

Neurocognitive outcome of monochorionic twins with different birth weights

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Declaration

I hereby declare that the work submitted in this thesis consists of a literature review and original research conducted by myself. Dr. M Korada assisted with data collection. I was supervised by Dr. N.D. Embleton and Professor H.R. McConachie throughout the study period. All the children included in this study were approached for enrolment by the relevant clinicians involved in their care but were recruited, consented and assessed by myself. This thesis has not been submitted by me for any other degree or qualification in this, or any other, institution.

Dr. Ravi Shankar Swamy

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Abstract

Background: Although the long term effect of intrauterine growth restriction has been assessed in a number of singleton studies, they all suffer from multiple confounding effects. A model that utilises monozygotic twins may markedly reduce the effect of confounders as monozygotic twins share the same gestational age length, family background, gender and genetic influences on growth and cognition. Comparison of monozygotic twins with birth weight discordance of 20% or more could be used as a model of in utero growth constraint. This model will still involve certain limitations and assumptions nevertheless; we used this to determine the level of cognitive function of in-utero growth discordant monozygotic twins in later childhood along with any differences in auxology and behavioural problems.

Methods: This was a retrospective cohort study. Eligible twins were identified from the Northern Survey of Twins and Multiple Pregnancies register. Cognitive function was assessed by a single observer using the British Ability Scales 2 to measure the general conceptual ability. Strength and Difficulties Questionnaire was used to identify behavioral problems. Height, weight, mid arm circumference, waist measurement and head circumference were also collected. Generalised estimating equations were used to determine the effect of birth weight on general conceptual ability scores. Statistical analyses were performed using SPSS v19.

Results: Between 2000 and 2004, a total of 51 twin pairs were assessed (n=23 female) with mean birth weight discordance 664gm and mean gestational age 34.7 weeks. The mean difference in the general conceptual ability score between the heavier and lighter twins was 3 points. Significant association between within pair differences in birth weight and general conceptual ability scores was found. Increasing birth weight discordance was not associated with a decrease of general conceptual ability scores. The differences in the size seen at birth between the twins were still detectable at the age of 5-8 years. There was a trend to increased

prevalence of behavioural problems in the lighter twin compared to the heavier twin as reported by both teachers and parents but this result was not statistically significant.

Conclusions: The smaller twin of a monochorionic growth discrepant pair was statistically significantly more likely to have a lower cognitive score compared to their co-twin at 5-8 years of age. This suggests that growth restriction in-utero is associated with lower cognitive scores in later childhood.

1. Introduction

Over the past two decades, the formulation of the ‘Developmental Origins of Health and Disease’ hypothesis has resulted in recognition that pre-natal influences have a longer-term effect on adult health. Birth weight is usually considered as a marker of prenatal influence and it has been recognised that newborns with lower birth weight are at an increased risk of certain physical and neurodevelopmental sequelae. Studies investigating the long term effects of intrauterine growth restriction in singletons are confounded by a number of variables that can modify the link between prenatal growth restriction and subsequent neurodevelopment. Twin studies involving monozygotic twins may be a useful model for developmental studies exploring the effects of growth restriction because monozygotic twins have identical genotypes and most environmental exposures are similar. Therefore, any differences in cognition can be attributed to effects of growth restriction secondary to poor intrauterine nutrition. This study is designed to explore this hypothesis, and aims to determine the cognitive effects of growth restriction using a birth weight discordant monozygotic twin model.

The introduction begins by examining the origin of twins, in particular, examining the monozygotic twins. This is followed by a chapter on neurodevelopmental outcomes of twins in general. The next chapter of this focuses on definition, etiology and effects of intrauterine growth restriction. The final chapters explore definition, mechanism and postnatal complications of birth weight discordance and how birth weight discordance has been used as a model to evaluate the effects of growth restriction by reviewing the available literature investigating the long term neurocognitive effects of birth weight discordant twins.

1.1 The genesis of twins

Twins can be monozygotic or dizygotic. Dizygotic twins develop when 2 ova are fertilized and have separate amnions and placentas. Monozygotic twins develop when a single fertilized ovum splits into 2 after conception. The division of monozygotic embryo takes place during the first 14 days following fertilisation and 4 categories can be distinguished depending on the time of division:

1. Early separation (Figure 1): In 18-36% of cases, separation occurs between the zygote and morula stage, which is up to 72 hours post-fertilisation. Such embryos are dichorionic-diamniotic. Splitting occurs very early when embryonic cells are totipotent, between the 1-cell and the 8-cell stage.(Blickstein and Keith, 2006)
2. Later separation (Figure 1): In 60-70% of cases, splitting occurs at the early blastocyst stage, after the formation of the inner cell mass which separates from the trophoblast before day 8, the resulting embryos are monochorionic-diamniotic
3. Rare separation (Figure 1): In 1% occurs after day 6 up to day 12. Splitting of inner cell mass takes place when the amnion has become distinct. The embryos are monochorionic-monoamniotic
4. The rarest type (Figure 1): Conjoined twins result from an even later stage 12-13 days after fertilisation. Their frequency is 1:200 monozygotic pairs and about 1:40000 births.

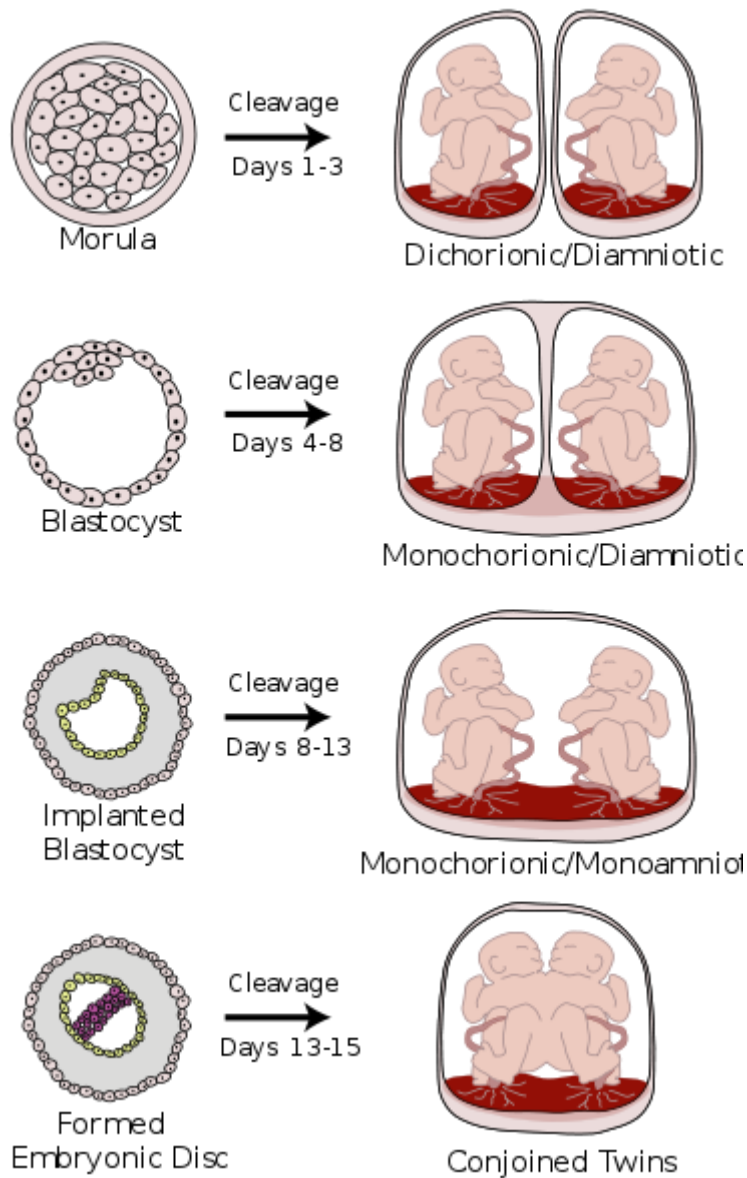


Figure 1: Showing the different chorionicity and amnionity occurring based on the time of separation of the zygote in the monozygotic twins. Reproduced from www.wikipedia.org

Table 1 shows the percentage of twins in each category for spontaneous pregnancies.

Type	Percentage of twins
Dizygotic	53%
Monozygotic dichorionic	12%
Monozygotic monochorionic	29%
Unknown/Conjoined	6%

Table 1: Percentage of twins according to their origin (Blickstein, 2009)

1.1.1 Monochorionic twins

Monozygotic twins can be monochorionic or dichorionic. Two thirds of monozygotic twins are monochorionic (Machin, 1996) and single placentas are generally characteristic of monochorionic pregnancies. Determining chorionicity is important as monochorionic pregnancies have a high mortality of 10-25% (Machin GA, 1997). Also, monochorionic twins are at substantially greater risk of miscarriage, perinatal death and intrauterine growth restriction than dichorionic twins (Sebire et al., 1997). This higher relative risk is likely to be the consequence of the underlying placental vascular communications (chorioangiopagus), which are present in virtually all monochorionic twins (Denbow et al., 2000). As a result, 15-20% of monochorionic twins develop specific problems that are apparent by 18-20 weeks of gestational age. These problems include haemodynamic imbalance leading to twin-twin transfusion syndrome, growth restriction and birth weight discordance; twin reversed arterial perfusion, fetal brain injury to the surviving twin if the co-twin dies in-utero and mono-amniotic intertwining of the umbilical cords. Placentation is hence considered generally a more important obstetric variable than zygosity. Determining chorionicity in a twin pregnancy is therefore thought to be important as it has a major impact on the outcome of twin pregnancies.

Determining zygosity, chorionicity and amnionicity: Zygosity refers to the type of conception and can be determined for most twins by placentation, gender, physical examination and blood group. Immunologic studies (HLA typing) or DNA fingerprinting analysis can also prove zygosity. Antenatally, this would require amniocentesis, chorionic villus sampling or cordocentesis.

Chorionicity refers to the type of placentation and amnionicity refers to the number of amniotic cavities in which the twins reside. They both can be determined early by vaginal ultrasonography with an accuracy of almost 100% (Tong et al., 2004). Between 6–9 weeks of gestational age, in dichorionic twins there is a thick septum between the chorionic sacs (Hill et al., 1996; Monteagudo et al., 1994). After 9 weeks, this septum becomes progressively thinner to form the chorionic component of the inter-twin membrane, but it remains thick and easy to identify at the base of the membrane as a triangular tissue projection called as lambda sign (Sepulveda et al., 1996; Finberg, 1992; Bessis and Papiernik, 1981). At the dating scan, which is done between 11–14 weeks of gestational age, sonographic examination of the base of the inter-twin membrane for the presence or absence of the lambda sign provides distinction between dichorionic and monochorionic pregnancies. In an study of 368 twin pregnancies at 10–14 weeks of gestational age, pregnancies were classified as monochorionic if there was a single placental mass in the absence of the lambda sign at the inter-twin membrane–placental junction, and dichorionic if there was a single placental mass but the lambda sign was present or the placentas were not adjacent to each other. In 81 (22%) cases, the pregnancies were classified as monochorionic and in 287 (78%) as dichorionic. All pregnancies classified as monochorionic resulted in the delivery of same-sex twins and all different-sex pairs were correctly classified as dichorionic (Sepulveda et al., 1996). It is recognised that if chorionicity is assessed before 14 weeks, the correct diagnosis is made in majority of the cases (Stenhouse et al., 2002).

Amnionicity depends on the membrane separating the twins when present. Dichorionic twins have a membrane comprised of two chorions and two amnions and on ultrasound it measures at least 2 mm thick due to four layers. Monochorionic-diamniotic twins, on the other hand, have a membrane that is only two layers thick, and usually measures at most 1.5 mm thick.



Figure 2: Dichorionic diamniotic twins with lambda sign. Reproduced from www.eimjm.com

1.2 Neurodevelopmental outcomes of twins

Neurodevelopment is a comprehensive evaluation of all brain functions including gross and fine motor skills, vision and hearing, social skills, speech and language, perception, learning, attention and cognition.

Neurodevelopmental assessment is conducted in order to understand how a child learns and how his or her brain functions.

Neurodevelopmental morbidity includes any abnormality in the above functions ranging from mild difficulties in motor skills to cerebral palsy.

Prior to examining the effect of growth restriction on neurodevelopmental outcome using the twin model, it is vital to examine the neurodevelopmental outcome of twins in general. If the cognitive abilities of twins are similar to that of singletons, then the results from twin studies can be applied to matched singletons. However, as this study focuses on effects of nutrition on cognition, only studies examining neuro-cognitive ability of twins are discussed. This section examines studies aiming to determine cognitive and behavioural development of twins.

Cognitive ability: Previous studies have shown that twins are associated with a variety of adverse outcomes, including delayed development and impaired sensorimotor function (Blickstein, 2002; Petterson et al., 1993). Several studies based on population cohorts of children born at least 50 years ago have found appreciable cognitive deficits for twins in childhood compared to matched singletons. In a study of 48,913 singletons, 1082 twins and 11 triplets born in Birmingham, between 1950 and 1954, verbal reasoning scores obtained by them in their 11 plus school examinations was compared. Twins had a deficit in verbal reasoning scores between 4 and 5 IQ (Intelligence Quotient) points when compared to singletons (Record et al., 1970). The mean standardised score for the singletons was 100, for twins 95.7 and for triplets 91.6. Attempts to correct for maternal age, birth weight, gestational age, zygosity and birth order were made, but these did not account for the differences seen. The authors

suggested that these differences were due to rearing practices. Similarly, when 9832 singletons and 236 twins born in Aberdeen between 1950 and 1956 were compared using school test results, twins had a cognitive deficit of more than 6 IQ points compared with singletons at ages 7 years and 9 years. This effect could not be explained by confounding due to socio-economic, maternal, family characteristics or by recruitment bias (Ronalds et al., 2005). Adjusting for the lower birth weight of twins and gestational age halved the difference at age 7 and reduced it by 30% at age 9. These differences were then no longer statistically significant. Reduced prenatal growth and shorter gestational age was thought to be more important than socioeconomic factors in explaining the differences.

Deary et al examined two whole population surveys of mental ability, one of which also provided information on social background. The total sample included 2000 twins. In both the surveys, twins scored lower on the Moray House Test of verbal reasoning, equivalent to a deficit of about 5 IQ points at the age of 11 years compared to singletons (Deary et al., 2005). Husen showed in a large study of Danish school age twins at 11-15 years, the mean IQ for twins was between a quarter and a third of one standard deviation below that of singletons (Husen, 1963).

However, the findings from a study based on the Netherlands twin registry showed different results from the above studies. A comparison was made between 260 adult twins with their 98 related singleton siblings. They showed no significant difference in cognitive ability on Wechsler Adult Intelligence Scale-III, even though a power analysis demonstrated that effects much lesser than those reported in previous studies could easily have been detected (Posthuma et al., 2000). Either the confounding factors explained the difference or early IQ differences became less apparent with age. The authors argued that significant disadvantages of twins in comparison with singletons seemed to be implied rather than observed.

Christensen et al compared the school performance of 3411 twins with 7796 singletons between the age of 15 and 16 in Denmark during 1986–1988. The sample was a random 5% of Danish 15-year-olds. They showed that twins had similar academic performance in adolescence as singletons and found no difference in cognitive ability between twins and singletons even though on average the twins were 908 g lighter at birth (Christensen et al., 2006). Similar results were obtained after controlling for birth weight, gestational age, parental age and educational level. A small but statistically significant association between birth weight and test scores was seen in both singletons and twins.

Wilson et al showed that although twins appeared to have delayed development at 18 months, no significant delay was noted at six years (Wilson, 1974). They hypothesised that the early delay in twins was probably due to the effect of another sibling at the same age. Morley et al. examined the growth and development of a group of 90 premature twins compared to 386 premature singletons at 18 months of age on the Bayley scales (Morley et al., 1989). After adjusting for confounding social and neonatal factors, twins were not found to be disadvantaged in their neurodevelopmental outcome. They suggested that the developmental disadvantage seen for twins in other studies may be due to the increased prevalence of preterm delivery.

Another study by Leonard et al looked into a group of twins born <1250 g (n=82) over a 10-year period between 1977 to 1987 compared to a group of singletons with similar weight (n=329) (Leonard et al., 1994). Infants were seen at 1 year of age and at school age. Morbidity was assessed by neurodevelopmental examinations and standard developmental tests. They found no difference in neurologic and neurosensory outcome between twins and singletons at 1 year age. There was also no difference in the cognitive outcomes at school age. Gestation type was not associated with cognitive outcome at school age but chronic lung disease and social risk factors were found to be associated with poorer cognitive outcome. A

similar study in the UK examined 280 infants born at less than 32 weeks of gestational age at seven years of age (Cooke, 2005). Sixty-three were twins. The IQ was determined by the short form Wechsler Intelligence Scale for Children- III. Multiple regression analysis showed that gestational age, presence of a patent ductus arteriosus and head circumferences at 7 years were independent predictors of IQ at 7 years of age. The mean IQ for twins and singletons was identical (89 points). The EPICure 1 study (in extremely preterm children) showed that cognitive scores of twins was not different from singletons (Costeloe et al., 2000).

Behavioural problems: Moilanen reported behaviour outcomes of 122 twins and 5455 singletons born in 1981 at the age of eight years using Rutter questionnaires and the Child Depression Inventory (Moilanen et al., 1999). Overall the teachers completing the forms reported fewer behavioural disturbances in twins compared to singletons. Parental and self-report data did not differ between the two groups. Another study in the Netherlands looked at 1363 twin pairs and 420 singletons using maternal ratings of problem behaviours in 2-3 year olds (Vandenoord et al., 1995). The Child Behaviour Check List was used. The results showed the level of problem behaviours to be similar in twins and singletons. Males, whether twins or not, had overall higher scores particularly for aggression and over activity.

In summary, there are a few studies especially the older ones that suggest that twins have a lower cognitive ability than singletons while recent studies suggest that there is no difference. The reasons for contrasting conclusions could be the following:

- 1) The lower cognitive abilities of twins found in some studies compared to singletons could be mainly be due to prematurity (defined as infants who are born before 37 weeks gestational age). During the last two decades, there has been a continuous increase in twinning rates due to a wider use of assisted reproductive technology (Office for National Statistics, 2006).

Twin pregnancies have a higher rate of premature delivery than singleton pregnancies and deliver on an average 2 weeks earlier, compared to singletons. The median gestational age is around 35 weeks (Keith et al., 1998; Ho and Wu, 1975). The reasons for higher rate of premature delivery in twins include spontaneous onset of labour, premature rupture of membranes and elective caesarean section due to maternal or fetal concerns. Major disabilities such as cerebral palsy and learning disabilities occur in 10-30% of premature twins (Pharoah and Cooke, 1996; Grether et al., 1993). Cognitive outcome correlates with prematurity and on average a decrease of 2.5 IQ points for each week below 33 weeks gestational age (Bhutta et al., 2002). This is consistent with the IQ data for children born at less than 26 weeks gestational age as reported in the EPICure study (Costeloe et al., 2000). Multiple births in this study did not have any independent effect on development after correction of other factors. Hence, the increased incidence of prematurity itself increases the number of disabilities and low IQ scores within the twins

2) Chorionicity, which is thought to have a major impact on the neurodevelopmental outcomes, was not ascertained in majority of the studies which showed a lower cognition in twins compared to singletons. It is known that monochorionic twins are at substantially greater risk of focal brain injury due to haemodynamic imbalance. Ong et al in their systematic review showed that following the death of one twin, the risk of monochorionic and dichorionic co-twin demise was 12% and 4% respectively. The risk of neurological abnormality in the surviving monochorionic twin was 18% (Ong et al., 2006). Perinatal morbidity and mortality is much higher in monochorionic twins as compared with dichorionic twins (Bagchi and Salihu, 2006). In a single-centre UK registry of neurodevelopmental outcome in twin gestational ages delivering at < 34 weeks of gestational age, monochorionic twins conferred a sevenfold increase in neurologic morbidity compared with dichorionic twins (Adegbite et al., 2004). It is possible that the low IQ scores seen in twins in some studies could be due to effect of chorionicity.

