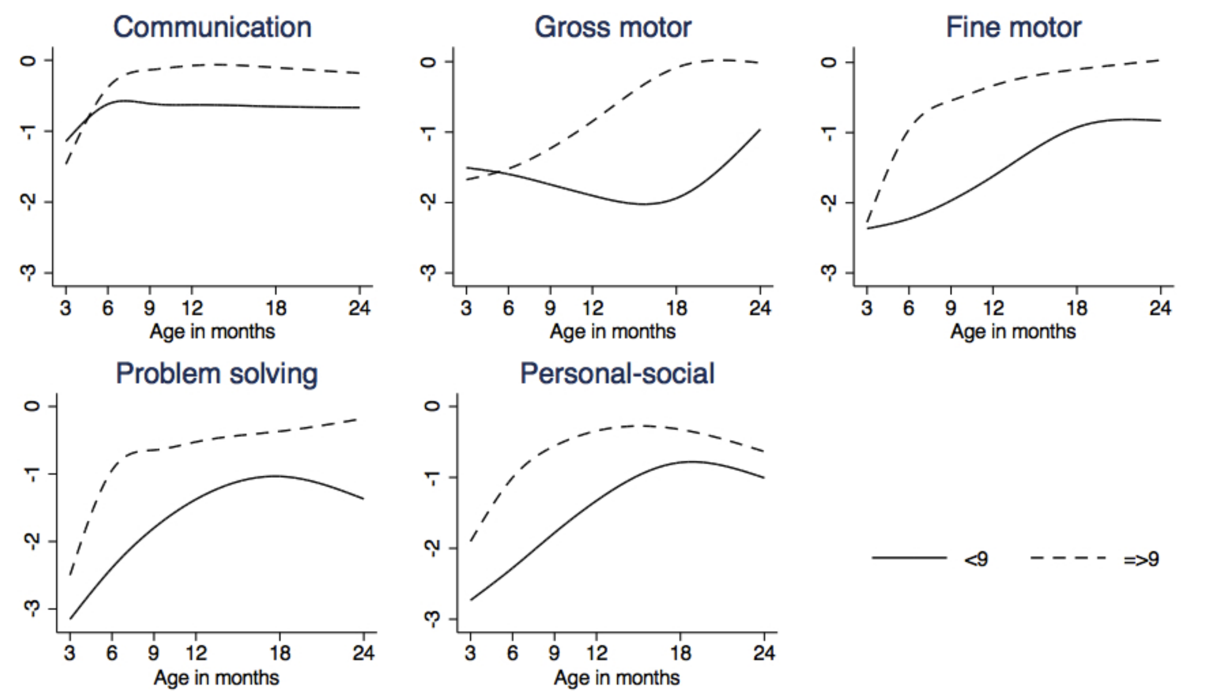

Figure V.ii Twins' developmental trajectories assessed with the Ages and Stages Questionnaires, stratified by 5-minute Apgar (<9 and =>9).

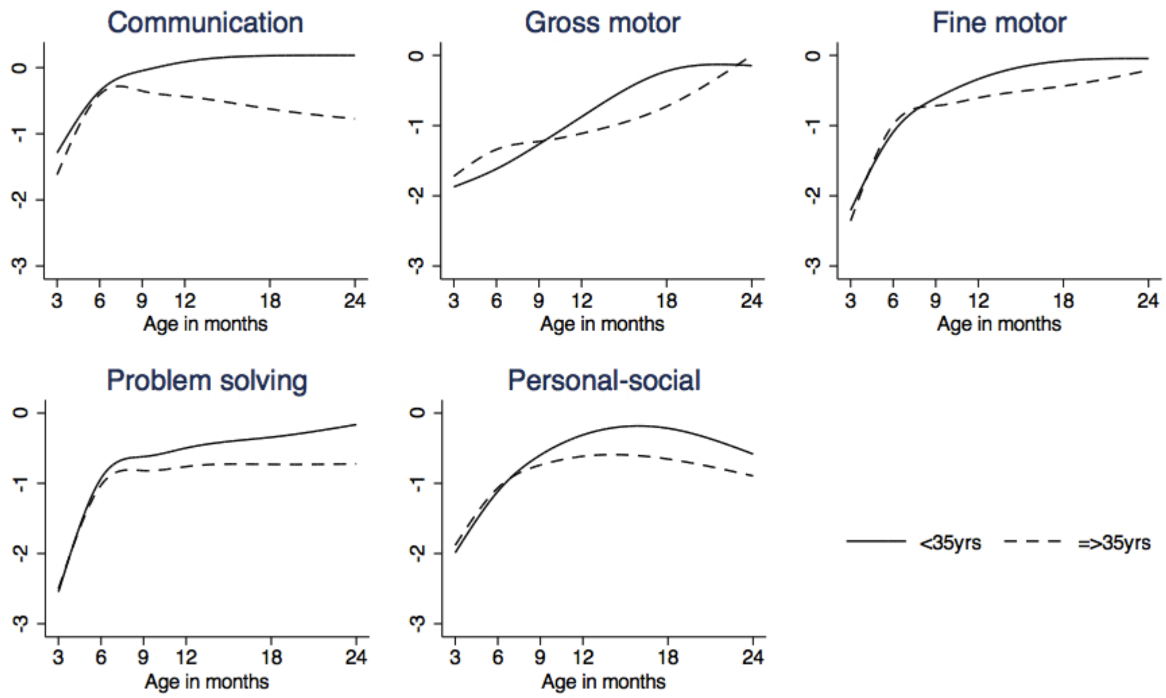

Figure V.iii Twins' developmental trajectories assessed with the Ages and Stages Questionnaires, stratified by maternal age (<35 years [yrs] and =>35 years).