3) Another limitation of the existing studies is that many were based on individuals born at least 35 years ago. The important question which then arises is whether there was a deficit in the cognitive abilities of twins and if so, whether these differences no longer exist for recent cohorts. The resolution of cognitive deficits may be explained by the considerable progress in nutrition and health care, both in the fields of obstetrics and neonatal medicine, especially in the last 2 decades. This may have reduced these cognitive differences between singletons and twins. It is also plausible that the education system has evolved to better deal with children with cognitive deficits.

To conclude, twins have IQ scores that are within the normal range and do not differ from those of unrelated singletons or singleton siblings. Although there is evidence that monozygotic twins are at risk of neurodevelopmental impairments, in those that escape focal brain injury, there is little consistent evidence of impaired neurodevelopment. This suggests that data from twin studies examining cognition can be generalised to matched singletons.

1.3 Intrauterine growth restriction

Several animal experiments, singleton and twin studies have shown that growth restriction can affect cognition, which may also explain the reason for lower level of cognitive function seen in some twin studies comparing singletons described earlier. This chapter hence explores the definition, etiology and finally effects of growth restriction, in particular on neurodevelopment.

1.3.1 Definition of intrauterine growth restriction in twins

Intrauterine growth restriction implies the fetus has failed to grow at the expected rate. Intrauterine growth restriction is defined antenatally in singletons as sonographic estimated fetal weight below the 10th percentile (Hadlock et al., 1991). This occurs in approximately 3-10% of singleton pregnancies (Lin and Santolaya-Forgas, 1998).

The singleton definition of sonographic estimated fetal weight applies to twins as well. This is seen in 9.1% of all twins, and in 9.9% of monochorionic twins (Ananth et al., 1998). Intrauterine growth restriction in monochorionic twins can affect only one of the fetuses and this event is known as selective-intrauterine growth restriction. In this case, the fetal weight difference becomes apparent. Estimated fetal weight difference between the twin pair has also been used as an approximation to the diagnosis of intrauterine growth restriction in twins.

Many parameters have been used to diagnose fetal weight discordance including intrapair differences in bi-parietal diameter, head circumference, abdominal circumference, femur length, humerus length and estimated fetal weight. The commonly accepted values are bi-parietal diameter difference > 6mm (Leveno et al., 1980), Abdominal Circumference difference >20mm (Barnea et al., 1985), Femur Length difference >5 mm (Storlazzi et al., 1987) and a difference in systole/diastole wave ratio in the umbilical artery of more than 15%

(Leveno et al.,1980). For example, Table 2 shows an example of how fetal weight difference is calculated at 26 weeks.

Table 2: Fetal measurements at 26weeks of gestational age

Measurements	Lighter twin	Heavier twin	Difference
Biparietal Diameter	54mm	62mm	8mm
Head circumference	200mm	225mm	25mm
Abdominal circumference	162mm	231mm	69mm
Femoral length	32mm	48mm	16mm
Humerus length	30mm	43mm	13mm
Estimated fetal weight	466gm	970gm	504gm

mm= millimetres, gm= grams

O'Brien et al showed that when birth weight discordance exceeded 20%, there was a prevalence of selective-intrauterine restriction in 50% of the twins (Obrien et al., 1986). Currently, the American College of Obstetricians and Gynaecologists suggest that intrauterine growth restriction in twins is usually diagnosed when there is discordance in estimated fetal weight of >20% between the twins (American College of Obstetricians and Gynecologists, 1998.). However, when fetal weight discordance is detected antenatally, it cannot be assumed that this difference is due to selective-intrauterine growth restriction in one twin, as both fetuses can be appropriately grown for gestational age, yet have an estimated fetal weight discordance >20%.

1.3.2 Differentiating selective-intrauterine growth restriction from twin-twin transfusion syndrome

Intrauterine growth restriction may be present in one or both twins. Selective-intrauterine growth restriction is the term used when only one of the twins is affected and is diagnosed antenatally when an estimated fetal weight < 10th percentile is detected (Quintero et al., 2001). Selective-intrauterine growth restriction occurs in about 12.5-25% of all monochorionic pregnancies (Quintero et al., 2001; Gaziano et al., 2000; Bjoro and Bjoro, 1985). The actual incidence is difficult to ascertain as the

distinction between twin-twin transfusion syndrome and pure selective-intrauterine growth restriction may not have been made previously. Pure selective-intrauterine growth restriction can be present in up to 15% of the monochorionic twins initially thought to have twin-twin transfusion syndrome (Quintero et al., 2001). Intrauterine growth restriction coexists with twin-twin transfusion syndrome in approximately 50% of patients (Russell et al., 2007). Inadequate placental sharing and presence of vascular anastomoses has been thought to be the cause of selective-intrauterine growth restriction (Valsky et al., 2010).

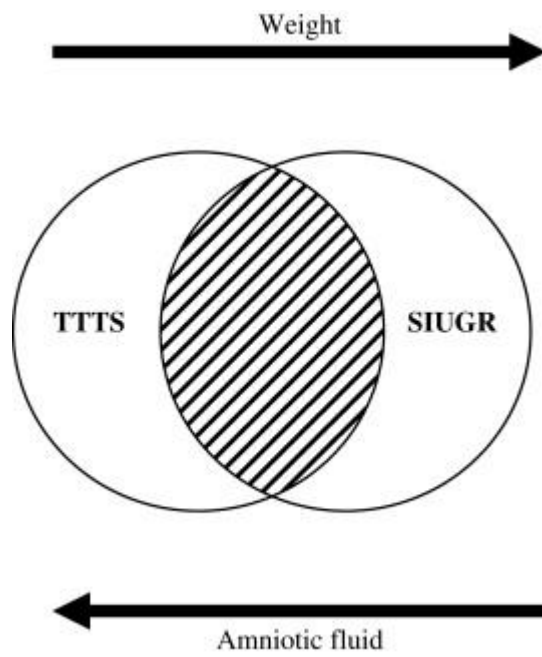
Twin-twin transfusion syndrome on the other hand is a condition that affects monochorionic twin pregnancies and is one of the serious complications. In almost all of these pregnancies, the placenta contains blood vessel connections between the twins. Discordant placental vascular pressure can result in transfer of blood from one twin to the other causing twin-twin transfusion syndrome. It is usually diagnosed around 20-24 weeks of gestational age, but can vary in rapidity of onset and severity. Severe twin-twin transfusion syndrome usually occurs before 24 weeks gestational age (Sebire et al., 1997). However, it is possible that discordant utero-placental function in later pregnancy results in inter-twin transfusion, thereby aggravating growth restriction and birth weight discordance. One of the most extreme scenarios is an intrauterine death of the co-twin resulting from acute or chronic twin-twin transfusion syndrome, which can lead to cerebral damage in the survivor.

It is important to distinguish selective-intrauterine growth restriction from twin-twin transfusion syndrome. In monochorionic twins, marked amniotic fluid volume discordance leads to the diagnosis of twin-twin transfusion syndrome, defined as a maximum vertical pocket of ≥ 8 cm in one sac and ≤ 2 cm in the other sac. It is possible that some of these severely discordant twin pairs also meet the sonographic criteria for twin-twin transfusion syndrome, although weight discordance is not required to make the diagnosis. Monochorionic pregnancies that do not meet

sonographic criteria for twin-twin transfusion syndrome but manifest intrauterine growth restriction of one of the twins are classified as selective-intrauterine growth restriction. The amniotic fluid volume discordances which exists in selective-intrauterine growth restriction, do not reach the level seen in twin-twin transfusion syndrome.

The relationship between twin-twin transfusion syndrome and selective-intrauterine growth restriction is shown in Figure 3 (Russell et al., 2007). When twin-twin transfusion syndrome is noted, intrauterine growth restriction occurs most often (85%) in the donor twin, 7% in the recipient twin, and in 14% of patients in both the donor and the recipient twin (Russell et al., 2007).

Figure 3: Relationship between selective-intrauterine growth restriction and twin-twin transfusion syndrome (Russell et al 2007)



1.3.3 Etiology of intrauterine growth restriction

There are numerous processes that may lead to growth restriction, but in many circumstances there are no attributable causes. The etiology can be broadly divided into 3 categories- Fetal, Maternal and Placental.

Fetal causes include chromosomal anomalies and infections.

Maternal causes can be nutritional, hypoxia related (lung or heart disease), vascular (e.g. pre-eclampsia, chronic hypertension), renal disorders or environmental (e.g. smoking, drugs, infections).

Placental causes include placental insufficiency and cord insertion abnormalities. Placental causes are the most important reason why monozygotic twins have a higher risk of growth restriction and this is explored further.

1.3.3.1 Role of placental insufficiency

Monozygotic twins are known to have a two-fold risk for significant discordance and intrauterine growth restriction compared to dizygotic twins (Gonzalez-Quintero et al., 2003; Hanley et al., 2002). Severe growth discordance in monozygotic twins cannot be explained by genetic factors as these twins share the same genes. Disproportionate allocation of blastomeres, which happens during the twinning process, may be responsible for discordant growth diagnosed in the first trimester (Machin, 1996) as these blastomeres may have been destined to become either trophoblast or embryo. Indirect proof of impaired trophoblastic invasion is supported by the finding of increased resistance in the spiral arteries of the selective-intrauterine growth restriction twin in monozygotic pregnancies discordant for growth. (Matijevic et al., 2002)

Also, placental weight measurements have suggested decreased total placental weight in severely discordant twins relative to concordant or mildly discordant counterparts. This suggests that growth restriction affects placental and fetal growth of both twins, but with different degrees

of severity (Victoria et al., 2001). In this study, vascular thrombotic lesions, infarcts, thromboses of fetal vessels, intraplacental hematomas and perivillous fibrin deposition were also found more frequently in the placentas of lighter, severely discordant twins which could be the aetiology of placental insufficiency. The effect of placental insufficiency is that it can induce redistribution of fetal blood flow with reduced resistance to the brain and increased resistance at the level of peripheral vessels. This can result in overall growth restriction with the sparing of brain.

1.3.3.2 Role of placental territory

Unequal placental sharing has also been implicated in the pathogenesis of severe birth weight discordance and intrauterine growth restriction (Quintero et al., 2003; Hecher et al., 1999; Ville et al., 1995). The concept of individual placental territory defined as the individual placental mass divided by the total placental mass to explain the unequal placental sharing was introduced by Quintero et al (Quintero et al., 2005). In this study, monochorionic placentas with twin-twin transfusion syndrome treated with laser therapy and controls (without twin-twin transfusion syndrome) were analysed by surgical pathology to determine the individual placental territory necessary for survival. Survival occurred with as little as 10% and 14% individual placental territory in non-twin-twin transfusion syndrome and twin-twin transfusion syndrome patients, respectively.

1.3.3.3 Role of cord abnormalities

Abnormalities of the cord, particularly velamentous cord insertion, have also been linked with severe discordance (Gonzalez-Quintero et al., 2003; Hanley et al., 2002; Victoria et al., 2001). The clinical consequences of cord insertion depend on the combination of insertion in any given twin pair. If a monochorionic twin pair has a combination of central and peripheral cord insertions, the centrally inserted twin commands a disproportionate amount of placental parenchyma, whereas the velamentous twin may have a very small territory. Selective-intrauterine growth restriction and

twin-twin transfusion syndrome are more likely to occur with velamentous cord insertion than in appropriately grown monochorionic twins. The odds of an monochorionic twin pregnancy developing twin-twin transfusion syndrome or selective-intrauterine growth restriction was higher in patients with velamentous cord insertion than in non-velamentous cord insertion placentas (Odds ratio =2.23, Confidence Interval: 1.12-4.5).(Martinez J, 2003).

1.3.3.4 Role of arterio-arterial anastomoses

Although traditionally, growth restriction of the donor twin has been attributed to placental insufficiency, recent evidence suggests that vascular anastomoses may also be related to the growth restriction of the donor twin (Ville et al., 1995). The presence, number and type of inter-twin vascular anastomoses have been correlated with growth restriction and birth weight discordance. Blood exchange can take place through two kinds of communications: deep (also known as AV) or superficial arterio-arterial or veno-venous communications. Deep anastomoses involve the sharing of one cotyledon by both twins. Arterio-arterial anastomoses consist of an artery at both ends with both twins pumping blood in opposite directions. Depending on the pressure gradient between the two fetuses and the presence or absence of arterial branches, arterio-arterial anastomoses may behave as functional deep unidirectional communications (Murakoshi et al., 2003). Multiple bidirectional deep anastomoses are more likely to be correlated with severe discordance without twin-twin transfusion syndrome than with twin-twin transfusion syndrome (Bajoria, 1998).

1.3.4 Monitoring intrauterine growth restriction: The value of umbilical artery Doppler velocimetry

Umbilical artery end diastolic velocities first appear around 10 weeks and are always present by 15 weeks. Absence of end diastolic velocity in the 2nd and 3rd trimesters is pathological. Umbilical artery Doppler abnormalities are associated with extensive fetoplacental vascular pathology, which

leads to utero-placental insufficiency and, as a consequence, to chronic fetal hypoxia and growth restriction. Increased resistance in the umbilical artery may be a sign of impaired placental perfusion, and thus reduced diffusion of nutrients and oxygen through placenta.

There is now consistent evidence that umbilical artery Doppler abnormalities such as absent or reversed end-diastolic velocity are predictive of intrauterine growth restriction and puts the fetus at increased risk of adverse perinatal outcome. Two reports also suggested that abnormal fetal aortic velocity waveform is the most significant predictor of minor neurological dysfunction and impaired intellectual development at 7 years of age (Ley et al., 1996a; Ley et al., 1996b).

The use of Doppler ultrasound umbilical artery velocimetry is associated with improvement in birth outcomes in high risk pregnancies by reducing perinatal mortality and stillbirth rate (Alfirevic and Neilson, 1995). There are a few studies investigating the relationship between Doppler velocimetry and fetal outcome in twin pregnancies (Hack et al., 2008; Gratacos et al., 2004a; Gratacos et al., 2004b; Joern and Rath, 2000; Giles et al., 1988) and in only few of these studies, chorionicity was taken into account (Hack et al., 2008; Gratacos et al., 2004a; Gratacos et al., 2004b). Gratacos et al (Gratacos et al., 2004a; Gratacos et al., 2004b) found that the incidence of intermittent absent and/or reversed end-diastolic flow was increased in pregnancies with selective-intrauterine growth restriction. They thereby identified a subgroup with an increased risk of intrauterine death in the lighter twin associated with a neurological damage in the larger twin, the latter even in the absence of intrauterine death of the lighter twin. Hack et al (Hack et al., 2008) showed a slightly increased risk of adverse outcome in cases with at least one abnormal Doppler finding in one or both fetuses during the course of pregnancy.

It is recognised that the absent end-diastolic velocity usually persists in majority of cases and occasionally deteriorates into a pattern of reversed

end-diastolic velocity, the most extreme form of increased vascular resistance in the placental bed. In the absence of intervention, this is usually followed by fetal distress and demise. Optimal management at this stage is a major dilemma for the obstetrician as the substantial majority of twins with absent or reversed end diastolic velocities are diagnosed in the late second or early third trimester.

1.3.5 Effects of Intrauterine growth restriction

Animal studies: Previous animal studies have shown that the brains of animals reared in nutritionally enriched environments have increases in cortical thickness brought about by a denser synaptic network (Nithianantharajah and Hannan, 2006). Suboptimal nutrition during rapid brain growth can affect brain structure and function permanently (Morgane et al., 1993; J, 1986). Effects of early under nutrition on animal brain structure include changes in cell number, growth of the cerebral cortex, and dendritic arborisation (Georgieff, 2007; Dobbing and Sands, 1971)

Short term effects: Intrauterine growth restriction is associated with postnatal occurrence of hypothermia, hypoglycaemia, pulmonary haemorrhage and death in extreme cases. Given the multiple antenatal and postnatal factors, it is often difficult to analyse the effect of intrauterine growth restriction in isolation.

Long term effects on cognition: Prenatal period is a time of rapid brain development, which includes marked changes in cortical folding (Battin et al., 1998), myelination (Counsell et al., 2002), and gray-matter distribution (Isaacs et al., 2001). Birth weight, a marker of prenatal growth therefore correlates with cognition (Richards et al., 2001). Hence if the birth weight is affected for any reason, neurodevelopment may also be affected.

Few studies in agreement with the above hypothesis have shown a negative relationship between intrauterine growth restriction and intelligence (Walker and Marlow, 2008; Geva et al., 2006; O'Keeffe et al.,

2003; Dobbing and Sands, 1971; Babson et al., 1964). Increased risk of cerebral palsy has been shown in small for gestational age infants born at term or moderately preterm (Stanley F, 2000). Differences between children with low birth weight and control children have been shown using a wide range of tests measuring cognitive functions and intelligence quotient (Anderson and Doyle, 2003; Hack et al., 2002).

Long term effects on behavioural problems: There is evidence that lower birth weight increases the risk for childhood psychopathology. Low birth weight children have been reported to be at increased risk of psychiatric disorders such as attention deficit hyperactive disorder (Botting et al., 1997; Gjone and Novik, 1995; McCormick et al., 1990; Szatmari et al., 1990), depressive symptomatology (Frost et al., 1999; Hoy et al., 1992) and behavioural problems (Horwood et al., 1998; Sommerfelt et al., 1996). Low birth weight has also been associated with adult psychiatric outcomes such as schizophrenia (Jones et al., 1998; Cannon et al., 1997; Rifkin et al., 1994). As adult psychiatric outcomes such as depression and schizophrenia are associated with childhood behavioural problems (Van Os et al., 1997; Jones et al., 1994), a causal pathway from low birth weight through child problem behaviour and adult psychiatric outcomes can be predicted.

To conclude, previous studies suggest that infants, who are subjected to growth restriction during the prenatal period, and therefore likely to be deprived of an optimal supply of nutritional substrates, are at risk of impaired neural and cognitive development. However, studies investigating effects of growth restriction in singletons are all complicated by a number of confounding factors. These include parental IQ, education, and social background (Robertson et al., 1992; Hawdon et al., 1990); infant gender (Matte et al., 2001); genetic effects on both birth weight and cognition (Chipuer et al., 1990; Loehlin, 1989); and gestational age (Hutton et al., 1997; Spinillo et al., 1997). All these variables can mediate or modify the link between prenatal growth restriction and subsequent

cognitive skills leading to differences in catch-up growth and psycho-motor development. Even in the best studies, it has been difficult to establish a representative control group. There are difficulties in separating intrauterine and postnatal environmental factors from genetic effects on neurodevelopmental outcome, due to complex interactions among them.

Therefore to assess the true effect of growth restriction, twins with birth weight discordance, especially monozygotic twins have been studied. The next section explores definition, mechanism of birth weight discordance and reviews all the studies which have used the discordant twin model to examine the effects of growth restriction.

1.4 Birth weight discordance

Unlike growth restriction, growth discordance is a complication unique to multiple gestational ages. It is expected that every set of fetuses will be accommodated within a given uterus, as the potential to increase uterine volume and nutritional capacity is limited. In the most extreme situation, the uterine milieu limits adequate growth for all fetuses. In less severe cases, growth is impaired for one fetus which results in birth weight discordance phenomenon. There may be constitutional variation between the twins and therefore the magnitude of the birth weight discordance is important.