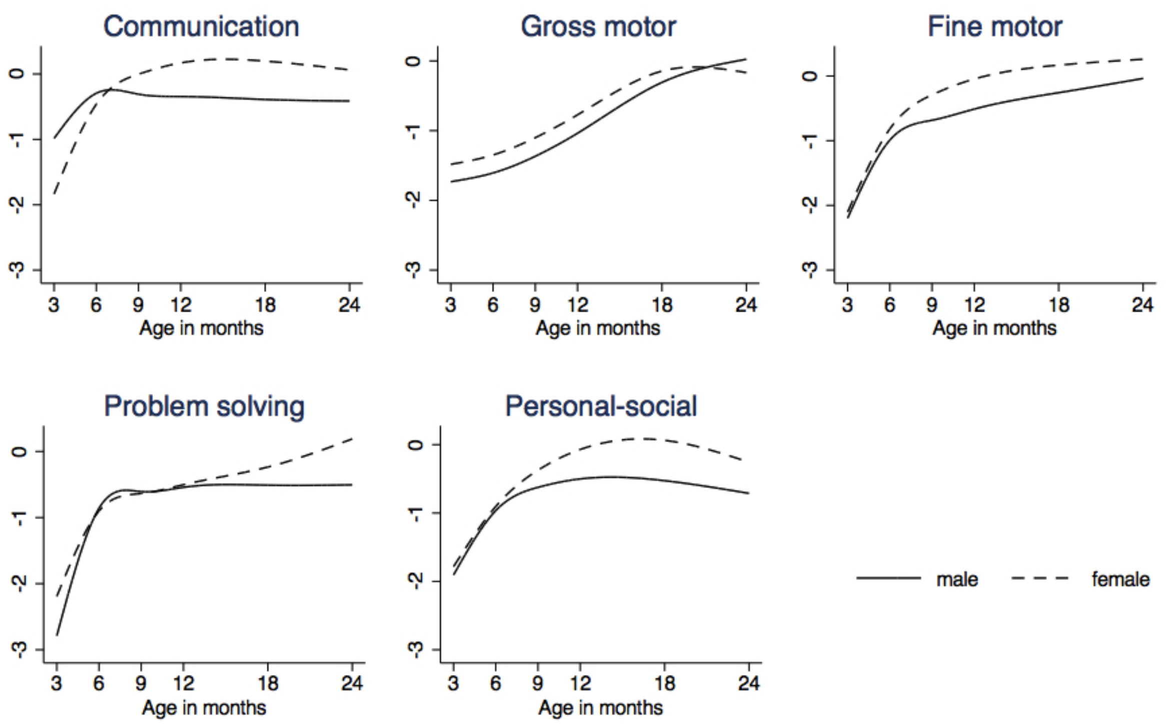

Figure V.iv Twins' developmental trajectories assessed with the Ages and Stages Questionnaires, stratified by gender.

APPENDIX VI

ADDITIONAL RESULTS FOR CHAPTER 5

Table VI.i Change in Ages and Stages Questionnaires z-scores for each millimetre increase in antenatal head circumference at 20, 28, 32 and 36 weeks.

	20 weeks Coef. (95% CI)	28 weeks Coef. (95% CI)	33 weeks Coef. (95% CI)	36 weeks Coef. (95% CI)
Communication				
3 months	0.06 (0.00 - 0.12)*	0.01 (-0.04 - 0.07)	0.01 (-0.04 - 0.06)	-0.01 (-0.05 - 0.01)
6 months	-0.03 (-0.06 - 0.01)	-0.03 (-0.06 - 0.00)*	-0.01 (-0.02 - 0.01)	0.00 (-0.01 - 0.01)
9 months	0.02 (-0.02 - 0.05)	0.02 (-0.01 - 0.05)	0.00 (-0.01 - 0.01)	0.00 (-0.01 - 0.01)
12 months	0.00 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	-0.01 (-0.02 - 0.01)	-0.01 (-0.02 - 0.00)*
18 months	-0.01 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	-0.01 (-0.02 - 0.00)	0.00 (-0.02 - 0.01)
24 months	-0.01 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	0.00 (-0.02 - 0.01)	0.00 (-0.03 - 0.04)
Gross motor				
3 months	-0.03 (-0.07 - 0.04)	0.01 (-0.04 - 0.06)	-0.02 (-0.07 - 0.02)	-0.03 (-0.06 - -0.01)**
6 months	0.01 (-0.04 - 0.03)	-0.01 (-0.04 - 0.02)	-0.01 (-0.02 - 0.01)	0.00 (-0.01 - 0.01)
9 months	0.03 (0.00 - 0.07)	0.03 (0.00 - 0.06)	-0.01 (-0.02 - 0.00)	0.00 (-0.01 - 0.01)
12 months	0.00 (-0.01 - 0.02)	0.00 (0.00 - 0.01)	-0.01 (-0.03 - 0.00)	-0.02 (-0.03 - 0.00)**
18 months	0.00 (-0.01 - 0.01)	0.00 (-0.01 - 0.01)	-0.01 (-0.02 - 0.00)	-0.02 (-0.03 - -0.01)***
24 months	0.00 (-0.02 - 0.02)	0.00 (-0.01 - 0.01)	0.01 (-0.01 - 0.02)	0.02 (-0.02 - 0.06)
Fine motor				
3 months	-0.01 (-0.06 - 0.04)	-0.01 (-0.05 - 0.04)	0.00 (-0.04 - 0.04)	-0.02 (-0.03 - 0.01)
6 months	0.01 (-0.03 - 0.06)	-0.02 (-0.05 - 0.02)	-0.01 (-0.03 - 0.01)	0.00 (-0.01 - 0.01)
9 months	0.04 (-0.01 - 0.09)	0.01 (-0.03 - 0.05)	0.01 (-0.01 - 0.02)	0.01 (-0.01 - 0.02)
12 months	0.00 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	-0.02 (-0.03 - 0.00)*	-0.01 (-0.03 - 0.00)*
18 months	-0.01 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	-0.01 (-0.02 - 0.00)	-0.01 (-0.02 - 0.00)
24 months	-0.01 (-0.02 - 0.00)	0.00 (-0.01 - 0.00)	0.00 (-0.01 - 0.01)	0.00 (-0.03 - 0.02)
Problem solving				
3 months	0.01 (-0.05 - 0.06)	-0.02 (-0.08 - 0.03)	-0.03 (-0.08 - 0.02)	-0.03 (-0.05 - 0.00)*
6 months	-0.02 (-0.05 - 0.02)	-0.03 (-0.06 - 0.00)	0.00 (-0.02 - 0.01)	0.00 (-0.01 - 0.01)
9 months	0.01 (-0.04 - 0.05)	0.02 (-0.02 - 0.06)	0.01 (0.00 - 0.02)	0.00 (-0.01 - 0.02)
12 months	0.00 (-0.01 - 0.02)	0.00 (-0.01 - 0.01)	-0.01 (-0.02 - 0.01)	-0.01 (-0.02 - 0.01)
18 months	0.00 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	-0.01 (-0.03 - 0.00)	-0.01 (-0.03 - 0.00)
24 months	-0.01 (-0.02 - 0.01)	0.00 (-0.01 - 0.00)	0.00 (-0.02 - 0.01)	0.01 (-0.02 - 0.04)
Personal-social				
3 months	-0.03 (-0.09 - 0.03)	0.04 (-0.02 - 0.09)	-0.03 (-0.08 - 0.02)	-0.04 (-0.07 - -0.02)***
6 months	0.00 (-0.04 - 0.04)	-0.02 (-0.05 - 0.01)	-0.01 (-0.03 - 0.01)	0.00 (-0.01 - 0.01)
9 months	0.01 (-0.02 - 0.05)	0.01 (-0.02 - 0.04)	0.00 (-0.01 - 0.01)	0.00 (-0.01 - 0.01)
12 months	0.00 (-0.01 - 0.02)	0.00 (-0.01 - 0.01)	-0.01 (-0.02 - 0.00)	-0.01 (-0.02 - 0.01)
18 months	-0.01 (-0.02 - 0.00)	0.00 (-0.01 - 0.00)	-0.01 (-0.02 - 0.01)	-0.01 (-0.02 - 0.00)
24 months	-0.02 (-0.03 - 0.00)*	-0.01 (-0.01 - 0.00)	0.00 (-0.02 - 0.01)	0.01 (-0.03 - 0.04)

Coef. = Regression coefficient, 95% CI = 95% Confidence interval.
Results have been adjusted for maternal age and gestational age at birth. Results for 20 and 36 weeks have been additionally adjusted for chorionicity. *p<0.05, **p<0.01 and ***p<0.001.

Table VI.ii Change in Ages and Stages Questionnaires z-scores for each millimetre increase in antenatal head circumference growth in 20-27, 28-32 and 33-36 weeks.