1.4.1 Definition and incidence of twin birth weight discordance

Various definitions have been used to define birth weight discordance and three of them have been used in the past studies (Blickstein and Lancet, 1988). The first was an “absolute” definition where the absolute birth weight difference is taken. The major limitation of this definition is that it assigns the same degree of discordance to a twin pair weighing 1500/1000g and to a pair weighing 3000/2500g.

The second definition used is the “percent” definition, where the birth weight discrepancy is calculated as a percentage of the larger infant. This definition is by far the most commonly used definition in practice.

However, even this does not refer to the actual size of the siblings. So, it may assign the same degree of discordance to a twin pair weighing 1500/1200g and to a pair weighing 3000/2400g.

The third definition is the “statistically derived” definition, which refers to the extremes of the distribution of discordance values (presented by the “percent” definition), such as the 95th percentile or one/two standard deviations above/below the mean. Despite its potential statistical relevance a large sample size is needed to derive these values which may not be possible.

Difference in birth weight standard deviation score (SDS) for sex and gestational age from singleton norms has been used to analyse catch up growth in few studies (Estourgie-van Burk et al., 2009; Ong et al., 2000). Standard deviation scores are derived by subtracting the population mean from an individual raw score and then dividing the difference by the population standard deviation. Catch-up growth is then calculated as the SDS for weight at the age of assessment minus the SDS for weight at birth. For example, when weight at 2 years is 0.5 SD below the mean reference value for that age and birth weight is 1.5 SD below the mean reference value, then the catch-up growth is +1 SDS. However, as twins usually have a low birth weight compared to singletons, using standard deviation scores may overestimate growth restriction.

Fifteen to twenty-nine percent of twin pregnancies are complicated by birth weight discordance, but this figure is dependent on the definition used (Cheung et al., 1995). Using the percent definition, about 75% of twins show < 15% discordance, an additional 20% are 15–25% discordant and only about 5% of twins are more than 25% discordant. Such differences are referred to as concordant, mildly discordant and severely discordant, respectively (Blickstein, 1991). The prevalence of monozygotic twins with birth weight discordance of more than 25% is around 11-19% (Valsky et al., 2010)

1.4.2 Mechanisms of twin growth discordance

1) Constitutional/Normal Variation: Some degree of discordance is likely to simply represent normal variation between siblings. Males weigh more than females, a difference possibly due to genotypic and phenotypic gender differences (Blumrosen et al., 2002). It has also been shown that the presence of a male fetus may alter the uterine growth due to male anabolic environment, and females in unlike-sex pairs tend to have higher birth weights compared with females in like-sexed pairs (Glinianaia et al., 1998). However, a later study by the Belgian East Flanders Prospective Twin Survey showed that the birth weight of the female fetus of the pair

was not influenced by the male co-twin but, the female twin enhances to a slight degree the birth weight of her male co-twin by prolonging the gestational age for a few days. (Derom et al., 2005)

2) Adaptive growth restriction: A possible reason for relative growth restriction in one twin is due to an adaptive measure of the uterus to promote maturity. The hypothesis is that within a limited uterine environment, a combination of one larger and one lighter twin may reduce uterine over-distension and thereby babies will be delivered at an advanced gestational age. Studies have shown that the mean gestational age of discordant pairs delivered spontaneously was significantly higher across the entire range of total birth weight intervals except for the top intervals (Blickstein and Goldman, 2003). The more favourable the uterine milieu, the lower the likelihood of discordant growth (Blickstein et al., 2002).

3) Placental origin of discordance: Growth discordance in monozygotic twins can be caused by placental abnormalities and some aspects have been discussed previously in the section of intrauterine growth restriction. Correlation between placental function and discordance show that growth discordance of twins exposed to the same maternal environment may be due to variations in fetal concentrations of insulin-like growth factor-I (IGF-I), IGF-II and insulin-like growth factor binding protein-1 (Westwood et al., 2001). The insulin-like growth factors and their binding proteins are essential for fetal growth and development. This is supported by studies using mice (Baker et al., 1993; Liu et al., 1993) in which ablation of either the IGF-I or IGF-II gene resulted in embryonic and neonatal mice becoming 40% lighter than their normal littermates. The IGF-II-deficient mice also had reduced placental growth but survived normally, whereas the mice lacking IGF-I had increased neonatal death. In humans, birth weight has been reported to correlate with serum IGF-I concentrations (Klauwer et al., 1997). Previous studies have shown that IGF-I concentrations are decreased in utero and at

birth in intrauterine growth restricted fetuses (Ogilvy-Stuart et al., 1998) and are increased in large for gestational age newborns (Giudice et al., 1995).

1.4.3 Postnatal complications of birth weight discordant twins

During the neonatal period, birth weight discordant twins are at high risk of needing medical care in view of either prematurity or growth restriction. Due to various complications in the neonatal period, birth weight discordance is thought to be a significant contributor to neonatal mortality and morbidity especially in the presence of increasing birth weight discordance (Branum and Schoendorf 2003; Demissie et al. 2002; Blickstein et al. 1987). Amaru et al (2004) have shown that birth weight discordance is independently associated with adverse neonatal outcomes. It is however not clear whether increased perinatal mortality and morbidity in twins with large birth weight discordance are mainly attributed to preterm birth and fetal growth restriction in the lighter twin (Cooperstock et al., 2000) or whether birth weight discordance itself is an independent factor for poor perinatal outcomes. However not all birth weight discordant twins have adverse neonatal outcomes (Cohen et al., 2001; Fraser et al., 1994).

It is therefore vital to establish that birth weight discordant twins participating in studies evaluating long term effects of growth restriction on cognition do not have any neurological impairment as a sequelae of complications in the neonatal period or due to any childhood illness.

1.4.4 Review of the Literature on long-term outcomes of birth weight discordant twins

Birth weight discordant twins provide a useful model to evaluate effects of growth restriction. Many studies have used this model in the past. There are studies investigating whether birth weight discordance and the degree of discordance (which will depend on growth restriction in one twin) itself are independent risk factors affecting neurodevelopmental outcomes. A literature search was performed using the key words twins, birth weight,

discordance, neurodevelopment, intrauterine growth restriction, and cognition in various combinations using the search engine Medline for all articles published from 1988 to 2007. A cut off of 20 years (1988 -2007) was chosen as obstetric and neonatal care has significantly changed in the last 2 decades. Four studies published in the last 20 years were identified and critically appraised. A further Medline search was undertaken in January 2012 and Scopus database was also used to identify articles which had cited the above 4 articles. Two additional articles were found investigating the long term outcome of birth weight discordant twins which have also been critically appraised. All these articles have been summarised in Table 3.

Critical appraisal of 6 studies

1) The effect of birth weight on childhood cognitive development in a middle-income country

Florencia Torche and Ghislaine Echevarra. International Journal of Epidemiology 2011;40:1008–1018

Clinical question: To use twin models to examine the hypothesis that in utero growth has a detrimental impact on cognitive development in childhood.

Methods

Type of study: Retrospective cohort study

Sample size calculation: No

Losses to follow up: No

Participants and location: A total of 2474 twin pairs born between 1998 and 1999 in Chile were tested around the age of 9 years.

Data collection: Birth registry information on birth weight was matched with standardized Maths and Spanish test scores for all twins.

Results: Lower birth weight was strongly associated with lower test scores especially in children whose mothers have less education relative to those who were well educated. This effect varied across family socioeconomic status and was seen strongly in the disadvantaged family than the affluent ones.

Strengths: The study was based in a middle-income country with wide social inequality. Twin fixed-effects models were used to estimate the causal effect of intra-uterine growth on test scores.

Weakness:

Gestation: No description on how the gestational age was determined.

Bias: The total number of births eligible for inclusion was not reported and therefore the extent of selection bias due to exclusion of children with incomplete information cannot be estimated.

Confounding factors: Whether any pregnancies were complicated by twin to twin transfusion syndrome, significant past medical history of the participants which can affect cognition was not mentioned.

Other comments: Zygosity status was estimated and not measured. No data on chorionicity was available. Cognition was based on the use of maths and Spanish fourth-grade results only. As intrauterine growth restriction differentially affects cognitive domains, it is vital that all cognitive data is reported.

2) The Effect of Intrauterine Growth on Verbal IQ Scores in Childhood: A Study of Monozygotic Twins

Caroline J. Edmonds, Elizabeth B. Isaacs, Tim J. Cole, Mary Haslinger Rogers, Julie Lanigan, Atul Singhal, Toni Birbara, Paul Gringras, Jane Denton and Alan Lucas. *Pediatrics* 2010;126;1095-1101

Clinical question: To examine whether suboptimal intrauterine growth relates to impaired cognitive outcome. This was done by relating within-pair differences in birth weight of monozygotic twins to the differences in IQ scores.

Methods

Type of study: Retrospective cohort study

Sample size calculation: No

Participants and location: A total of 71 monozygotic twin pairs aged between 7 years 11 months and 17 years 3 months participated in this study. They were recruited from Multiple Birth Foundation and other twin support groups.

Data collection: The Wechsler Intelligence Scale for Children was administered, and verbal IQ and performance IQ scores were calculated.

Results: Verbal IQ was affected in the lighter twins with a mean advantage of half a standard deviation for heavier twins. In twin pairs with minimal birth weight discordance, heavier twins had a lower verbal IQ scores than their lighter twins.

Strengths: Zygosity was determined using molecular genetics methods. The number of participants was large compared to other studies. Preterm twins below 32 weeks were excluded.

Weakness:

Gestation: No description on how the gestational age was determined.

Exclusion criteria: Although exclusion criteria states that twins with twin to twin transfusion syndrome were excluded, 6 twin pairs with this condition apparently participated in the study.

Bias: Possibility of sampling bias as recruitment was made via advertisements and newsletters resulting in only motivated parents/children agreeing to participate. The setting where the cognitive tests were administered was not described as this may affect the scores.

Confounding factors: Significant past medical history which may impact cognition was not ascertained.

Blinding: The assessors were not blinded to the lighter twin.

Reliability: This study was not population based and cognitive tests were administered by different people but there were no comments on inter

observer variability.

Other comments: The age at assessment was quite wide ranging from 7 years to 17 years. Chorionicity was not ascertained. A short form of Wechsler Intelligence scale for children was used to calculate the IQ.

3) Influence of intrauterine and extra uterine growth on neurodevelopmental outcome of monozygotic twins

R.K. Reolon¹, N.T. Rotta¹, M. Agranonik¹, A.A. Moura da Silva² and M.Z. Goldani. Brazilian Journal of Medical and Biological Research (2008) 41: 694-699

Clinical question: To determine the influence of intrauterine and early postnatal growth on neurocognitive development of monozygotic twins, using intrapair and interpair differences in anthropometric measurements collected at birth and at the corrected age of 12 to 42 months.

Methods

Type of study: Retrospective cohort study

Sample size calculation: Yes

Participants and location: A total of 601 twin sets born in Porto Alegre between January 2000 and September 2002 were identified from the Brazilian Information System. 36 monozygotic twin pairs participated in the study.

Data collection: Bayley Scales of Infant Development, 2nd edition was used to measure neurodevelopmental outcome.

Results: No effect of intrauterine growth was found on cognition and only postnatal head growth was associated with mental but not with motor outcomes. An increase of 1 cm in current head circumference of one twin compared to the other was associated with 3.2 points higher in Mental Developmental Index.

Strengths: Zygosity and gestational age were determined. The assessors were not aware of the birth weight of the twins. A standardized neurological examination was performed to determine the presence of neurosensory impairment by a neuropsychiatrician.

Weakness:

Bias: Possibility of sampling bias as only 36 twin pairs recruited out of possible 65 twin pairs. The setting where the cognitive tests were administered was not described as this may affect the scores.

Reliability: This study was not population based and cognitive tests were administered by different people but there was no description on how inter observer variability was assessed.

Other comments: Canadian-based intrauterine growth curves were used as standards due to lack of Brazilian curves of in twin gestational ages. This may have underestimated fetal growth. The extent of birth weight discordance was not mentioned.

4) Cognitive and verbal development of discordant twins without neurological morbidity

M Bellido-Gonzalez, S Defior-Citoler, M Diaz-Lopez

Journal of Reproductive and Infant Psychology, VOL. 25, NO. 2, May 2007, pp. 161–168

Clinical question: To examine the early cognitive and verbal development of discordant twins without neurologic morbidity.

Methods

Type of study: Prospective cohort study

Sample size calculation: No

Losses to follow up: Yes

Participants and location: Twins with a discordant birth weight of 15% or more were selected. They assessed these twins at four time points: at

birth, at 1 year, at 2 years and at 4 years. The study was conducted at the Virgen de las Nieves' Hospital, Granada, Spain.

Data collection: The Bayley Scales were used to assess children at the age of 1 and 2 years. The Kohen-Raz System was used to score items on the mental scale in five areas: eye/hand coordination, manipulation, conceptual ability, imitation/comprehension, and vocalization/socialization. At 4 years of age the children were evaluated with the McCarthy Scales of Children's Abilities.

Results: The differences in height, weight and head circumference persisted at the age of 1 year. At 2 years, differences were only found in the weight while no statistically significant differences were found at the age of 4 years for any of the growth parameters.

Regarding cognition, at 1 year of age, the larger twins scored significantly higher in cognitive skills with a median difference of 23 points ($p < 0.01$). At 2 years of age, the difference was 18 points and remained significant ($p < 0.01$). At 4 years of age, the General Cognitive Index showed median scores to be 24 points higher among the larger twins ($p < 0.01$). The larger twins also scored higher on both subscales of verbal skills at age 1 and 4 years ($p < 0.05$).

Strengths: This was a prospective study and no children with neurological morbidity were included. This was a longitudinal follow-up of the same group of twins by measuring at four time points of age. Clear inclusion and exclusion criteria were defined and birth weight centiles were also noted.

Weakness

Gestation: No description on how the gestational age was determined.

Bias: Possibility of sampling bias as only single centre twins included and attrition bias as few twins dropped out of the study.

Blinding: The assessors were not blinded to the lighter twin.

Reliability: Psychological tests were administered by different people but there was no description of inter observer variability.

Other comments: This study had a very small sample size with only 9 pairs evaluated as 2 twin pairs were lost for follow-up. This study showed a huge difference in developmental scores between the birth weight discordant twins and the results are markedly different from similar studies.

5) Developmental outcome of discordant premature twins at 3 years

T Goyen, M Veddovi, K Lui

Early Human Development 73 (2003) 27–37

Clinical question: Outcome of preterm twins discordant for birth weight at 3 years in order to examine the role of intrauterine growth restriction on the developing infant compared with the corresponding co-twin

Methods

Type of study: Prospective cohort study

Sample size calculation: No

Losses to follow up: Yes

Participants and location: Twin pairs with >15% birth weight discordance and with one or both twins below 1500 grams who were born between 1987 and 1994 at the Westmead Hospital, Australia. A control group of non-discordant twin pairs who weighed below 1500 grams were also followed up in the same study period.

Data collection: Follow- up by a multidisciplinary team at 4, 8 and 12 months corrected age and 3 years. At 3 years of age, growth parameters (weight, height and head circumference) for all twins were measured and

neurodevelopmental outcome determined. Development was assessed using the Griffiths mental development scales.

Results: In paired comparisons, the lighter twins at birth remained lighter at the time of assessment and had a slight lower Griffiths' developmental quotient than the heavier co-twins [mean 100 versus 104 $p=0.002$].

Strengths of the study: This was a prospective study and a separate control group was recruited to compare results. Clear inclusion and exclusion criteria were defined. Twins with cerebral palsy were not included while assessing development.

Weakness:

Gestation: No description on how the gestational age was determined.

Bias: Possibility of sampling bias as only single centre twins included and attrition bias as few twins dropped out of the study.

Reliability: Psychological tests were administered by different people but there was no description of inter observer variability.

Other comments: A cut-off of 15% birth weight discordance between twins might not have ascertained true effects of growth restriction. There were only 7 discordant monochorionic twins in the study.

6) Early developmental progress of preterm twins discordant for birth weight and risk

A. Stauffer, W Burns, K Burns, J Melamed, C Herman
Acta Genet Med Gemellol 37:81-87 (1988)

Clinical question: Examine the developmental outcomes of birth weight discordant premature twins.

Methods

Type of study: Prospective cohort study

Sample size calculation: No

Losses to follow up: Yes

Participants and location: A total of 45 twin pairs born between 26 and 37 weeks were followed up at birth, 3, 6, 9, 12, 24 and 36 months at the North Western Memorial Hospital Developmental Evaluation clinic between 1979 and 1983.

Data collection: The birth weight discordance between the twins was 15%. All the twins in this cohort were classified as high-risk with many medical complications by the Postnatal Complications Scale. Brazelton exams and Bayley exams were administered by psychologists and graduate students.

Results: No difference in the developmental outcome in discordant twin pairs was found but prematurity affected developmental outcomes.

Strengths: This was a prospective study and the gestational age was accurately assessed by Dubowitz exam at the delivery. Twins were serially followed up to assess their development.

Weakness:

Bias: Possibility of sampling bias as only single centre twins included and attrition bias as few twins dropped out of the study.

Confounding factors: Postnatal complications that may impact on cognition as these twins were classified as high risk infants.

Blinding: The assessors were not blinded to the lighter twin.

Reliability: This study was not population based and psychological tests were administered by different people but there was no description on inter observer variability.

Other comments: Not all twins were examined at all ages as the follow up rates decreased as the twins got older. Zygosity or chorionicity was not

determined. True growth restriction is usually diagnosed at birth weight discordance of 20% and above. Therefore a cut-off of 15% might have included twins with minimal growth restriction.

Author, date	Gestation	BWD	Outcome measures	Age at assessment	Numbers	Key Results	Weakness
Torche, 2011	Mean 36.2 weeks	Not available	Math and Spanish test scores	4 years	2474 twin pairs	Lower birth weight was strongly associated with lower test scores	Sampling bias and all cognitive data was not reported
Edmonds 2010	Mean 36.5 weeks	Not available	Short form of the Wechsler Intelligence Scale, 3 rd edition	7 years 11months to 17years 3months	71 Monozygotic twin pairs	Within-pair difference in birth weight correlated with within pair-difference in verbal IQ scores.	Sampling bias and wide age range at assessment
Reolan, 2008	Mean-35.6 weeks	Not available	Bayley scales of infant development-II	Between 12-42 months	36 Monozygotic twin pairs	Significant association between postnatal head growth and MDI	Sampling bias & overestimation of growth restriction
Bellido-Gonzalez 2007	7 – term 4- preterm	≥15% (15-41%)	Bayley scales of infant development-II	0, 1, 2 & 4 years	11 twin pairs, 10 Monozygotic 7Monochorionic	Growth difference disappears but cognitive differences persist at age 4	Small sample size. Biologically difficult to explain results
Goyen, 2003	27 to 34 weeks	>15%	Griffiths developmental scales	4,8,12 months & 3 years of corrected age	20 twin pairs 7 Monochorionic	Lower Griffiths Quotient in lighter twin but within normal range for age	Data from single centre and discordance of 15% used as cut off
Stauffer, 1988	27 to 46 weeks	>15%	Bayley scales and Stanford Binet scales	Term,3,6,9,12,24,36 months	45 twin pairs	Preterm twins had lower mental scores. No difference in discordant twins	Inter-observer variability and discordance of 15% used as cut off

Table 3: Shows summary of the 6 critically appraised studies

BWD- Birth weight discordance, IQ- Intelligence quotient, MDI- Mental developmental index

1.4.5 Conclusions and rationale for this study

Although the long term effect of intrauterine growth restriction has been assessed in a number of singleton studies, they all suffer from multiple confounding effects. A model that involves monozygotic twins can markedly reduce the effect of confounders as they share gestational age length, gender, family background, parental IQ, gender, and genetic influences on growth and cognition. However, most twin studies investigating the influence of birth weight on cognition described above have several limitations. Some studies have analysed the data as difference in values within pairs. This analysis accounts for within-pair effects (characteristics such as different fetal nutrient supply lines) but not for between-pair effects (shared characteristics such as socioeconomic status, maternal diet, maternal smoking, pregnancy risk factors and genes for monozygotic twins). Therefore, analysing data as difference in values within pairs does not provide information on both shared and individual factors. This information would be available by using more specialised regression methods for twins like mixed model estimation or generalised estimating equations (Carlin et al., 2005). Hence we designed this study to address the limitations of the previous studies and used generalised estimating equations as it provides a more robust estimation of error for population based data than mixed models.