	20 - 27 weeks Coef. (95% CI)	28 - 32 weeks Coef. (95% CI)	33 - 36 weeks Coef. (95% CI)
Communication			
3 months	0.02 (-0.03 - 0.07)	0.02 (-0.04 - 0.08)	-0.04 (-0.07 - 0.00)
6 months	-0.03 (-0.06 - 0.01)	0.01 (-0.01 - 0.03)	-0.01 (-0.03 - 0.01)
9 months	0.03 (0.00 - 0.06)*	0.00 (-0.02 - 0.01)	-0.01 (-0.02 - 0.01)
12 months	0.00 (-0.01 - 0.01)	-0.01 (-0.02 - 0.00)	-0.01 (-0.03 - 0.01)
18 months	-0.01 (-0.02 - 0.01)	-0.01 (-0.02 - 0.00)	0.01 (-0.02 - 0.03)
24 months	0.00 (-0.02 - 0.02)	0.00 (-0.02 - 0.01)	0.01 (-0.04 - 0.06)
Gross motor			
3 months	0.02 (-0.04 - 0.07)	-0.02 (-0.08 - 0.03)	-0.04 (-0.08 - -0.01)**
6 months	0.00 (-0.04 - 0.04)	-0.01 (-0.03 - 0.02)	0.00 (-0.02 - 0.03)
9 months	0.02 (-0.01 - 0.06)	-0.01 (-0.02 - 0.00)*	-0.01 (-0.04 - 0.01)
12 months	0.00 (-0.01 - 0.02)	-0.02 (-0.03 - 0.00)*	-0.01 (-0.04 - 0.01)
18 months	0.00 (-0.02 - 0.01)	-0.01 (-0.02 - 0.00)	-0.03 (-0.05 - -0.01)**
24 months	0.01 (-0.02 - 0.03)	0.01 (-0.01 - 0.03)	0.03 (-0.04 - 0.09)
Fine motor			
3 months	0.01 (-0.04 - 0.06)	0.00 (-0.05 - 0.05)	-0.02 (-0.06 - 0.01)
6 months	-0.02 (-0.06 - 0.03)	0.00 (-0.03 - 0.02)	0.01 (-0.02 - 0.03)
9 months	0.01 (-0.03 - 0.06)	0.00 (-0.01 - 0.02)	0.00 (-0.02 - 0.03)
12 months	0.00 (-0.02 - 0.01)	-0.02 (-0.03 - 0.00)**	0.00 (-0.02 - 0.03)
18 months	0.00 (-0.02 - 0.01)	-0.01 (-0.02 - 0.00)	0.00 (-0.02 - 0.02)
24 months	0.00 (-0.01 - 0.02)	0.00 (-0.01 - 0.01)	0.01 (-0.03 - 0.04)
Problem solving			
3 months	-0.02 (-0.08 - 0.04)	-0.01 (-0.06 - 0.05)	-0.03 (-0.07 - 0.00)
6 months	-0.03 (-0.06 - 0.01)	0.01 (-0.01 - 0.03)	0.01 (-0.02 - 0.03)
9 months	0.03 (-0.01 - 0.07)	0.01 (0.00 - 0.02)	-0.01 (-0.03- 0.02)
12 months	0.00 (-0.02 - 0.01)	-0.01 (-0.03 - 0.00)	-0.01 (-0.03 - 0.02)
18 months	0.00 (-0.02 - 0.02)	-0.01 (-0.03 - 0.00)	0.01 (-0.02 - 0.03)
24 months	0.01 (-0.01 - 0.03)	0.00 (-0.02 - 0.01)	-0.01 (-0.06 - 0.03)
Personal-social			
3 months	0.06 (0.00 - 0.11)*	-0.05 (-0.11 - 0.00)	-0.05 (-0.09 - -0.02)**
6 months	-0.01 (-0.05 - 0.02)	0.00 (-0.02 - 0.02)	0.00 (-0.02 - 0.02)
9 months	0.01 (-0.02 - 0.04)	0.00 (-0.01 - 0.01)	-0.01 (-0.03 - 0.02)
12 months	0.00 (-0.01 - 0.00)	-0.01 (-0.03 - 0.00)	0.00 (-0.02 - 0.02)
18 months	0.00 (-0.01 - 0.02)	-0.01 (-0.02 - 0.01)	0.01 (-0.01 - 0.04)
24 months	0.01 (-0.01 - 0.03)	0.00 (-0.02 - 0.02)	0.00 (-0.05 - 0.05)

Coef. = Regression coefficient, 95% CI = 95% Confidence interval.
Results have been adjusted for maternal age, gestational age and size at the start of the age window. Results for 33-36 weeks have been additionally adjusted for chorionicity. *p<0.05, **p<0.01 and ***p<0.001.

APPENDIX VII

ADDITIONAL RESULTS FOR CHAPTER 6

Table VII.i The association of below (<=) and above (>) median growth in 0-3, 3-9, 9-12 and 12-18 month with developmental skills at 24 months.

	Communication Coef. (95% CI)		Gross motor Coef. (95% CI)		Fine motor Coef. (95% CI)		Problem solving Coef. (95% CI)		Personal-social Coef. (95% CI)	
	<=median	>median	<=median	>median	<=median	>median	<=median	>median	<=median	>median
Weight										
0 - 3 months	-0.03 (-0.15 - 0.09)	-0.16 (-0.32 - -0.01)*	-0.17 (-0.40 - 0.06)	-0.02 (-0.14 - 0.10)	-0.14 (-0.25 - -0.03)*	-0.10 (-0.22 - 0.01)	-0.11 (-0.23 - 0.01)	-0.19 (-0.34 - 0.04)*	-0.06 (-0.16 - 0.05)	-0.05 (-0.17 - 0.07)
3 - 9 months	-0.24 (-0.54 - 0.07)	-0.10 (-0.18 - -0.02)*	-0.05 (-0.50 - 0.40)	0.01 (-0.06 - 0.09)	-0.13 (-0.35 - 0.09)	-0.04 (-0.14 - 0.07)	0.08 (-0.28 - 0.44)	-0.07 (-0.13 - 0.00)*	-0.12 (-0.39 - 0.16)	-0.4 (-0.16 - 0.07)
9 - 12 months	0.03 (-0.17 - 0.22)	-0.01 (-0.06 - 0.05)	0.06 (-0.22 - 0.34)	-0.03 (-0.06 - 0.00)*	-0.03 (-0.19 - 0.14)	0.12 (-0.07 - 0.18)***	-0.13 (-0.35 - 0.09)	-0.04 (-0.15 - 0.06)	-0.13 (-0.30 - 0.03)	0.17 (0.05 - 0.29)**
12 - 18 months	0.08 (-0.27 - 0.44)	0.05 (-0.21 - 0.31)	0.40 (-0.07 - 0.87)	0.00 (-0.19 - 0.18)	0.21 (-0.10 - 0.51)	0.15 (0.08 - 0.23)***	0.06 (-0.33 - 0.46)	-0.11 (-0.17 - -0.04)**	-0.04 (-0.33 - 0.25)	0.29 (0.00 - 0.58)*
Length										
0 - 3 months	-	-	-	-	-	-	-	-	-	-
3 - 9 months	-0.04 (-0.09 - 0.02)	-0.02 (-0.05 - 0.01)	0.04 (-0.03 - 0.12)	-0.06 (-0.16 - 0.03)	-0.02 (-0.7 - 0.02)	-0.08 (-0.13 - -0.03)***	-0.03 (-0.09 - 0.03)	-0.07 (-0.09 - -0.05)***	-0.02 (-0.07 - 0.02)	-0.08 (-0.12 - -0.05)***
9 - 12 months	-0.05 (-0.09 - 0.00)	-0.01 (-0.05 - 0.03)	-0.02 (-0.07 - 0.03)	0.00 (-0.10 - 0.10)	-0.04 (-0.08 - 0.00)	-0.01 (-0.07 - 0.04)	-0.06 (-0.12 - -0.01)**	-0.01 (-0.06 - 0.05)	-0.07 (-0.10 - -0.04)***	0.04 (-0.02 - 0.10)
12 - 18 months	-0.09 (-0.16 - -0.01)*	0.06 (-0.02 - 0.13)	-0.01 (-0.13 - 0.11)	0.08 (0.04 - 0.12)***	-0.07 (-0.14 - -0.01)*	0.06 (0.00 - 0.13)	-0.12 (-0.21 - -0.04)**	0.02 (-0.08 - 0.12)	-0.12 (-0.20 - -0.04)**	-0.01 (-0.08 (0.06)
Head circumference										
0 - 3 months	-	-	-	-	-	-	-	-	-	-
3 - 9 months	-0.06 (-0.24 - 0.11)	0.01 (-0.01 - 0.04)	0.24 (-0.04 - 0.52)	0.05 (0.03 - 0.07)***	-0.04 (-0.19 - 0.11)	0.00 (0.00 - 0.01)	-0.11 (-0.30 - 0.08)	-0.02 (-0.03 - 0.00)*	-0.05 (-0.21 - 0.10)	-0.05 (-0.05 - 0.04)***
9 - 12 months	0.00 (-0.04 - 0.04)	-0.15 (-0.32 - 0.03)	-0.06 (-0.21 - 0.08)	-0.31 (-0.74 - 0.13)	-0.01 (-0.08 - 0.07)	-0.19 (-0.42 - 0.03)	-0.02 (-0.11 - 0.07)	-0.14 (-0.33 - 0.05)	0.01 (-0.07 - 0.09)	-0.05 (-0.11 - 0.10)
12 - 18 months	-0.08 (-0.27 - 0.11)	-0.03 (-0.05 - 0.01)***	-0.25 (-0.55 - 0.05)	-0.03 (-0.04 - -0.02)***	-0.08 (-0.30 - 0.13)	0.00 (-0.03 - 0.02)	-0.12 (-0.34 - 0.10)	-0.09 (-0.24 - 0.06)	-0.13 (-0.33 - 0.07)	0.00 (-0.19 - 0.19)

Coef. (95% CI)=Regression coefficient (95% Confidence interval).
Results have been adjusted for gestational age at birth. All significant results are displayed with *p<0.05, **p<0.01 and ***p<0.001.

APPENDIX VIII

ADDITIONAL RESULTS FOR CHAPTER 7

Table VIII.i Average metabolic equivalents (METs) by occupation category, derived from the International Standard Classification of Occupations (ISCO-88).