We chose monozygotic twins for 2 reasons. Monozygotic twins are at substantially greater risk of intrauterine growth restriction (Sebire et al., 1997) and therefore weight discrepancies are larger and the lighter twin is likely to be genuinely growth restricted. Monozygotic twins can therefore provide a better model to evaluate the cognitive effects of growth restriction and may therefore aid antenatal care in pregnancies complicated by intrauterine growth restriction which is of global medical and social importance.

Another reason for choosing monozygotic twins was the lack of studies investigating the long term neurodevelopmental outcomes of birth weight

discordant monochorionic twins. The study results could help advancing this under researched topic. The information provided by the study might be of importance for health professionals and parents in planning antenatal management of monochorionic twin pregnancies and the future care of the children from such pregnancies.

We chose monochorionic twins with birth weight discordance of 20% and above as per the American College of Obstetricians and Gynaecologists' report which showed that intrauterine growth restriction in twins is usually diagnosed when there is discordance in estimated fetal weight of >20% between the twins. Cognitive scores obtained from the study might provide a proxy for early cognitive development and subsequent educational attainment.

2. Clinical study: Aims and Hypotheses

2.1 Aims

To assess the growth and neurocognitive outcomes in early childhood for monozygotic twins born with $\geq 20\%$ inter-twin birth weight discordance in the Northern region of England. The study is designed to determine the cognitive effects of restricted intrauterine growth using monozygotic twin pair's model.

2.2 Specific Hypotheses to be tested

- 1) There is a difference in cognitive outcome within pairs of discordant twins i.e. Growth restriction affects cognition
- 2) Differences in cognitive outcome between pairs of discordant twins are correlated with the degree of discordance.
- 3) Differences in cognitive outcomes between pairs of discordant twins remain significant even when accounting for the degree of fetal concern i.e. the results hold true when the analysis is conducted in twins where there was no evidence of fetal compromise due to abnormal umbilical artery Doppler waveforms. (Sensitivity analysis)
- 4) There will be differences in size and behaviour problems between the twin pairs at the time of assessment.

3. Methods

3.1 Study design and Subjects

This was a retrospective cohort study of monochorionic twins and all twin births were identified using records held by the Northern Survey of Twins and Multiple Pregnancy (NorSTAMP).

3.2 Inclusion criteria

Monochorionic twins born between the years 2000-2004 and alive at the time of the study were eligible to participate.

3.3 Exclusion criteria

Pairs where one twin had a neurosensory impairments (Cerebral palsy, deafness and blindness) were excluded from the analyses as we wanted to understand the effect of birth weight discordance and thereby the effect of intrauterine growth restriction on cognition. Children with the above impairments would not have been able to undertake the several tests in the British Ability Scales. Moreover, the British Ability Scales standardisation sample did not include any children with neurological impairment and was not designed to test cognition in these children.

3.4 Participants

Gestation was determined antenatally on the ultrasound scan and the median gestational age was 12 weeks (Range 7 -22 weeks). Chorionicity was determined on antenatal scan at a median gestational age of 13 weeks (Range 11-22 weeks). The NorSTAMP is notified of the diagnosis of chorionicity upon receipt of multiple pregnancy notification cards which are sent when a woman with a multiple pregnancy is booked into a hospital. NorSTAMP is then notified of the final chorionicity diagnosis at delivery when the delivery forms are sent. A copy of the placental pathology report is also sent to NorSTAMP where available. For this study group, a copy of placental report confirming monochorionicity was available for 39 (76%) twin pairs.

Details of any deaths which occurred within the first year of life were obtained from the Perinatal Mortality Survey (PMS) database. PMS, also part of RMSO, collects information on all infant deaths as part of its routine data capture procedure. This is a well validated database (Northern Regional Health Authority Coordinating Group, 1984) and records are complete with accurate data capturing.

The NHS tracing system was then used to identify the details of surviving children including details of their general practitioner. The General Practitioner (GP) was subsequently contacted to establish absolutely that both the twins were alive before approaching parents. Thus, all the surviving twins in this group were identified and approached.

The GP was also informed about the study at the same time and contact details to approach parents were confirmed. Medical history including diagnosis, medications and any ongoing medical follow-up was also ascertained.

Parents were then approached by:

- (1) The principal obstetrician involved in the antenatal management of these children (NorSTAMP lead at each unit) or
- (2) The lead paediatrician if they are currently under follow-up.

Those parents who agreed to participate in the study were asked to return the information form (Appendix) in the enclosed prepaid envelope with their contact details. The Principal Investigator (PI) then contacted them and an appointment was made to assess the twins.

3.5 Measures

3.5.1 Information obtained from case records and parents

The following data were collected through interview with parents and case records:

- Birth weight
- Sex
- Gestational age at birth
- Inter-twin birth weight discordance: The percentage of birth weight discordance was calculated as follows:
[(Weight of the heavier twin at birth – Weight of the lighter twin at birth/Weight of the heavier twin at birth)] X100
- Chorionicity and when it was diagnosed.
- Pregnancy outcome: reason for preterm birth, intrauterine growth restriction and its cause, any congenital anomalies and admission to neonatal unit.
- Type of delivery: Caesarean section or spontaneous/induced normal delivery
- Maternal information - age, parity, obstetric history, medical/social history and smoking history
- Umbilical artery Doppler waveforms ultrasound parameters along with the gestational age. This was obtained after linking with a fetal medicine database and looking through antenatal records. Umbilical artery Doppler waveforms were considered abnormal if absent or reversed end diastolic flow was detected. The gestational age at which this abnormality was first seen were also noted.

- Sociodemographic information from parents which included educational level and occupation of both parents, language spoken at home, and family structure
- Hearing and vision problems (If applicable): The type of hearing and vision problems and its management were noted

3.5.2 Direct assessment - Cognition

Cognitive function was assessed using the revised British Ability Scales: Second Edition (BASII). The BAS II is a battery of individually administered tests to measure cognitive abilities and educational achievement. It is suitable for use in children from age 2 years and 6 months to 17 years and 11 months. The battery provides a comprehensive means of assessing different aspects of children's intellectual function.

The British Ability Scales is considered to be a reliable measure of cognitive functioning over a wide age range. These are divided into two batteries: Early Years and School Age. The BAS II comprises several short tests, each of which is used to assess particular types of knowledge, thinking and skills. Cognitive scales measure mental abilities that are the outcome of interaction between a child's innate capabilities and his or her experiences, both at home and at school. There are also some tests known as the Achievement scales that measures educational level, number skills, spelling and word reading. These scales were not used in this study due to time constraints of the study and probable risk of participants fatigue due to prolonged examination.

Reason for choosing British Ability Scales II

The twins who were participating in this study were between the ages of 4 and 9 years and were hypothesised to have subtle difficulties (We expected only few to have a severe cognitive or motor impairment). Therefore it was appropriate to use cognitive assessment tools that had not included children with impairments in their standardisation samples.

The main reason for choosing British Ability Scales II was because it can be used for the cognitive assessment of children from the age of 2.5 years and the standardisation sample did not include children with neurological impairments. Also, British Ability Scales II provides separate normed scores for verbal and non-verbal abilities, and it includes scales for speed of information processing and recall of digits which are used to assess memory and distractibility.

Apart from this, it has norms that are derived from assessments of over 1600 individuals drawn from over 200 educational establishments across England, Wales, Scotland and Northern Ireland and the sampling paid detailed attention to ethnic-and gender-representativeness. Finally, in order to minimise testing times and reduce the risk of fatigue or demoralisation, the British Ability Scales has age-related start and stop points which enables the assessment to be completed as soon as sufficient information is gathered.

Organisation of BAS II

The BAS II consists of 2 batteries: Early year's battery and School age battery. Early year's battery is appropriate for preschool children while School age battery is appropriate for school years. The Early years battery is composed of cognitive scales while the school age battery comprises both cognitive and achievement scales. The Early year's battery scale has attractive artwork and flexible objects to assess reasoning, perception and memory, along with understanding of basic quantitative concepts. The school age battery includes a variety of scales that assess reasoning, perception, processing speed and memory using numerical, verbal and figural methods.

3.5.2.1 The Cognitive Scales

The cognitive scales are designed to assess clearly identifiable abilities that are important for learning and educational performance. These scales are divided into 2 groups. The first set are known as 'Core Scales' and they

contribute to the General Conceptual Ability score (General Conceptual Ability). The second set is known as the Diagnostic scales and they provide additional information on specific abilities.

The core scales are further subdivided into three clusters that relate to verbal ability, non-verbal reasoning and spatial ability. The total score is made up as the calculated mean of these 3 clusters. This composite score of cognitive element reflects general conceptual and reasoning abilities. Each cluster consists of two subtests. The cluster scores measures aspects of the general abilities involving particular type of information while the individual scale scores cover a diversity of well defined specific abilities. The BAS has an ability score (general conceptual ability, General Conceptual Ability) standardised to a mean of 100 and an SD of 15. The verbal and non-verbal scales are generally administered alternately to provide regular variation in the nature of the tasks the child has to do.

Early years Battery

There are 2 levels in this battery; the lower level is suitable for ages 2:6 to 3:5 years whilst the upper level covers ages 3:6 to 5:11 years. The latter was used in this study. Cluster scores and some more challenging scales are introduced at the upper level. The composition of early year's battery is shown in Figure 4

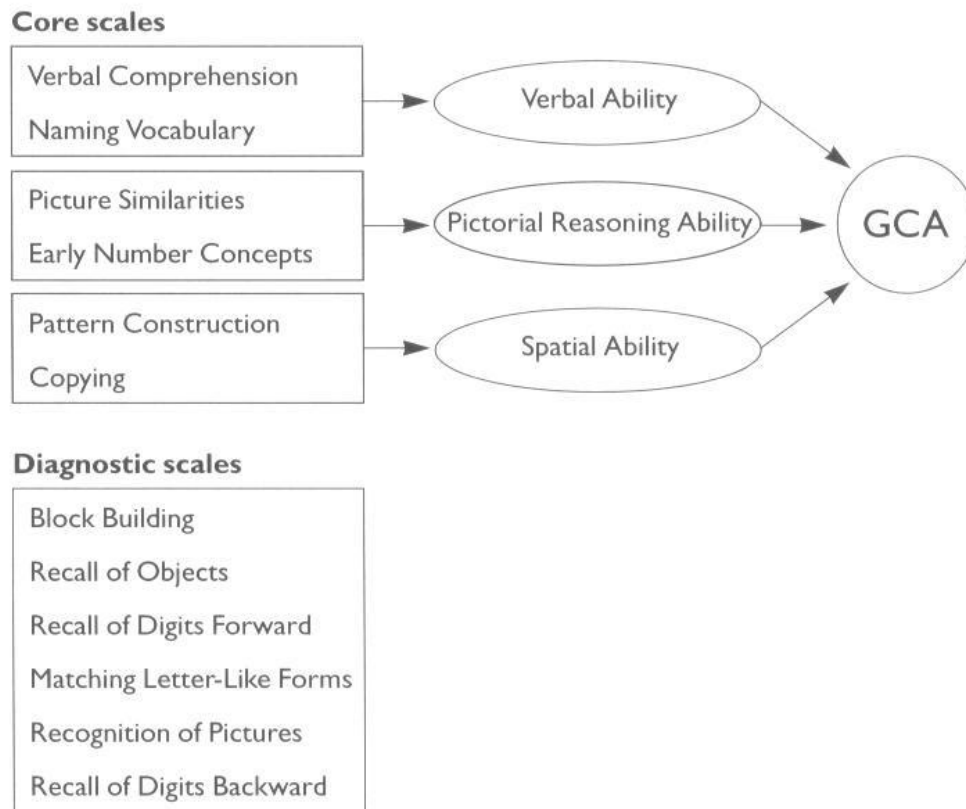


Figure 4: Composition of Early year's battery

School age battery

This has only one level which covers ages from 6 years to 17:11 years. The composition of School age battery is shown in Figure 5

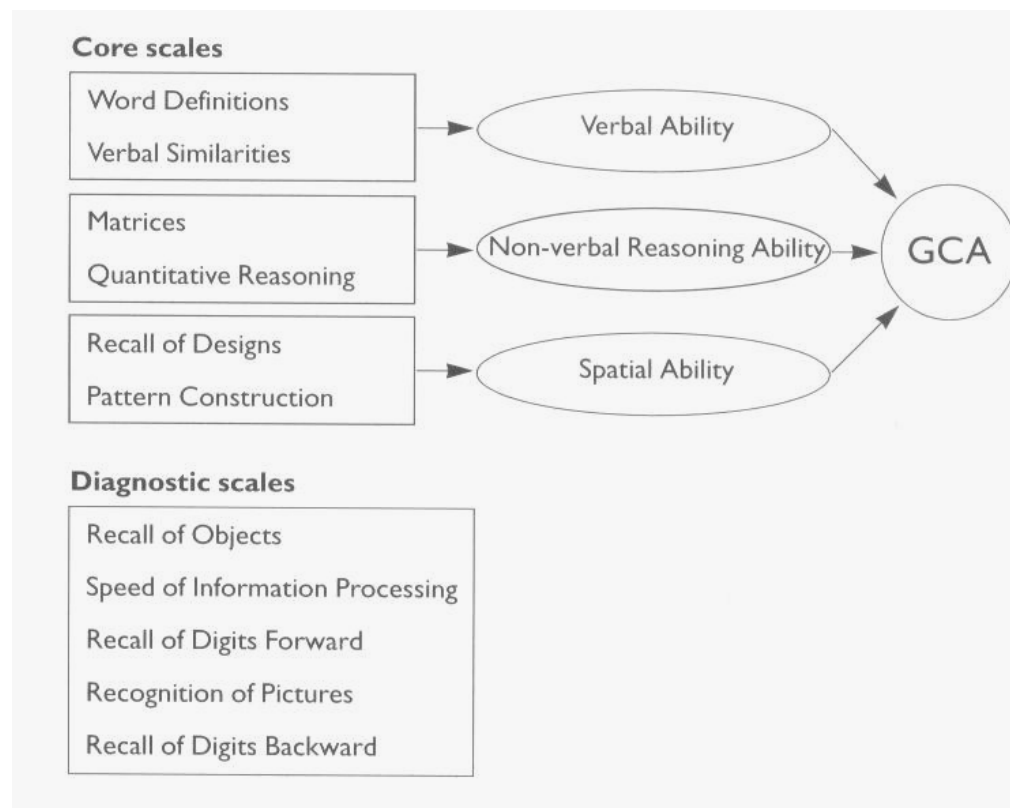


Figure 5: Composition of School age battery

3.5.2.1.1 Item Selection

The aim of BAS II is to obtain an accurate score by administering adequate number of items appropriate for the child's level of ability. Items that are moderately difficult for the child provide the most information about his or her capability. Little can be learned from administering items that are extremely easy or extremely difficult.

To meet this aim, children of different ages have different starting and stopping points on most of the scales. In this way, the child will be presented with items that are likely to be appropriate for their level of ability. The starting and stopping points are flexible which allowed the PI to adapt the item administration sequence in response to the child's performance.

In contrast to the traditional system of basils and ceilings, the BAS 2 method does not assume that the child would pass all items before a starting point or fail all items after a usual stopping point. Instead, the estimate of the child's ability is based on his or her performance on the targeted set of items. As different children take sets of items that span different ranges of difficulty, their raw scores cannot be directly compared with one and other. Therefore, raw scores are first converted to ability scores by using tables. The ability score reflects both the number of items the child answered correctly and the difficulty of the items taken. For example, a raw score of 5 on a set of easy items might correspond to an ability score of 30, whereas a raw score of 5 on a set of difficult items might correspond to an ability score of 115. Ability scores are like raw scores in that they reflect the absolute level of the child's performance but they are not norm' referenced scores.

The general principle of BAS 2 is based on the fact that for an accurate measurement of the ability, the administrator should attempt to present a set of items on which the child would have at least 3 passes and at least 3 failures.

The starting point and decision point for each age are designated on the record booklet by an arrow symbol and an arrow symbol followed by a question mark respectively. If the child failed a string of items between the starting point and the decision point, an alternative stopping point rule was applied which was designated by a stop sign on the booklet.

Starting Points

These have been chosen so that most children will find the initial items of a scale fairly easy or only moderately difficult and will pass several of them. If the administrator suspects that the child will have difficulty with the initial items at the normal starting point for the child's age, an earlier starting point was used.

Decision points

The PI presented all items up to the decision point for the child's age. If the child had passed and failed at least 3 items from all the items administered the scale was stopped. However, if the child had failed less than 3 items the administration of more difficult items was continued until the next decision point. If the child had failed 3 items by then, testing was stopped. However, if the child had less than 3 failures testing was continued on. If the child failed to pass 3 items, an earlier starting point was chosen. This usually had easier items and was administered in a forward sequence. All items were administered in blocks and within each block the items were given in a forward sequence. A block is usually defined by a starting point and a decision point. However, if the child had dropped back to an earlier starting point, the end of this block of items would coincide with the child's original starting point. Once a decision was made to administer a block of items, all of the items up to the end of the block were given unless the child reached an alternative starting point.

Alternative stopping points

Usually, all items up to the appropriate decision points were administered. However, on certain occasions when the child failed so many items in

succession that was not valuable, an alternative stopping point rule was followed according to the BAS 2 manual. The alternative stopping point rule is based on failure of a specified number of consecutive items or failure of certain proportion of a set of consecutive items. If an alternative point was reached, harder items were not administered. Generally this rule was applied when the child had passed at least 3 items but then failed the specified number of consecutive items. If the child failed the specified number of consecutive items but had not passed 3 items, the PI dropped back to an earlier starting point. Whenever a child encountered an alternative stopping point, the remaining items up to the next decision point were scored as if they had been given and failed.

3.5.2.1.2 Ensuring that the child understands the task

To make a valid inference about a child's ability from his or her performance, the PI felt that we must be confident that the child understood the nature of the task and what he or she was being asked to do. Children may obtain low scores simply because they misunderstood the instructions. Four methods have already been incorporated into the BAS2 administration procedures to ensure that the child understands the instructions. They are:

- Repeating or rephrasing the directions
- Demonstration the task with examples
- Providing additional instruction through teaching after failure on designated items
- Questioning or encouraging more elaborate responses

Repetition of directions

The PI followed the instructions on repeating questions. When a child asked for repeating the question, the PI rephrased the task and no additional information was provided. In most scales a specific question was repeated. However, in certain short term memory scales, for example, recall of digits backwards and forwards and the timed scales such as speed

of information processing and pattern construction, repetition was not allowed.

Examples

A number of scales started with examples that clarified the task. Examples were not scored and usually incorporated more elaborate instructions. The PI generally had the opportunity to repeat the instructions until the child understood the task before proceeding to the scored items.

Teaching

This was done after completion of examples. The record booklet and the manual clearly identified examples of items designed for teaching. The purpose of teaching was to provide additional instruction after the child had failed one of the initial items of the scale in order to help the child do as well as they can on the later items. As the first items were easier, failure on one of them may reflect a mere misunderstanding of the instructions. Teaching usually included repeating and rephrasing the question, providing clues and on some occasions demonstrating or saying the correct response.