Legislators & managers

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
		1.9

Professionals

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
11610	standing - light/moderate, patient care	3.0
11805	walking, pushing a wheelchair	4.0
11875	teach PE, exercise, sports classes (non-sport play)	4.0
11876	teach PE, exercise, sports classes (participate in the class)	6.5
		2.2

Technicians & associate professionals

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
11610	standing - light/moderate, patient care	3.0
11805	walking, pushing a wheelchair	4.0
11875	teach PE, exercise, sports classes (non-sport play)	4.0
11876	teach PE, exercise, sports classes (participate in the class)	6.5
		2.2

Clerks

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
		1.9

Skilled agricultural & fishery workers

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
04001	fishing, general	3.0
04010	digging worms, with shovel	4.0
04020	fishing from river bank and walking	4.0
04030	fishing from boat, sitting	2.5
04040	fishing from river bank, standing	3.5
04050	fishing in stream, in waders	6.0
04060	fishing, ice, sitting	2.0
04070	hunting, bow and arrow or crossbow	2.5
04080	hunting, deer, elk, large game	6.0
04090	hunting, duck, wading	2.0
04100	hunting, general	5.0
04110	hunting, pheasants or grouse	6.0
04120	hunting, rabbit, squirrel, prairie chick, raccoon, small game	5.0
04130	pistol shooting or trap shooting, standing	2.5
11140	farming, baling hay, cleaning barn, poultry work, vigorous effort	8.0
11150	farming, chasing cattle, non-strenuous (walking), moderate effort	3.5
11151	farming, chasing cattle or other livestock on horseback, moderate effort	4.0
11152	farming, chasing cattle or other livestock, driving, light effort	2.0
11160	farming, driving harvester, cutting hay, irrigation work	2.5
11170	farming, driving tractor	2.5
11180	farming, feeding small animals	4.0
11190	farming, feeding cattle, horses	4.5
11191	farming, hauling water for animals, generaly hauling water	4.5
11192	farming, taking care of animals	6.0
11200	farming, forking straw bales, cleaning corral or barn, vigorous effort	8.0
11210	farming, milking by hand, moderate effort	3.0
11220	farming, milking by machine, light effort	1.5
11230	farming, shoveling grain, moderate effort	5.5
11250	forestry, ax chopping, fast	17.0
11260	forestry, ax chopping, slow	5.0
11270	forestry, barking trees	7.0
11280	forestry, carrying logs	11.0
11290	forestry, felling trees	8.0
11300	forestry, general	8.0
11310	forestry, hoeing	5.0
11320	forestry, planting by hand	6.0
11330	forestry, sawing by hand	7.0
11340	forestry, sawing, power	4.5
11350	forestry, trimming trees	9.0
11360	forestry, weeding	4.0
11370	furriery	4.5
11510	orange grove work	4.5
11010	bakery, general, moderate effort	4.0
11015	bakery, light effort	2.5
		3.9

Craft and trade workers

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
06010	airplane repair	3.0
06020	automobile body work	4.0
06030	automobile repair	3.0
06040	carpentry, general, workshop	3.0
06050	carpentry, outside house, installing rain gutters, building a fence	6.0
06060	carpentry, finishing or refinishing cabinets or furniture	4.5
06070	carpentry, sawing hardwood	7.5
06080	caulking, chinking log cabin	5.0
06090	caulking, except log cabin	4.5
06100	cleaning gutters	5.0
06110	excavating garage	5.0
06120	hanging storm windows	5.0
06130	laying or removing carpet	4.5
06140	laying tile or linoleum, repairing appliances	4.5
06150	painting, outside home	5.0
06160	painting, papering, plastering, scraping, inside house, hanging sheet rock, remodeling	3.0
06165	painting	4.5
06170	put on and removal of tarp - sailboat	3.0
06180	roofing	6.0
06190	sanding floors with a power sander	4.5
06200	scraping and painting sailboat or powerboat	4.5
06210	spreading dirt with a shovel	5.0
06220	washing and waxing hull of sailboat, car, powerboat, airplane	4.5
06230	washing fence, painting fence	4.5
06240	wiring, plumbing	3.0
11020	bookbinding	2.3
11040	carpentry, general	3.5
11130	electrical work, plumbing	3.5
11420	locksmith	3.5
11480	masonry, concrete	7.0
11720	tailoring, cutting	2.5
11730	tailoring, general	2.5
11740	tailoring, hand sewing	2.0
11750	tailoring, machine sewing	2.5
11760	tailoring, pressing	4.0
11765	tailoring, weaving	3.5
11790	using heavy tools (not power) such as shovel, pick, tunnel bar, spade	8.0
11870	working in scene shop, theater actor, backstage employee	3.0
		3.3