Teaching after failure on a scored item does not affect the child's score on that item. If the child failed an item on which teaching is permitted, the PI scored the item as failure and then attempted to guide the child towards the correct response.

The sole purpose of teaching according to BAS2 is to help the child do as well as possible on subsequent items.

Questioning

Children who occasionally gave responses that were of borderline quality or too brief for the PI to evaluate, were asked to elaborate or give further explanation. The child was not given clues nor directed towards the correct

solution. This was done in a non threatening manner and without making the child feel that the first response was entirely wrong.

3.5.2.1.3 Timing

Most of the BAS2 scales are untimed. Only 2 scales, speed of information processing and pattern construction incorporate the response time in scoring. The 5 memory scales (recall of designs, recall of digits forward, recall of digits backward, recall of objects, and recognition of pictures) required timing to control the exposure to each stimulus and, in the case of recall of objects, the time allowed for recall. Scales that required timing were indicated with a clock symbol on the record booklet. A stopwatch (Tissot, 2007) was used for all of the above named scales which enabled the PI to record the time.

On scales that did not have a time limit, the PI used professional judgement to decide whether or not the child was going to respond appropriately after a relatively protracted time interval. The PI proceeded from item to item and scale to scale at a smooth but brisk pace in order to maintain the attention of the child while still allowing sufficient time for the child to respond.

3.5.2.1.4 Scoring procedures

The BAS2 scoring rules were designed to be clear and objective. Scoring criteria for each scale were always included in the administration directions for that scale. For most of the scales, the content rather than the form of the response was scored as instructed by the BAS2 manual. Thus, in most verbal tests, a response was not scored 0 because of grammatical or pronunciation errors. Similarly, in recall of designs the child was not penalised for clumsy or unrefined drawing as long as the essential features of the response were present.

Most BAS2 items were scored as either correct (1 point) or incorrect (0 points). However, several other scales used multiple points scoring so that the PI could derive more information from each item.

3.5.2.1.5 Extended selection of scales

In this study, school age level battery of tests was used on all children who were assessed at the age of 5 and above. Early year's level was used on all children who were below the age of 5. As a part of standardisation complete norms are available from the BAS 2 to support the use of many of the scales outside the age range at which they are usually given.

3.5.2.1.6 General principles for testing

Testing environment

All assessments were carried out in a quiet room with adequate lighting. The PI and the child were usually seated on a desk or a table and the PI was positioned to make full observation of the child's behaviour while completing the task as per the BAS 2 manual. Manipulation of the blocks, shapes and all other materials was carried out by the PI in the child's view but not writing scores on the record booklet.

Rapport

A good rapport was established with the participants throughout the assessments. The child was made comfortable and encouraged for their efforts. The PI always introduced the tasks as games and puzzles which the children enjoyed. Children were also made aware that some of the tasks were easy and some hard and the degree of difficulty increased as the child moved along the task. Children were told that they are not expected to answer every question perfectly, but they are expected to do their best on all items. Breaks were given after the delayed trial for recall of objects. A parent stayed with the child if the child wanted them to stay.

Record booklet

BAS 2 has 2 separate record booklets, one for early year's battery and the other for school age battery. The greater part of the booklet consists of spaces for recording the child's responses and scores. Tables for converting raw scores to ability scores are available on each scale. The last pages

consist of summary of all the scales, calculations for composite scores and profile analysis.

3.5.2.1.7 Calculating the child's chronological age

The BAS2 method of determining a child's age is unlike that of most other tests. The child's exact age on the date of testing was computed before testing began as this affects the starting point for many of the scales. The date of testing and the child's date of birth were recorded in the year, month and day format and subtracted. Where necessary, 30 days were borrowed from the month column and 12 months from the year column. Age was expressed in years and months and days were disregarded.

3.5.2.2 How was the score obtained

The standard scores were obtained using the following method:

1. The beginning and the ending item numbers were recorded and the raw scores for each scale were obtained.
2. The raw scores were then converted to ability scores using the tables in the record booklet
3. The ability scores were then converted to T scores.
4. Sum of T scores in a composite was then converted to a standard score.

3.5.2.3 Classification of General Conceptual Ability scores

Table 4 provides the category for describing the child's General Conceptual Ability score. These General Conceptual Ability score ranges are numerically the same as those reported for other cognitive batteries such as Weschler Scales. Children scoring below 80 are not classified to have 'moderate learning difficulties' as it would be poor practice to categorise solely on the basis of BAS II test. These scores need to be supplemented by other information on child's behaviour and development.

General Conceptual Ability score	Category
130 and above	Very high
120-129	High
110-119	Above average
90-109	Average
80-89	Below average
70-79	Low
69 and below	Very low

Table 4: Categories of general conceptual ability score

3.5.3 Direct Assessment - Auxology

At the time of the child’s assessment, following details were collected:

3.5.3.1 Height

For the purpose of height measurement, the participants were asked to stand in front of the Leicester Portable Height Measure with the feet together and chin up looking straight ahead. The height was measured twice and the average of these measurements was recorded and used for analysis. The Leicester height measure can measure heights between 0 to 2.07 meters (0 to – 81.5 inches) and was manufactured by Medisave Figure 6.



Figure 6: Leicester portable height measure

3.5.3.2 Weight

In order to measure weight, the subjects were asked to stand on the Tanita, Baby & Mommy weighing machine (Model 1582) until the exact weight was indicated by the machine. Each child was asked to stand on the weighing machine twice and 2 weights were recorded. The average of these weights was taken for analysis.

3.5.3.3 Head circumference

Head circumference was measured using 'Lasso measuring tape' supplied by the Child Growth Foundation. Two recordings of the head circumference were taken and the average of these 2 readings was taken for analysis.

3.5.3.4 Mid-Arm Circumference and Waist/Hip ratio

Mid-Arm Circumference (Mid arm circumference) and Waist/Hip ratio was measured using 'Acomplia measuring tape'. Again, 2 recordings of these measurements were taken and the average of these 2 readings was taken for analysis.

3.5.4 Direct assessment - Quick Neurological Screening Test

Neuromotor function, balance, and coordination were assessed with the Quick Neurological Screening Test (Quick Neurological Screening Test-II). The Quick Neurological Screening Test has been designed for use in screening for early identification as young as 5 years old who have minor neurological signs that are frequently associated with learning disabilities. It is a 20 minute test which looks at neurological integration.

The Quick Neurological Screening Test consists of a series of 15 observed tasks. These tasks are simple in nature and are adapted primarily from a typical paediatric neurological examination. However, a few tasks are derived from developmental scales or neuropsychological tests. It is designed so that it is easy for administrators and is non-threatening to the children. Typically, neuromotor function tasks or performance that are age dependent and merely reflect development are scored 1 point, but tasks or

performance that reflect a clear neuromotor dysfunction are scored 3 points. A score of 25 or less on the Quick Neurological Screening Test is considered in the normal range, 26–49 is considered a moderate discrepancy, and 50 or more is considered a severe discrepancy.

The Quick Neurological Screening Test does not provide enough detailed information to justify a neurological diagnosis. However the data collected could be used as a basis for referral to a neuropsychologist or a paediatric neurologist. The Quick Neurological Screening Test allows the examiner to assess how the child monitors and integrates sensory information from visual, tactile, auditory and proprioceptive or kinesthetic sources. Using the Quick Neurological Screening Test, the examiner could assess the child's control of muscles, both large and small, as they are used to maintain position and for voluntary motion. The examiner could also assess the child's ability to organize that motion in time and space for purposeful output.

Using this information, the examiner is then able to take a rapid look at the child's fine-motor control, gross-motor control, balance, rhythm, strength, motor planning and sequencing, sensory awareness, spatial orientation, visual perception, auditory perception, distractibility, impulsiveness, left-right differences, and visual-motor skills.

The Quick Neurological Screening Test attempts to identify three populations

1. Children who demonstrate no failures in age-related tasks and no abnormal neurological signs.
2. Children who have distinct, even if minor, neurological signs as clear-cut differences from one side to the other in sensation or motor control, or disorders of control of movement, such as tremor, ataxia, or nystagmus

3. Children with frank organic neurological signs who, even so, are not able to perform at the level predicted for their age – often called neurologically immature but often labeled as learning disabled.

Reason for choosing Quick Neurological Screening Test

The purpose of using a neurological assessment was to confirm that the participants did not have any neurological impairment that could affect cognition. Although many of these children were born premature and were admitted in the neonatal unit, according to their general practitioner, these children did not have any significant neurological impairments. We confirmed the same by using Quick Neurological Screening Test as it can identify minor neurological signs that are frequently associated with learning disabilities.

3.5.4.1 Tasks involved in Quick Neurological Screening Test

Hand Skill

The way a child picks up and holds a pencil was noted.

Figure Recognition and Production

This subtest assesses attention, visual discrimination, visual perception, motor planning, fine-motor control, eye-hand skills, and motor maturity. The geometric forms selected were chosen because normal children can complete these figures by age 6 although mastery of the diamond may be delayed to age 7. Performance on this task in part relates to cerebellar-vestibular function. It also predicts computation skills and reading success or failure.

Rapidly Reversing Repetitive Hand Movements

Rate, rhythm, symmetry and accuracy are all components of this subtest.

Palm Form Recognition

In older children, this task corresponds with IQ and reading success.

Finger to Nose

Smoothly executed excursions are accomplished by unimpaired children by the age of six.

Thumb and Finger Circles

95% of children between 6 and 7 1/2 years of age can perform this successfully.

Double Simultaneous Stimulation of Hand and Cheek

Displacement (when a subject indicates that the stimulus occurred at a spot other than the one touched by the examiner) and extinction (failure to indicate a spot touched) are common in young children.

Hand, Foot, Eye Preference

Cerebral dominance, resulting in hand, foot, and eye preference is a natural proclivity. However very bright, highly coordinated children often demonstrate little difference in accuracy or skill between preference tests of right or left hand, foot or eye. However, lack of dominance may result in delayed development of a clear sense of direction. Where hemispherical injury or local lesions are present, one may see a large variety of choices or preferences, resulting in mixed dominance, ambidexterity, or shift of dominance to the side opposite the one that has been destined genetically.

Eye Tracking

Jerkiness, asymmetry of movement, rapid alternating uncontrolled movement is abnormal at any age.

Sound Patterns: Rhythm, Rate, and Sequencing Discrimination

Observation of badly scarred eardrums and ear infections are related to failure on this subtest even without failure on pure tone audiometry. Thus failure is not a hearing impairment but some type of auditory inattention or apraxia.

Tongue Protrusion – Arm and Leg Extension

Considered abnormal are random quick irregular movements most often

appearing in fingers during arm-finger extension. Boys with this problem have more reading and spelling difficulties. Unusual posture of wrist flexion (wrist dip) and finger hyperextension is related to cortical dysfunction. The test is particularly effective in demonstrating subtle differences between right and left side gross- and fine-motor control.

Tandem Walk

Heel-toe walking is performed satisfactorily in 100% of normal school aged children. Backward tandem walking is skill not acquired until 7. Failure is an indicator of cerebellar-vestibular dysfunction.

Stand and Skip

90% of normal subjects are able to stand on one foot for 10 seconds without external support and without unusual posturing by age 6 on 2 out of 3 tries.

Behavioral Irregularities

Toe or finger tapping, excessive talking or making noises, fidgeting, impulsiveness, withdrawal, and defensiveness were noted. Hyperactive patterns and hypokinetic behaviors were also noted.

3.5.4.2 Medical Interpretations

Subjective scoring is required for handwriting ability, perceptual ability for numbers written on the palms of the hands, eye tracking, finger to nose coordination, rapidly reversing repetitive hand movements, tandem walk, and arm and leg extension. Success on Quick Neurological Screening Test activities indicate the child does not have neuromotor problems.

3.5.5 Strength and Difficulties Questionnaire

Behavioural screening questionnaires provide balanced coverage of children and young people's behaviours, emotions, and relationships. Behaviour was rated using the Strengths and Difficulties Questionnaire (SDQ). The SDQ was completed by parents and teachers. The questionnaire consisted of 25 items. The total behaviour deviance score was calculated as the sum of four of the five subscales: emotional

symptoms, conduct problems, hyperactivity, and peer problems. For questionnaires completed by parents, a total score between 0–13 was considered normal, a total score between 14–16 was considered borderline, and a score between 17–40 was considered abnormal. For questionnaires completed by teachers, 0–12 was considered normal, 13–15 as borderline score, and 16–40 as abnormal score.(Goodman, 1997)

Reason for Choosing Strength and Difficulties Questionnaire

A variety of methods have been used to assess behaviour. We considered the following 2 methods and each has its own advantages and drawbacks. The behavioural measure most frequently used in the previous studies was the Child Behaviour Checklist (CBCL). However, the Strengths and Difficulties Questionnaire (SDQ; adapted from the Rutter behaviour scales) was first published in 1997 so would not have been available to the studies before that. Strengths and Difficulties Questionnaire has been evaluated appropriate for gestational age against the benchmark set by the Rutter's parent and teacher questionnaires by Goodman.(Goodman, 1997). He noted that there was a high correlation between the total scores generated by the SDQ and Rutter questionnaires which provided sufficient evidence for the concurrent validity of the SDQ to be used to assess behaviour.

We also considered the fact that both SDQ and CBCL have parent and teacher formats, and are appropriately normed for the age group. However, the main advantage of the SDQ was its brevity (25 items) while the advantage of the CBCL (118 items) was subscales (including DSM diagnostic scales). For practicality, comparability with a previous study, and not needing to identify diagnostic subscales, we chose SDQ. As previous studies have shown that CBCL and Rutter parent questionnaire scores are highly correlated (Berg et al., 1992), and that these two sets of questionnaires are of comparable predictive validity (Berg et al., 1992), it is likely that the SDQ and CBCL would also be highly correlated and have comparable validity.

3.6 Procedures

3.6.1 Location

Children were assessed either at the children's out-patient department at the Royal Victoria Infirmary in Newcastle upon Tyne or at their home depending on parental preference. The research team paid car parking expenses for those parents who came to Royal Victoria Infirmary.

3.6.2 How the study was conducted

The PI was blind to the child's birth weight. Children were assessed either at home or at the children's outpatients department at the Royal Victoria Infirmary. The cognitive assessment was divided into 2 sessions of 30-40 minutes each to ensure that the children did not get tired during these assessments. This division allowed the assessors to maximise the children's motivation, whilst minimising any possible reduction in their performance resulting from initial worries about the testing situation or fatigue towards the end of the test session. The non-verbal scale was always administered at the start of the assessment, so that children could settle in to the session and get some positive feedback before they had to start giving verbal responses.

In an attempt to make each child feel as comfortable as possible, they were given the choice of doing the tests with or without their parent. Auxology and Quick Neurological Screening Test assessments were done in parallel on the same day or on a different day depending on parental preference but within the same calendar age.

The BAS early years battery was used when assessing children under the age of 5 years and school age battery was used on all children above the age of 5 years.

3.6.3 Inter observer variability

The auxology measurements and Quick Neurological Screening Test were administered by PI on all twins seen at home and by Dr. Korada on all twins seen at the hospital. This is a potential source for bias and to

minimise it, all the measurements and Quick Neurological Screening Test were administered on a control child by both assessors and it was found that the inter observer variability was minimal. This procedure was repeated every 4 months.

3.7 Statistical analysis and sample size

Independent and qualified statisticians were consulted for statistical analysis.

Sample size: A previous study has suggested that infants born with growth restriction and abnormal umbilical Doppler flow studies may have a cognitive outcome of one standard deviation (Mean=100 IQ points, SD=15 IQ points) below the mean (Schreuder et al., 2002). In our study, we hypothesised that the growth retarded twin will have a cognitive outcome of half a SD (equivalent to 7.5 IQ points) lower than their twin pair with or without abnormal umbilical artery Doppler flow. Using a paired analysis, 34 twin pairs would be able to document a difference of half a SD at a significance level of 5% with 80% statistical power.

The Kolmogorov–Smirnov test was used to check normality of data distribution. Means and standard deviation were used for parametric variables. Minimum and maximum points of the data were noted. The difference between the individual test score means between the lighter and heavier twin groups and the confidence intervals for this difference was also calculated. The t test was used to compare General Cognitive Ability scores between the 2 groups.

The associations between general conceptual ability and birth weight were performed using generalised estimating equations. Robust standard errors and confidence intervals for estimates have been produced (Morley et al., 2005). The general conceptual ability values were transformed to achieve adequate normality by squaring the values. We then estimated the association between general conceptual ability and birth weight within twin pairs by fitting a model with general conceptual ability (square

transformed) as the dependent variable. An independent variable representing the difference between the individual birth weight and the twin pair mean birth weights as well as a term representing pair mean birth weight was included in the model. This approach allowed estimates of the association between intrauterine growth restriction and general conceptual ability to be adjusted for factors shared within twin pairs (intrapair) while also allowing examination of possible independent effects of between twin pair (interpair) differences. Gestation and gender were included in this model and we also tested for effect modification using appropriate interaction terms. Maternal smoking was excluded as a variable as only seventeen mothers smoked and all of them stopped smoking when they learnt they were pregnant. All parents had GCSE or university qualifications apart from one mother. This was therefore deemed as not a significant variable to be included in the regression analysis. Similarly, the Townsend index was used to assess socioeconomic status. However this was based on 2001 census was thought not to represent the true socio-economic status during the study period and was also excluded from the analyses. A sensitivity analysis was also performed excluding twin pairs who had umbilical artery Doppler waveform abnormalities using the above model.

Linear regression analysis was used to assess relationships between general conceptual ability difference and birth weight difference. Gestation and gender were included in this model as other independent variables. A paired t-test was used to compare auxology data between the 2 groups. A p-value <0.05 was accepted for statistical significance.

Kappa statistics were used for the analysis of the Strength and Difficulties questionnaire. All analyses were conducted using SPSS v19 and Minitab v16.

3.8 Ethical issues and Confidentiality

This study was given a favourable ethical opinion by the County Durham & Tees Valley REC 1 Research Ethics Committee.

This study was also registered with the Research and Development Department, Royal Victoria Infirmary, Newcastle Upon Tyne Hospitals NHS Trust (Registration number - 4410). We also had Caldicott guardian's approval from the same trust.

3.9 Data Storage

All electronic data obtained in this study were kept password protected on NHS trust PCs or server. Data were not shared with those outside the NHS or held on University or personal computer.

3.10 Grant application and funds

The study was successful in obtaining a grant £4,000 from 'The Children's Foundation'. This grant was utilised to purchase tools required for assessments and cover travel expenses.

4. Results

During the period between January 2000 to December 2004, 66 pairs of monochorionic twins had a birth weight discordance of $\geq 20\%$. Five of these twins did not agree to participate. Seven did not respond to our invitation letters and in three pairs, one of the twins was confirmed to have cerebral palsy by the general practitioner. As a result, a total of 51 pairs of monochorionic twins were assessed.

4.1 Description of the study group

The total number of live births during the study period was 148,914 of which 4277 births were twins. Eight hundred and five twins were monochorionic of which 66 twin pairs had $\geq 20\%$ birth weight discordance. This amounts to a birth prevalence of 0.04% for total live births and 1.5% for the total number of twin live births.

There were 28 male twin pairs and 23 female twin pairs. The mean gestational age was 34.7 weeks (Range 26 to 40 weeks). Figure 7 shows the breakdown of the gestational age for the study group. The mean birth weight of the lighter twins was 1701gms (Range 670gms – 2680gms) and the mean birth weight of the heavier twins was 2366 grams (Range 1030 grams – 3800 grams). Figure 8 shows the distribution of the birth weight in the two groups. The mean birth weight discordance between the lighter and the heavier twins was 664 grams (Range 245gms – 1250gms) and the percentage of discordance ranged from 20% to 56%. The lighter twin was first born in 26 twin pairs. This is graphically shown in Figure 9. The mean age at assessment was 6years and 4months (Range 4years - 8years 9months). Table 5 shows the summary statistics of the participants.