Plant & machine workers

ISCO-88 CODE	**Example**	**METs**
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
11080	coal mining, drilling coal, rock	6.5
11090	coal mining, erecting supports	6.5
11100	coal mining, general	6.0
11110	coal mining, shoveling coal	7.0
11120	construction, outside, remodeling	5.5
11430	machine tooling, machining, working sheet metal	2.5
11440	machine tooling, operating lathe	3.0
11450	machine tooling, operating punch press	5.0
11460	machine tooling, tapping and drilling	4.0
11470	machine tooling, welding	3.0
11640	steel mill, fettling	5.0
11650	steel mill, forging	5.5
11660	steel mill, hand rolling	8.0
11670	steel mill, merchant mill rolling	8.0
11680	steel mill, removing slag	11.0
11690	steel mill, tending furnace	7.5
11700	steel mill, tipping molds	5.5
11710	steel mill, working in general	8.0
11766	truck driving, loading and unloading truck	6.5
11780	using heavy power tools such as pneumatic tools (jackhammers, drills etc)	6.0
16050	driving heavy truck, tractor, bus	3.0
		3.8

Service workers

ISCO-88 CODE	Example	METs
07050	reclining - writing	1.0
07060	reclining - talking or talking on phone	1.0
07070	reclining - reading	1.0
09030	sitting - reading, book, newspaper etc	1.3
09040	sitting - writing, desk work, typing	1.8
09050	standing - talking or talking on the phone	1.8
09055	sitting - talking or talking on the phone	1.5
09060	sitting - studying, general, including reading and/or writing	1.8
09065	sitting - in class, general, including note-taking or class discussion	1.8
09070	standing - reading	1.8
09071	standing - miscellaneous	2.0
11580	sitting - light office work, general, sitting, reading, driving at work	1.5
11585	sitting - meetings, general, and/or with talking involved, eating at a business meeting	1.5
11770	typing, electric, manual or computer	1.5
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
11240	fire fighter, general	12.0
11245	fire fighter, climbing ladder with full gear	11.0
11246	fire fighter, hauling hoses on ground	8.0
11485	masseur, masseuse (standing)	4.0
11525	police, directing traffic (standing)	2.5
11510	police, driving squad car (sitting)	2.0
11527	police, riding in a squad car (sitting)	1.3
11528	police, making an arrest (standing)	4.0
		2.8

Elementary occupations

ISCO-88 CODE	Example	METs
09071	standing - miscellaneous	2.0
11791	walking on job, less than 2.0mph, very slow	2.0
11792	walking on job, 3.0mph, in office, moderate speed, not carrying anything	3.3
11793	walking on job, 3.5mph, in office, brisk speed, not carrying anything	3.8
11796	walking, gathering things at work, ready to leave	3.0
16010	riding in a car or truck	2.0
16015	riding in a bus	1.0
16020	flying airplane	2.0
16030	motor scooter, motorcycle	2.5
11600	standing - light, standing and talking at work, changing clothes when teaching PE	2.3
11030	building road (incl hauling debris, driving heavy machinery)	6.0
11035	building road, directing traffic	2.0
11070	chambermaid, making bed	2.5
11121	custodial work - buffing the floor with electric buffer	3.0
11122	custodial work - cleaning sink and toilet, light effort	2.5
11123	custodial work - dusting, light effort	2.5
11124	custodial work - feathering arena floor, moderate effort	4.0
11125	custodial work - general cleaning, moderate effort	3.5
11126	custodial work - mopping, moderate effort	3.5
11127	custodial work - take out trash, moderate effort	3.0
11128	custodial work - vacuuming, light effort	2.5
11129	custodial work - vacuuming, moderate effort	3.0
11530	shoe repair - general	2.5
11540	shoveling, digging ditches	8.5
11550	shoveling, heavy (more than 16 pounds/minute)	9.0
11560	shoveling, light (less than 10 pounds/minute)	6.0
11570	shoveling, moderate (10 to 15 pounds/minute)	7.0
		3.5

Armed forces

ISCO-88 CODE	Example	METs
11495	skindiving or SCUBA diving as a frogman (Navy Seal)	12.0
11240	fire fighter, general	12.0
11245	fire fighter, climbing ladder with full gear	11.0
11246	fire fighter, hauling hoses on ground	8.0
11525	police, directing traffic (standing)	2.5
11510	police, driving squad car (sitting)	2.0
11527	police, riding in a squad car (sitting)	1.3
11528	police, making an arrest (standing)	4.0
		6.6

Table VIII.ii Estimated means and odds ratios for birth outcomes and risk for neonatal admission in each light and medium category of maternal occupational physical activity and psychological strain.