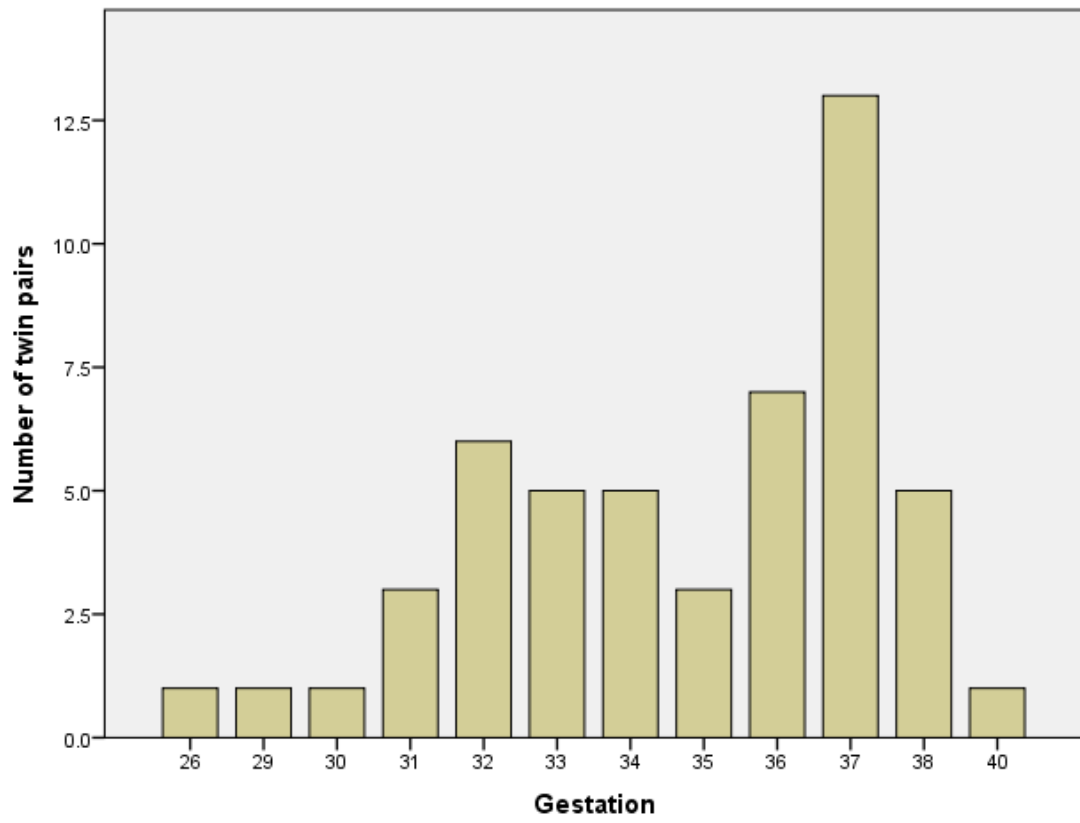


Figure 7: Distribution of gestational age

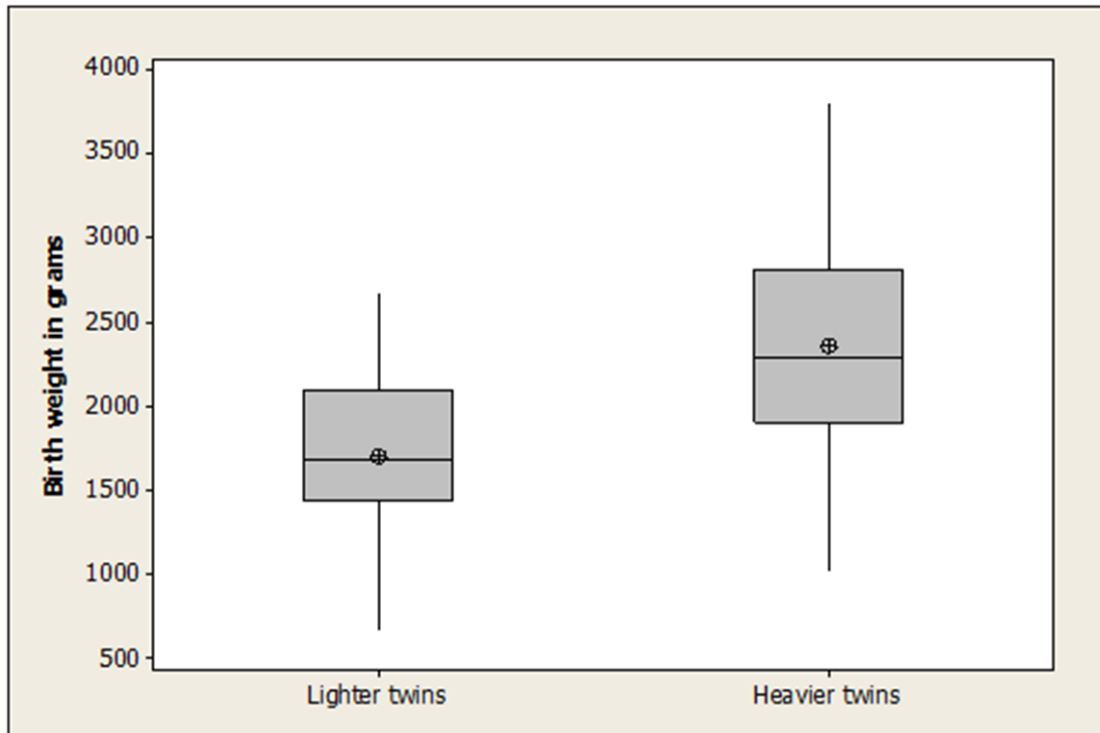


Figure 8: Birth weight distribution of the study group. The box represents inter-quartile range and whiskers the minimum and maximum values. The circles with cross represents mean value and straight line within the box represents median value.

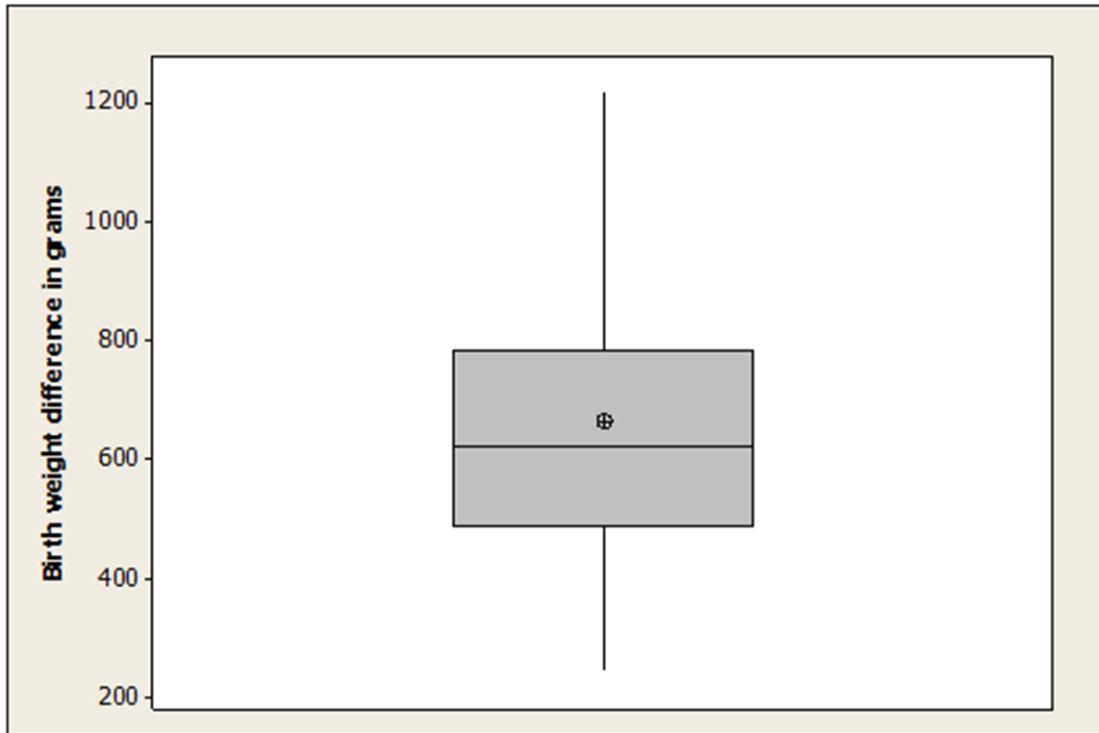


Figure 9: Distribution of birth weight difference between lighter and heavier twins. The box represents inter-quartile range and whiskers the minimum and maximum values. The circle with cross represents mean value and straight line within the box represents median value.

Table 5: Summary Statistics of 51 monochorionic twin pairs

		All	Heavier twin	Lighter twin	Difference
		n	n	n	
Gender	Male	28			
	Female	23			
Neonatal unit admission		67	31	36	
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Gestation		34.7(2.8)			
Birth weight (gm)		2033 (652)	2366 (628)	1701 (489)	664 (241)
Age at assessment		6y 4m*			
General Conceptual Ability		106.8 (14.7)	108.3 (14.1)	105.3 (15.1)	3 (7.2)

*y=years, m=months, SD-Standard Deviation

Mode and Reason for delivery

Thirty five (69%) of the twin pairs were born by caesarean section and the remaining sixteen (31%) were born by normal vaginal delivery. The reason for delivery is shown in Table 6.

Reason	n	Percentage
Fetal complications	19	37.3
Maternal	5	9.8
Spontaneous	27	52.9
Total	51	100.0

Table 6: Reason for delivery

Nineteen sets of twins were delivered early in view of fetal concerns. Of these, 10 twin pairs had absent or reversed end-diastolic flow velocity waveforms in the umbilical artery Doppler ultrasound examination. Three of the lighter twins had absent end diastolic flow and seven had reversed end diastolic flow waveforms in umbilical artery Doppler. These abnormalities were diagnosed at a mean gestational age of 23 weeks (Range 17–27 weeks). Other fetal reasons expediting the delivery were fetal distress, worsening of oligohydramnios in lighter twin and worsening of birth weight discordance. The rest were delivered by normal delivery at term or prematurely if the mother went into spontaneous labour. Five sets of twins were delivered in view of maternal reasons. Figure 10 shows the reason for delivery according to gestational age and birth weight discordance.

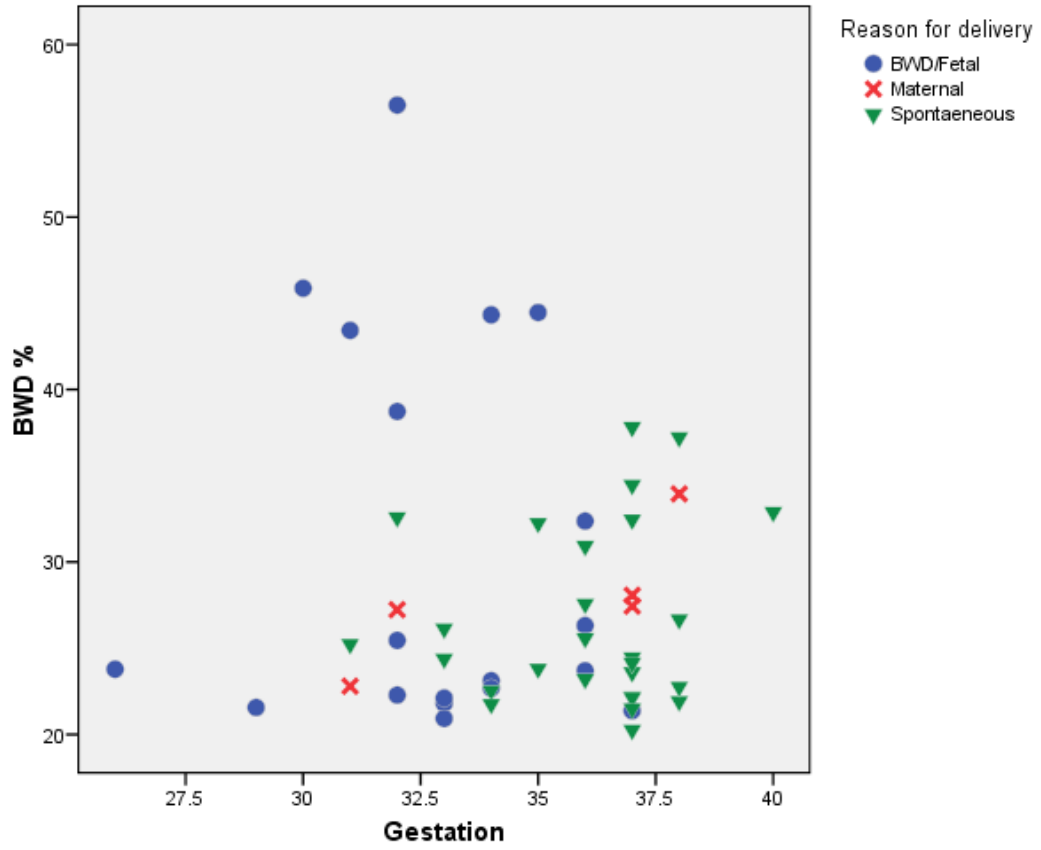


Figure 10: Reason for delivery according to gestational age and birth weight discordance
 BWD- Birth weight discordance

Reason for Neonatal unit admission:

Seventy percent of the lighter twins needed admission to Neonatal unit. In the majority of cases this was to maintain normal temperature and blood glucose (either nasogastric tube feeding or intravenous fluids). In the heavier twins group, 12 babies required continuous positive airway pressure support and 2 babies required ventilation while in the lighter twin group, 15 babies required continuous positive airway pressure support and 4 babies required ventilation. The mean duration of admission was 4 weeks and 6 days (range- 3 days to 8 weeks). All these twin pairs were discharged and followed up by paediatricians and none had any significant neurological impairment.

4.2 British Ability Scales test results

Fifty one twin pairs were split into 2 groups, the lighter twins at birth group and the heavier twins at birth group. Mean values with standard deviation and 95% confidence intervals for the mean difference in the above scores between the 2 groups were also calculated for the individual subtests, individual clusters and the general conceptual ability. There were 45 twin pairs in the school age group and 6 twin pairs in the early year's battery group. Certain tests were common in both the groups and so all 51 twin pairs undertook the same test. On couple of occasion, few twins in the early year's group refused to take the test and as a result only 48 or 49 twin pairs results were analysed for these tests. There was a mean difference of three general conceptual ability points between the twin groups and this result was statistically significant. However, results for individual cluster [Verbal standard score, Spatial standard score, Non verbal reasoning (School age battery- 45 twins)/pictorial reasoning (Early years battery-6 twins)] were not statistically significant. The difference in special non verbal score which combines spatial standard score and non verbal reasoning/pictorial reasoning was also not statistically significant. Amongst the individual subtests, difference in mean scores for quantitative reasoning and recall of objects-immediate verbal were significant. These results are shown in Table 7, Table 8, Table 9 and Table 10. Figure 11 shows the distribution of the general conceptual ability scores between the lighter and the heavier twins and Figure 12 shows the distribution of the difference in the general conceptual ability scores between the 2 groups.

Table 7: Results of individual subtests

Test	Groups	N	Mean (SD)	Mean Difference (95% CI)
Recall of design	Lighter twin	45	48.22 (10.75)	-0.53 (-3.01, 1.94)
	Heavier twin	45	48.76 (10.94)	
Word definition	Lighter twin	45	54.80 (8.44)	-0.31 (-2.04, 1.42)
	Heavier twin	45	55.11 (6.96)	
Pattern construction	Lighter twin	51	49.22 (11.21)	-1.17 (-2.90, 0.55)
	Heavier twin	51	50.39 (9.60)	
Matrices	Lighter twin	45	57.24 (11.69)	0.51 (-2.32, 3.34)
	Heavier twin	45	56.73 (9.48)	
Verbal similarities	Lighter twin	45	56.91 (7.90)	-1.60 (-3.58, 0.38)
	Heavier twin	45	58.51 (8.87)	
Recall of objects - Immediate Spatial	Lighter twin	49	47.73 (10.11)	0.16 (-2.32, 2.64)
	Heavier twin	49	47.57 (9.63)	
Recall of objects - Delayed verbal	Lighter twin	49	56.78 (13.01)	-1.37 (-4.98, 2.25)
	Heavier twin	49	58.14 (12.22)	
Recall of objects - Delayed spatial	Lighter twin	49	51.98 (9.82)	0.96 (-1.33, 3.24)
	Heavier twin	49	51.02 (9.55)	
Recall digits forward	Lighter twin	49	44.47 (10.25)	-1.14 (-3.47, 1.19)
	Heavier twin	49	45.61 (8.85)	
Recognition of pictures	Lighter twin	48	50.42 (9.48)	-0.17 (-3.07, 2.74)
	Heavier twin	48	50.58 (8.64)	
Quantitative reasoning	Lighter twin	45	53.40 (9.44)	-3.69 (-6.14, -1.24)
	Heavier twin	45	57.09 (8.91)	
Recall of objects - Immediate verbal	Lighter twin	49	50.55 (10.83)	-3.69 (-6.60, -0.79)
	Heavier twin	49	54.24 (8.80)	
Recall of digits backward	Lighter twin	45	50.29 (11.98)	-2.78 (-5.88, 0.33)
	Heavier twin	45	53.07 (11.72)	

SD- Standard deviation, CI- Confidence intervals

Table 8: Results of individual subtests continued

Test	Groups	N	Mean (SD)	Mean Difference (95% CI)
Speed of information processing	Lighter twin	45	59.22 (12.44)	0.93 (-1.79, 3.66)
	Heavier twin	45	58.29 (10.60)	
Verbal Comprehension	Lighter twin	6	51.67 (13.28)	-3.67 (-18.2, 11.1)
	Heavier twin	6	55.33 (5.92)	
Picture Similarities	Lighter twin	6	53.33 (10.42)	-3.00 (-10.85, 4.85)
	Heavier twin	6	56.33 (7.84)	
Naming Vocabulary	Lighter twin	6	53.00 (14.52)	-2.33 (-13.86, 9.19)
	Heavier twin	6	55.33 (6.59)	
Early number concept	Lighter twin	6	49.83 (7.28)	-6.00 (-16.04, 4.04)
	Heavier twin	6	55.83 (11.05)	
Copying	Lighter twin	6	42.17 (4.67)	-3.50 (-8.04, 1.04)
	Heavier twin	6	45.67 (4.03)	
Matching letter	Lighter twin	6	52.00 (7.48)	-3.75 (-19.0, 11.5)
	Heavier twin	6	55.75 (3.77)	
Pictorial reasoning	Lighter twin	6	102.33 (11.15)	-7.83 (-16.42, 0.75)
	Heavier twin	6	110.17 (15.88)	

SD- Standard deviation, CI- Confidence intervals

Table 9: Results of individual clusters

Test	Groups	N	Mean (SD)	Mean Difference (95% CI)
Verbal Standard score	Lighter twin	51	108.47 (13.47)	-2.49 (-5.09, 0.10)
	Heavier twin	51	110.96 (11.54)	
Spatial standard score	Lighter twin	51	96.63 (17.43)	-1.65 (-4.21, 0.92)
	Heavier twin	51	98.27 (15.99)	
Non verbal reasoning	Lighter twin	45	108.71 (15.28)	-2.91(-6.32, 0.50)
	Heavier twin	45	111.62 (14.10)	
Special Non verbal	Lighter twin	6	103.29 (16.77)	-1.96 (-4.69, 0.76)
	Heavier twin	6	105.25 (14.97)	

SD- Standard deviation, CI- Confidence intervals

Table 10: General conceptual ability scores of twins

	N	Mean	SD	SE Mean
General conceptual ability- Lighter twin	51	105.37	15.08	2.11
General conceptual ability- Heavier twin	51	108.37	14.16	1.98
Difference		-3.00	7.27	1.02

95% CI for mean difference: (-5.04, -0.96)

SD- Standard deviation, CI- Confidence intervals, SEM- Standard error

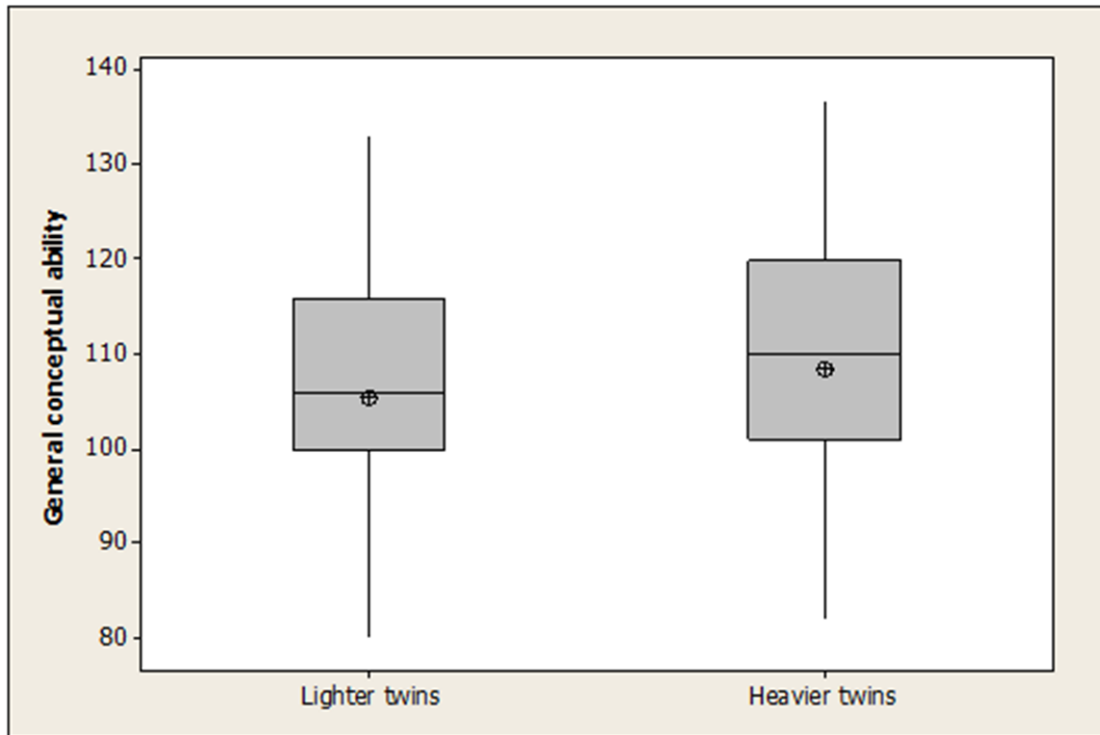


Figure 11: Distribution of the general conceptual ability between the 2 groups. The box represents inter-quartile range and whiskers the minimum and maximum values. The circles with cross represents mean value and straight line within the box represents median value.

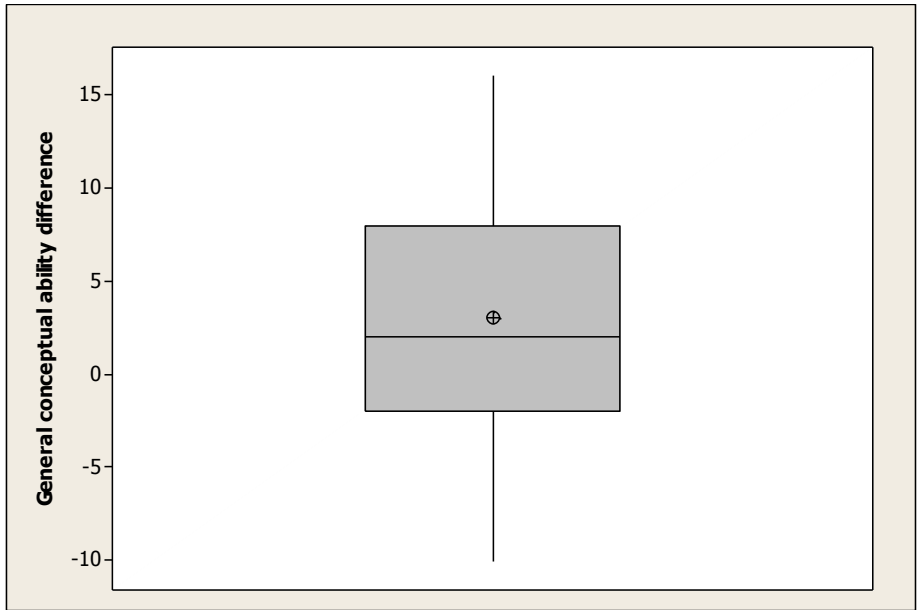


Figure 12: Distribution of the general conceptual ability score difference between the heavier and lighter twins. The box represents inter-quartile range and whiskers the minimum and maximum values. The circle with cross represents mean value and straight line within the box represents median value.

4.3 Results for specific hypotheses

4.3.1 Effect of birth weight discordance on cognition

Generalised estimating equations were used to test the effect of birth weight within and between twin pairs on general conceptual ability. Gender and gestational age were included in the model as covariates with general conceptual ability (square transformed) as the dependent variable. All 51 pairs (102 children) were included in the model. Males were coded as -1 and females were coded as 1. The results are shown in Table 11. This analysis showed that there is a significant association between within pair differences in birth weight and general conceptual ability scores. The general conceptual ability increases by half a point for every increase in 100 gram in weight.

We then tested for effect modification in this model using appropriate interaction terms for gestation and gender. Adding interaction terms to a regression model can greatly expand understanding of the relationships among the variables in the model. An interaction may arise when considering the relationship among gender, gestation and growth restriction as simultaneous influence of any two variables can affect the third variable. Table 12, model 1 shows a two-way interaction between the within pair effect and gestational age while model 2 shows a two-way interaction between the within pair effect and gender. There was no statistically significant interaction between the within-pair differences in birth weight with gender or gestational age.

Table 11: Effect of within pair and between pair differences in birth weight on cognition

Parameter	B	95% Confidence Interval
Gender	-1865.636	-3306.814; -424.458
Gestation	501.969	27.669; 976.269
Mean birth Weight	-1.730	-4.456; 0.995
Birth weight –Mean weight	0.593	0.022; 1.165

Table 12: Interaction of within pair difference in birth weight with gender and gestational age

Parameter	B	95% Confidence Interval
Model 1		
Gender	-1865.636	-3306.814; -424.458
Gestation	501.969	27.669; 976.269
Mean birth Weight	-1.730	-4.456; 0.995
Birth weight –Mean weight	7.882	-2.718; 18.481
Gestation * Birth weight –Mean weight Interaction	-0.203	-0.497; 0.090
Model 2		
Gender	-1865.636	-3306.814; -424.458
Gestation	501.969	27.669; 976.269
Mean birth Weight	-1.730	-4.456; 0.995
Birth weight –Mean weight	0.423	-0.456; 1.302
Gender * Birth weight –Mean weight Interaction	0.351	-0.746; 1.448

4.3.2 Effect of birth weight discordance on general conceptual ability difference

To determine the influence of birth weight discordance on general conceptual ability score difference, multiple linear regression was performed. Variables entered into the model were percentage birth weight difference, gestational age and gender. Difference in General conceptual ability score was the dependent variable. All 51 twin pairs were included in the analysis. The results are shown in Table 13. This analysis shows that except gestational age, none of the other variables had a statistically significant effect on cognition.

Table 13: Multiple regression analysis of various independent factors and inter-twin general conceptual ability difference

Parameter	B	p-value
Gender	-0.875	0.650
Gestation	-1.043	0.004
Birth weight difference	-0.182	0.139

4.3.3 Effects of umbilical artery Doppler waveform abnormality and birth weight discordance on cognition

The mean and standard deviation of general conceptual ability of the 10 twin pairs with abnormal Doppler flow in the umbilical artery during the fetal life is shown in Table 14 along with the difference in the mean scores. Sensitivity analysis excluding mothers with concerning Doppler's was performed and 41 twin pairs were included in the model after excluding the 10 pairs with fetal concerns. Generalised estimating equations were used to test the effect of birth weight within and between twin pairs on general conceptual ability. Gender and gestational age were included in the model as covariates with general conceptual ability (square transformed) as the dependent variable. The results are shown in Table 15. The analysis shows that when 10 twin pairs with abnormal umbilical Doppler's were removed, the effect of within pair weight discordance on general conceptual ability disappeared.

Table 14: general conceptual ability of twins with abnormal umbilical artery Doppler flow

	N	Mean	SD	SE Mean
General conceptual ability- Lighter twin	10	92.60	12.98	4.104
General conceptual ability- Heavier twin	10	99.70	13.83	4.374
Difference		-7.10	9.527	3.012

95% CI for mean difference: (-13.91, -0.28)

SD- Standard deviation, CI- Confidence intervals, SEM- Standard error

Table 15: General Conceptual Ability of twins excluding twins with abnormal umbilical artery Doppler flow

Parameter	β	95% Confidence Interval
Gender	-1959.180	-3597.912; -320.449
Gestation	448.628	-186.200; 1083.455
Mean birth Weight	-2.352	-5.313; 0.610
Birth weight –Mean weight	0.455	-0.147; 1.058

4.3.4 Differences in size

All the twin pairs (51) had their height, weight, head circumference, mid-arm circumference, waist, hip circumference measurements and body mass index (BMI) recorded at the time of assessment. A paired t-test was used to compare these measurements. The results are shown in Table 16. The difference in size persists at school age and all these results were statistically significant. There was considerable catch up growth in the lighter twin. The average difference between the birth weights between the twins was 28% and was only 8% at the time of examination.

Measurement	Groups	Mean (SD)	Difference (95% confidence interval)
Height	Lighter twin	115.6 (7.0)	-2.1 (-2.8, -1.3)
	Heavier twin	117.7 (7.9)	
Weight	Lighter twin	20.7 (3.6)	-1.9 (-2.5, -1.3)
	Heavier twin	22.6 (4.3)	
Head circumference	Lighter twin	51.0 (1.8)	-0.7 (-1.0, -0.4)
	Heavier twin	51.7 (1.9)	
Mid arm circumference	Lighter twin	17.9 (1.4)	-0.6 (-0.8, -0.3)
	Heavier twin	18.5 (1.5)	
Waist	Lighter twin	53.9 (5.2)	-2.7 (-3.7, -1.7)
	Heavier twin	56.6 (6.0)	
Hip	Lighter twin	62.7 (4.4)	-1.8 (-2.7, -0.9)
	Heavier twin	64.5 (5.2)	
Body mass index	Lighter twin	15.4 (1.6)	-0.84 (-1.13, -0.55)
	Heavier twin	16.2 (1.8)	

Table 16: Differences in size between the twins. SD-Standard deviation

4.3.5 Behaviour

Strengths and Difficulties Questionnaire analysis

Both parent and teacher questionnaire results were available for 45 twin pairs. Five twins had only parent questionnaire and neither parent or teacher questionnaires were available for one twin pair. These six twin pairs were therefore excluded from the analysis.

Parental questionnaire analysis

Six lighter twins and three bigger twins were classified to have borderline behavioural abnormalities. Eight lighter and eight bigger twins were classified to have abnormal levels of behaviour problems.

Teacher questionnaire analysis

Eight lighter twins and five bigger twins were classified to have borderline behavioural abnormalities. Seven lighter and six bigger twins were classified to have abnormal levels of behaviour problems.

Kappa statistics was used for teacher and parent classification cross tabulation. The results are shown in Table 17. The value of kappa is 0.708. This suggests that the parent and teacher ratings are largely similar, with some exceptions.

Table 17: Analysis of Strength and Difficulties questionnaire

		Parent Classification		
		Normal	Borderline/Abnormal	Total
Teacher Classification	Normal	62	6	68
	Borderline/Abnormal	4	18	22
Total		66	24	90
Measure of Agreement- Kappa		0.708		

4.4 Further analysis

4.4.1 Mathematical skills

The difference in mean score for quantitative reasoning (Mathematical score) between the twin pairs was statistically significant (Table 18). Therefore, generalised estimating equations model was used to test the effect of birth weight within and between twin pairs on quantitative reasoning scores. Gender and gestational age were included in the model as covariates with quantitative reasoning scores (square transformed) as the dependent variable. Forty five twin pairs were included in the model. The results are shown in Table 19. We found that there was a significant association between within pair differences in birth weight and quantitative reasoning score.

Table 18: Mathematical skills test results

	N	Mean	SD	SE Mean
Quantitative reasoning- Lighter twin	45	53.40	9.44	1.41
Quantitative reasoning- Heavier twin	45	57.09	8.91	1.33
Difference		-3.69	8.15	1.22

95% CI for mean difference: (-6.14, -1.24)

SD- Standard deviation, CI- Confidence intervals, SEM- Standard error

Table 19: Effect of within pair and between pair differences in birth weight on maths score

Parameter	β	95% Confidence Interval
Gender	-1.618	-6.412; 3.176
Gestation	1.302	-0.694; 3.299
Mean birth Weight	-0.007	-0.017; 0.003
Birth weight –Mean weight	.004	0.000; 0.007

4.4.2 Memory test

Early years upper level and School age Battery: Recall of objects- Immediate verbal

The difference in mean score for recall of objects (Memory score) between the twin pairs was statistically significant (Table 20). Therefore, generalised estimating equations model was used to test the effect of birth weight within and between twin pairs on recall of objects scores. Gender and gestational age were included in the model as covariates with recall of objects scores (square transformed) as the dependent variable. Forty nine twin pairs were included in the model as 2 twin pairs did not undertake this test. The results are shown in Table 21. We found that there was a significant association between within pair differences in birth weight and recall of objects scores.

Table 20: Results of memory test in twins

	N	Mean	SD	SE Mean
Recall of objects-immediate verbal- Lighter twin	49	50.55	10.83	1.55
Recall of objects-immediate verbal- Heavier twin	49	54.24	8.80	1.26
Difference		-3.69	10.10	1.44

95% CI for mean difference: (-6.60, -0.79)

SD- Standard deviation, CI- Confidence intervals, SEM- Standard error

Table 21: Effect of within pair and between pair differences in birth weight on memory scores

Parameter	β	95% Confidence Interval
Gender	-7.285	-11.568; -3.003
Gestation	-0.195	-1.794; 1.403
Mean birth Weight	0.001	-0.007; 0.010
Birth weight –Mean weight	0.005	0.002; 0.009

5. Discussion and Conclusions

5.1 Principal findings

The main finding of this study is that the smaller twin of a monochorionic growth discrepant pair was significantly more likely to have a lower cognitive score compared to their co-twin at 5-8 years of age. There was a relationship between a within-pair difference in birth weight and a subsequent within pair-difference in general conceptual ability. However factors shared between the twins did not have any effect on cognition. The mean difference in the general conceptual ability between the heavier and lighter twins was 3 general conceptual ability points. Although the amount of variation explained by our models is small, and the effect on an individual is small, our analysis indicates that intrauterine growth has an important long term effect on cognitive development. Mathematical skills and memory skills were more affected in the lighter twin than the heavier twin. However when twin pregnancies with fetal concerns (abnormal umbilical artery Doppler flow) were excluded from the analysis, within pair differences in birth weight did not have any effect on cognition.

The difference in the general conceptual ability score did not increase with the increasing degree of birth weight discordance. The difference in the size seen at birth between the twins persisted at the age of 5-8 years. There was a non-significant increase in prevalence of behavioural problems in the lighter twin than the heavier twin as reported by both teachers and parents.

5.2 Strengths and Weakness in relation to other studies

5.2.1 Cognition

A difference in the general conceptual ability scores was noted between the lighter and heavier twins in this study. This result is similar to that of studies done by Torche et al (2011), Edmonds et al (2010), Bellido-Gonzalez et al (2007) and Goyen et al (2003) but in contrast to Reolan et al

(2007) and Stauffer et al (1988). These studies are compared individually with our study.

Torche et al showed that intrauterine growth has a substantial effect on children's cognitive development, as measured by test scores in primary school (Torche and Echevarria, 2011). However cognitive outcomes were based on the use of maths and Spanish fourth-grade results only. It has been shown in previous studies that intrauterine growth restriction differentially affects cognitive domains and therefore we considered it important that all aspects of cognition were examined. We examined several aspects of cognition (verbal, Spatial and non-verbal) and found that cognition was affected in the growth restricted twin. Torche et al's study population was based in a middle-income country with wide social inequality and therefore the results may be considered applicable to countries where the primary reason for intrauterine growth restriction is more likely to be due to poor maternal nutritional status rather than due to placental reasons. Also, not all eligible twins were approached for the study by Troche et al and therefore the extent of selection bias due to exclusion of children with incomplete information was not estimated. Confounding factors like significant past medical history (any illness which can affect child's cognitive ability) of the participants was not determined. We had included all these information in our study. Nonetheless, the finding that growth restriction affects cognition was found in both studies.

Edmonds et al found birth weight discordance only affects verbal IQ. Our results do suggest that the general conceptual ability was lower in the lighter twin and the verbal scores were just below statistical significance (Edmonds et al., 2010). Although the number of participants was larger than our study they were recruited via advertisements and newsletters which may result in sampling bias as there is a possibility that only motivated parents/children agree to participate. Moreover, these children were assessed between 7 years to 17 years of age which is a wide range

using a short form of Wechsler Intelligence scale for children. Our study was population based and assessed all domains of the British Ability Scales. In addition, the age range at assessment was just 4 years. Again, significant past medical history which may impact cognition was not ascertained in Edmonds et al study but we ascertained this information. The difference in the verbal IQ scores between the twins were more pronounced as the degree of discordance increased in their study but we did not identify this result in our study. This could possibly be due to the fact that severe discordance is usually noted when the pregnancy is allowed to continue. As twins are born at an advanced gestational age they escape the complications of prematurity and its effects on neurodevelopment. Twins in our study were more mature and were probably not influenced by problems of prematurity. One other explanation could be that the heavier twin exposed to the same intrauterine environment may have a lower cognition. According to Riese (2001), in severe birth weight discordance, both the twins are likely to be cognitively delayed.

Bellido-Gonzalez et al (2007) showed that the cognitive and verbal domain differences persist consistently throughout the ages 1, 2 and 4 years. We did not measure serially the cognition and are therefore unable to compare. Like our study, no children with neurological morbidity were included. In contrast to our results, most of the lighter twins in their study had an IQ score below normal range. General conceptual ability scores in our study were within the normal range for the lighter twin with one exception. This difference could be due to the small sample in their study group and possibility of sampling bias as only twins from a single centre were included. Bellido- Gonzales et al also found that 4 children who had severe birth weight discordance and were small for gestational age at birth had a very low IQ. However, we did not find a similar observation in our study. It is however interesting to note that this finding of lower IQ scores in lighter twin with severe discordance did not occur with all twins. In one twin with severe discordance, the lighter co-twin developed well. Bellido-

Gonzales et al suggested that although this small twin was subjected to severe birth weight discordance, the birth weight of this twin was not very low. Therefore, the low IQ in the lighter twins with severe birth weight discordance could be due to effect of intrauterine growth restriction rather than severe birth weight discordance per se.