	Estimated mean (95% confidence interval (CI))				Estimated odds ratios (95% confidence interval (CI))					
					Delivery mode			Neonatal admission		
	Birth weight (kilograms)	Birth weight SDS	Gestational age (weeks)	5-minute Apgar	Vaginal or Elective caesarean (N)	Emergency caesarean (N)	Odds ratio	No (N)	Yes (N)	Odds ratio
Occupational physical activity										
Light activity	2.39 (2.15 - 2.62)	-0.85 (-1.21 - -0.49)	35.77 (33.91 - 37.63)	9.16 (8.97 - 9.35)	88	80	1.00	170	52	1.00
Medium activity	2.40 (2.17 - 2.63)	-0.62 (-0.98 - -0.27)	35.43 (33.57 - 37.28)	8.94 (8.75 - 9.13)*	84	73	0.67 (0.37 - 1.23)	116	52	0.98 (0.51 - 1.89)
Perceived occupational demand										
Low	2.43 (2.18 - 2.68)	-0.72 (-1.16 - -0.30)	35.80 (33.93 - 37.67)	9.02 (8.77 - 9.27)	33	39	1.00	53	21	1.00
High	2.38 (2.16 - 2.61)	-0.74 (-1.08 - -0.40)	35.54 (33.75 - 37.34)	9.05 (8.88 - 9.22)	139	114	0.82 (0.65 - 1.04)	233	83	1.12 (0.52 - 2.43)
Perceived occupational control										
Low	2.37 (2.14 - 2.60)	-0.71 (-1.09 - -0.34)	35.41 (33.53 - 37.30)	9.09 (8.89 - 9.28)	57	69	1.00	86	48	1.00
High	2.41 (2.19 - 2.64)	-0.75 (-1.10 - -0.40)	35.72 (33.85 - 37.59)	9.02 (8.85 - 9.19)	115	84	0.82 (0.65 - 1.04)	200	56	0.47 (0.25 - 0.89)*
Perceived occupational strain										
Low	2.36 (2.14 - 2.59)	-0.68 (-1.05 - -0.32)	35.32 (33.53 - 37.12)	8.99 (8.82 - 9.16)	77	84	1.00	111	64	1.00
High	2.44 (2.20 - 2.67)	-0.81 (-1.20 - -0.42)	35.99 (34.17 - 37.80)*	9.14 (8.94 - 9.33)	95	69	0.82 (0.45 - 1.52)	175	40	0.59 (0.29 - 1.19)

SDS=standard deviation score. Significant effects with p<0.05 are marked with *. Occupational physical activity, perceived occupational demand, control and strain categories are based on the sample median.

APPENDIX IX

ADDITIONAL RESULTS FOR CHAPTER 8

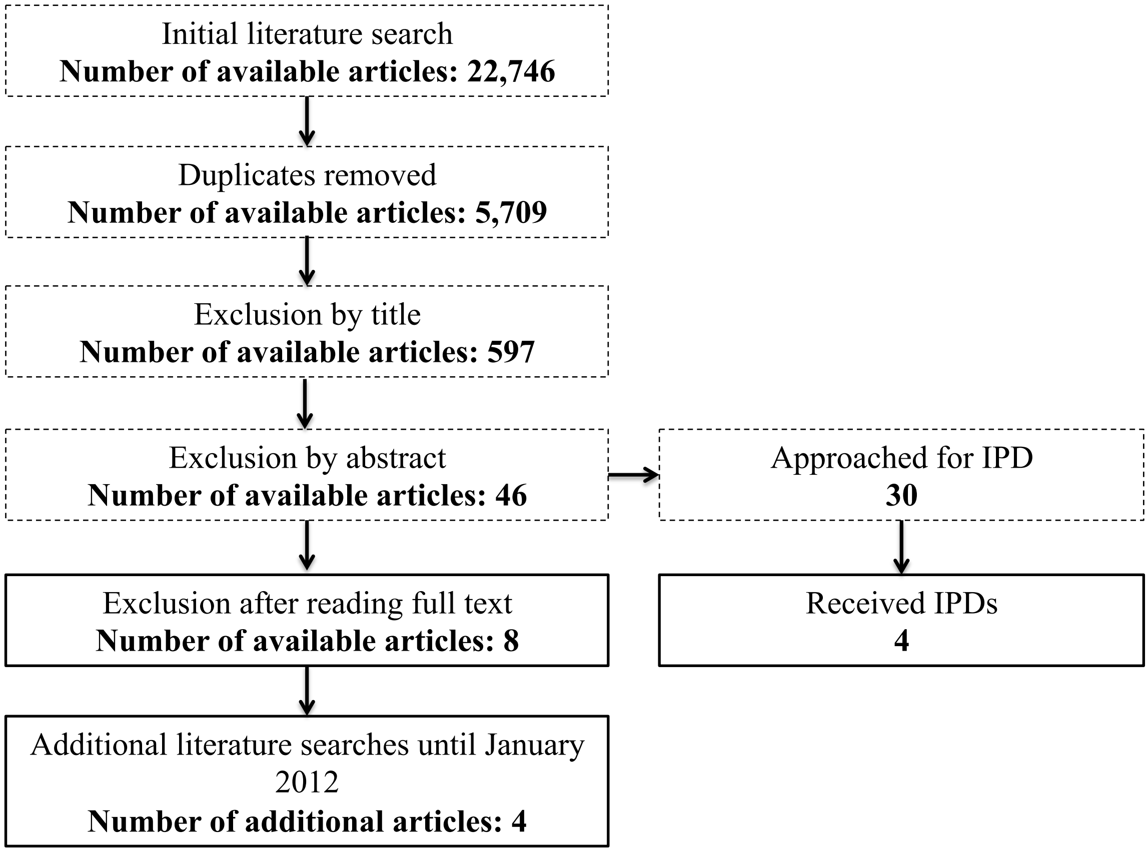

Figure IX.i Results for the initial literature search, request for individual participant datasets (IPDs) and the additional literature search.

APPENDIX X

PUBLICATIONS

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