Goyen et al (2003) found that the mean GQ of the lighter twins was lower than the larger twins at the age of 3 years. Significantly lower scores were observed for the locomotor, hearing and speech, and practical reasoning subscales. There was no unfavorable developmental outcome for the lighter twin. Like our study, twins with neurological impairments were not included in the developmental assessment. Subgroup analysis by Goyen et al (2003) showed a trend towards greater GQ difference (mean - 7 GQ points) between the discordant co-twins of >30% discordance. In the multiple regression analysis, lower gestational age and higher percentage discordance contributed to lower GQ at age 3. We did not find this result in our study. Probable reason for different findings could be related to physical aspects of growth. The main reason for low scores in Goyen's study was related to the mean 9 point difference in the locomotor scale and not related to other domains of Griffith's scales which relate to cognition. Although there was significant catch-up growth in the lighter twin, they remained lighter than the heavier twin (at birth) at the time of assessment. The reduced muscle bulk may have affected ability to perform motor tasks which involved strength and hence the low scores. Other possible reasons for contrasting findings include those mentioned earlier while discussing similar finding in Edmond's et al study. There was also sampling bias as Goyen et al study was done in a single centre and many dropped out of the study but our study was population based.

In contrast to the results from our study, Reolan et al showed a significant association between postnatal head growth and mental developmental index but intrauterine growth restriction in the lighter twin did not have any influence of on cognition at 12 to 42 months

corrected age (Reolon et al., 2008). However, in our study, cognition was affected by growth restriction. The different findings could be because of the poor sampling in their study as of the 65 twin pairs eligible for the study, only 36 were recruited and inter-observer variability while assessing the development. However, like our study, they also performed a neurological examination to determine the presence of neurosensory impairments, and used mixed effect linear regression models to analyse data.

Stauffer et al (1988) also found no differences in developmental outcome in discordant twin pairs but prematurity affected developmental outcomes. One possible explanation for this dissimilar finding from our study could be that the twins in this cohort had many medical complications and it is therefore possible that any differences between the twins were too small to be apparent. Other explanation could be due to sampling bias as this was a single centre study and not all twins were examined at 36 months. Also, true growth restriction is usually diagnosed at a birth weight discordance of 20% and above. Therefore a cut-off of 15% used in this study might not have ascertained true effects of growth restriction.

Twins with abnormal Umbilical artery Doppler blood flow:

There are only a few follow up studies looking at the neurodevelopmental outcome of children who were born following pregnancies complicated by absent or reversed end diastolic blood flow in the umbilical artery Doppler. Absent or reversed end diastolic blood flow in the umbilical artery Doppler in singletons has been attributed to increased placental impedance (Divon and Ferber, 2001). Rising ratios of the systolic/diastolic frequency in a cardiac cycle reflect an increasing amount of impedance to flow in the placenta and this is usually due to increased placental circulatory resistance as a result of a reduced number of tertiary villous arteries. In monozygotic twins, the aetiology of abnormal UA Doppler in one twin is not very clear but is thought to be secondary to intermittent absent and/or

reversed end-diastolic flow because of the large arterio-arterial anastomoses. (Gratacos et al., 2004b)

In our study there was a mean difference of 7 General Conceptual Ability points between the twins with normal and abnormal Doppler flows. When these 10 twin pairs with abnormal antenatal Doppler's were removed from the model, the within pair effect on general conceptual ability disappeared. The absence of within pair effects on general conceptual ability could be due to reduced power in this "subgroup" analysis or because these 10 twin pairs were driving the significant within-pair effect.

In a study done by Schreuder et al, comparing singletons who had reversed end diastolic blood flow in the umbilical artery with absent end diastolic blood flow; there was a difference of 13 points on the British Ability Scale General Conceptual Ability score. The mean gestational age of this group was 31.6 weeks (Range 26-38 weeks) and the mean birth weight was 1319gms (Range 585-3206gms). However, comparing twins and singletons with absent or reversed end diastolic blood flow in the umbilical artery is open to criticism as the groups may contain pregnancies with different pathologies which themselves will have a major impact on outcome. However, it is important to note that absent or reversed end diastolic blood flow in the umbilical artery represented a gradient of fetal insult which may affect neurological development. Comparison of outcome between the absent or reversed end diastolic blood flow in the umbilical artery and normal end diastolic flow twins may be a better indicator of the long term sequelae of placental vascular compromise.

Memory and mathematical skills:

In our study, the immediate verbal recall score (short term memory) was affected in the lighter twin but the score for delayed verbal recall (long term memory) was not affected. Similarly, the quantitative reasoning score (mathematical skills) was affected in the lighter twin. It is

recognised that different parts of the brain are responsible for different functions. As intrauterine growth restriction affected memory and mathematical skills in our study, this result was further explored.

Previous studies have shown that fetuses with intrauterine growth restriction have long term cognitive impairments and learning difficulties in school (Geva et al., 2005; Hollo et al., 2002; Low et al., 1992). Two areas are thought to be altered due to intrauterine growth restriction which affects memory. The first is the hippocampal region. Animal studies of intrauterine growth restriction have shown specific susceptibility and alterations of the hippocampal formation and its related neural structures. Intrauterine growth restriction in these models was induced by a period of reduced placental blood flow during the second half of pregnancy. Further examination of hippocampal area showed reduced numbers of neurons in the hippocampus and the cerebellum in conjunction with retarded dendritic and axonal growth within these structures (Dieni and Rees, 2003; Mallard et al., 2000; Cintra et al., 1997). Histological and anatomical findings in primates and humans have indicated that the hippocampus matures early during pregnancy (Kostovic et al., 1989) and is susceptible to prenatal compromise (Isaacs et al., 2003). Alterations in hippocampal formation causes a difficulty in declarative memory, such as a reduced capacity for acquisition and recall of word lists (Cohen et al., 1993).

The second area is the limbic and frontal lobe. Studies suggest that this area are susceptible to intrauterine growth restriction (Makhoul et al., 2004). Limbic and frontal susceptibility would predict executive-attention related memory difficulty that predominantly impedes short-term memory functions (Geva et al., 2006; Vakil et al., 2004). Geva et al showed that memory profile of children born with intrauterine growth restriction is characterised primarily by a short-term memory deficit that does not necessarily comply with a typical hippocampal deficit, but rather may reflect an executive short-term memory deficit characteristic of anterior hippocampal–prefrontal network (Geva et al., 2006).

Regarding quantitative reasoning, Westwood et al (1983) reported significantly lower IQ scores among 13 to 19 year old children who had severe intrauterine growth restriction but this difference was not significant after controlling for socio-economic status of the families. There were however, significant differences on arithmetic achievement scores (Westwood et al., 1983). Similarly, Lagerstrom examined the outcome of intrauterine growth restriction children at 13 years of age. Seven children in their cohort of 780 children were born at term gestational age and weighed less than 2.5 kg at birth. At 13 years of age, these 7 children had significantly poorer scores on measures of school performance, including intelligence, language, and mathematics (Lagerstrom et al., 1991). The reason why mathematical skills could be affected is probably because growth restriction affects the intraparietal sulcus, which is responsible for numerosity. Whenever we engage in calculation, the left and right intraparietal regions of the brain are systematically activated (Dehaene et al., 2003; Eger et al., 2003; Dehaene et al., 1999).

The above findings suggest that perhaps, intrauterine growth restriction may not affect cognition globally but certain parts like prefrontal cortex and intraparietal sulcus are more vulnerable than the others parts.

5.2.2 Auxology

In our study, differences in weight between the twins persisted. Although the lighter twin remained small, considerable catch-up growth had decreased the intra-twin weight discrepancy from a mean of 28% at birth to 8% at the time of examination. We were unable to investigate the differences in measures of length and head size, as we did not have these measurements robustly recorded at birth.

Our results are similar to the results from previous studies (Goyen et al, 2003, Ylitalo et al 1988, Reolan et al 2007). However, our findings are in contrast to the findings from Bellido-Gonzalez et al (2007) who showed that the differences existing at birth between the co-twins in weight,

height and head circumference diminished from the age of 2 years and disappeared by the age of 4 years. This different finding could possibly be due to a small sample in their cohort and selection bias.

5.2.3 Behaviour

Although the lighter twin was on average reported to have more borderline behavioural abnormalities as compared to the heavier twin, there was no difference in the number of twins with abnormal behaviours. Lower birth weight was not found to have a significant effect on behaviour. This result is different from the result obtained by Van Os et al (2001) and Hultman et al (2007). Van Os et al in the Netherlands, examined 324 monozygotic twins using Child Behaviour Check List at a mean age of 10 years (Van Os et al., 2001). Low birth weight was found to have a negative relationship with child behavioural problems. They therefore concluded that low birth weight is a causal risk factor for child behavioural problems. The possible reasons for contrasting findings between this study and our study are that the child problem behaviour was assessed using only parental reporting, which only is one dimension of problem behaviour. A useful addition would have been teacher-derived like our study and different pattern of associations with birth weight could have emerged. The age range of the children was wide, from 6 to 17 years and there was an element of selection bias as the sample represented only 50% of all eligible individuals. Finally, paired analysis was used to analyse data and the sample size was too small when the group with significant levels of Child Behaviour Check List discordance was analysed.

In another study, Hultman et al studied 1,480 twin pairs born between 1985-1986 at age 8 to 9 years and 13 to 14 years (Hultman et al., 2007). They used a dichotomous approach for birth weight discordance either >400 g or 15% difference between twins. The lighter twin in birth weight discordant pairs had on average a 13% higher attention deficit hyperactive disorder symptom score at age 8 to 9 years ($p = 0.006$) and 12% higher attention deficit hyperactive disorder score at age 13 to 14 years ($p = 0.018$)

compared with the heavier twin. They concluded that low birth weight is associated with the development of attention deficit hyperactive disorder symptoms, and fetal growth restriction seemed to represent a modest but fairly consistent environmental influence on the development of attention deficit hyperactive disorder symptoms. Again the reasons for contrasting findings could be due to the reliance on the parental report only for the diagnostic assessment of attention deficit hyperactive disorder symptom score rather than multiple informants. The sample size was too small when the group with higher attention deficit hyperactive disorder symptom score were analysed.

5.3 Outcome of non participants

Cases with Cerebral palsy: There were 3 children (lighter twins) who were known to have cerebral palsy during our study period. We excluded these twin pairs where one of them had cerebral palsy from our study analysis as we were interested to determine effects of growth restriction and children with neurological impairments were unable to be tested using British Ability Scales. However, in order to determine the outcome of growth discordant monochorionic twins, we used this information. It was interesting to note that out of 66 twins with more than 20% birth weight discordance, only 3 children developed cerebral palsy and all 3 pregnancies were complicated by twin-twin transfusion syndrome. Two children with cerebral palsy were not severely discordant for birth weight. It is therefore likely that this was related to a combination of twin to twin transfusion syndrome and premature birth.

Eligible twins not recruited in the study: It is impossible to speculate about the general conceptual ability scores of twins who did not take part in the study. However none of them had any documented neurological impairments or significant medical history. Therefore based on our study results, we assume that the general conceptual ability scores for these children would probably be within the normal range for their age. Moreover, there were no differences regarding gestational age, birth

weight, and sex and it is unlikely that the missing data introduced sufficient bias to alter our study results.

5.4 Strengths and limitations of the current study

Strengths

There are several strengths of this study in relation to other similar studies. The cognitive assessment of only monochorionic twins who are prone for growth restriction using twin specific regression analysis was able to truly quantify effects of growth restriction. By undertaking a population based study we avoided selection bias. The sample size was adequate. Cognition was assessed by a single assessor who was blinded to the study groups. Auxology was assessed by two people and inter observer variability was minimal. Antenatal details including accurate determination of gestational age, diagnosis of chorionicity and details of umbilical artery Doppler abnormality details were available. We also examined the course in the neonatal period and ruled out any significant medical history via the general practitioner that may affect the cognitive outcome apart from birth weight. Moreover, Quick Neurological Screening Test was also used to confirm that participants did not have any neurological impairment at the time of cognitive assessments. The age range at the time of assessment was not wide. Generalised estimating equations were used to analyse both within and between twin effects on cognition.

Another important strength of this study is the use of population based registers which are valuable as a case identification mechanism. As population based information on the long-term outcome of growth discordant monochorionic twins is lacking, there is little accurate information for parents or health professionals. The information provided by the study will be of great importance for health professionals and parents in planning antenatal management of twin pregnancies and the future care of children from twin pregnancies and family. We believe that our study has advanced this under-researched topic.

Limitations

Since this was a retrospective study, we were not able to assess growth and neurodevelopment of these twins sequentially from birth. Also we did not have good measures at birth of length, head circumference, or mid-arm circumference. There were only 51 pairs in total and 41 for sensitivity analyses which might have underpowered the analysis. Comparison group consisting of monozygotic twins with <20% discordance would have elicited the effects of genetics on cognition. However, due to time constraints, we are unable to recruit these cohorts and were unable to gain further information.

Potential for bias also existed during assessments as the PI was aware of the hypothesis and although blind for birth weight, the lighter twin remained somatically lighter at the time of assessment. In many cases this difference was not easily apparent and in every case, measurements were taken by the PI or by a different person, only after the cognitive assessment had been completed. However, in some cases, it was easier to identify the lighter twin. To minimise bias, all the tasks in the study were administered according to the rules of testing.

Finally, it is important to note that monozygotic twins do not share all the genetic characters and this is one of the limitations of any study which uses twin model.

5.5 Meaning of the study: possible mechanisms and implications for clinicians

Cumulative risk models of infant development are guided by two central propositions. First, early risk inherent in the infant's biology or environment carries a lasting effect on the developmental outcome (Rutter, 1987). For example, a compromised neurological profile or environmental adversity, such as poverty or domestic violence, bears long-term negative consequence on children's growth. Secondly, all these risk factors usually exert both cumulative and interactive effects on the development, and the impact of intertwined risk is greater than the sum of each risk experienced independently. Given the multiple antenatal and postnatal factors, it is often difficult to analyse the effect of intrauterine growth restriction in isolation. We therefore examined the effect of growth restriction using a model of discordant twins without congenital anomalies or neurological impairments. In doing so, the underlying factor would much more likely relate to placental nutritional compromise and its consequences. The outcome is also likely controlled for many (but not all) in-utero factors, genetic and environmental factors.

We chose early childhood to assess cognition as the period between 2 and 5 years marks a stage of significant growth in children's cognitive, social and emotional skills (Sternberg, 1999; Case, 1992). Global cognitive development is complemented at the same time by the development of neuropsychological skills. The development of executive functions, the integrative aspects of the neuropsychological skill, is particularly important before school entry and reflects the maturation of the prefrontal cortex during the preschool years (Posner, 2002). Children's interactions with their parents increase as they grow old, and preschool-aged children also start adapting to the systems and rules of the society (Feldman and Eidelman, 2009). The preschool years also signify an important time in the

development of premature infants, as they often show significant catch-up of physical and mental growth by school age (Sullivan et al., 2008; Hack et al., 2005). In light of these developments, it was important to assess whether any abnormalities noted in twins across infancy persist into later childhood or whether they attenuate as children mature, gain independence, and acquire new cognitive and social competencies.

The time when nutrition has the greatest effect on brain development, is during the perinatal period. This is usually considered in humans to include the third trimester of pregnancy and the first few months of postnatal life (Dobbing and Sands, 1973). During this period of growth, neural events occur according to a well-established (de Graaf-Peters and Hadders-Algra, 2006) so that the effects of under nutrition will depend, to some extent, on when they take place. For example, during the first months of human gestational age, the brain cells that are being produced are almost all neurons whilst, after 25 weeks, glial cells predominate (Herschkowitz, 1988). Nutrition may also have a role to play in brain physiology by affecting both the level and operation of various neurotransmitters. Greenwood et al showed that there are at least three important ways in which diet may affect neurochemistry (Greenwood and Craig, 1987). First, nutrition affects the availability of the precursors required for the synthesis of neurotransmitters. Second, nutrition is the source of the vitamins and minerals that are essential co-factors for the enzymes that synthesize neurotransmitters. Third, dietary fats alter the composition of the nerve cell membrane and myelin sheath, and that in turn, influences neuronal function. Glucose, the main metabolic fuel of the brain could also influence cognitive function as well (Benton et al., 2003). Therefore any changes to the basic neural architecture brought about by under nutrition are likely to be long-term.

Other possible mechanisms through which under nutrition can affect cognition includes a direct effect on brain growth or through some other intervening factor like lack of certain essential amino acids. Other

mechanism is through damage to the developing brain might induce an abnormal growth pattern through endocrine or other pathways. Finally, growth restriction increases the child's vulnerability to other extrinsic factors such as perinatal hypoxia or postnatal hypoglycaemia.

Herschkowitz also showed that the basic mechanisms underlying specific events in the course of neural development during the prenatal period are genetically determined (Herschkowitz, 1988). However, epigenetic and environmental factors can modulate brain development at every stage. Monozygotic fetuses with identical genetic make-up exposed to different nutritional regimes during gestational age, can start to diverge in their neural development. Our study suggests that intra uterine nutrition acts as such a factor as we found that growth restriction significantly affects general conceptual ability.

To determine the effects of nutrition on neurodevelopment, Lucas et al randomised 926 preterm infants to either a high-nutrient formula designed to meet the increased needs of prematurity by fuelling more rapid somatic and brain growth or standard-nutrient diet (term formula or banked donor breast milk) for an average of 4 weeks in infancy. They found that children fed the high-nutrient diet outperformed those receiving the standard-nutrient diet on measures of neurodevelopment and IQ at age 9 months, 18 months, and 7.5 to 8 years (Lucas et al., 1998; Morley and Lucas, 1993; Lucas et al., 1990; Lucas et al., 1984). The major effect of early nutrition on cognition was seen in males and there was also a selective effect on Verbal IQ than Performance IQ. They therefore suggested that early nutrition had a long-term impact on cognitive performance. This study was designed to test the vulnerability of the human brain to suboptimal nutrition and showed that even a short period of dietary intervention after preterm birth was related to significant effects on intelligence scores at adolescence. Analysis of the brain MRI scans in a subset of the above cohort demonstrated significant differences in the volume of the caudate nuclei between those fed a high-nutrient diet

and those fed a standard-nutrient diet (Isaacs et al., 2008). This study illustrates that a brief period of dietary intervention in infancy, has major effects on IQ even in adolescence. This suggests that early nutrition has permanent effects on cognition concurring with our study results.

However, it is important to also note that the human brain is fairly resistant to the effects of under-nutrition and the fact that despite being below the 10th centile for birth weight, the majority of the twins had their general conceptual ability within the normal range. Hammond put forward in his theory of “priority of partition of nutrients” that fetuses as a whole have a first priority because of their high metabolic rate (Hammond, 1944.). According to this theory, a nutritional state in which the blood content of specific nutrients is reduced to a fetus will cause the maternal organism to mobilize nutrients from its own tissues to meet the fetal requirements for maintenance and growth. However, the fetus can be parasitic on the mother only to a certain extent. The somatic tissues of the fetus are perhaps the most sensitive to under-nutrition, being the first to show effects by a reduction in body weight. The reaction of various visceral organs to under-nutrition is more complex. In Wallace’s experiments (Wallace, 1948) on pregnant sheep in which one group was grossly underfed, he found some fetal tissues more severely affected than others. The central nervous system and the heart competed more effectively for available nutrients compared to liver and muscular tissue. This was supported by perinatal death collaborative study data (Fujikura and Froehlich, 1972) in which they found brain weight to be least affected in conditions interfering with somatic growth such as in twinning and preeclampsia, whereas the liver showed a marked reduction in weight concurrent with low body weight. Although brain weight cannot be equated with intelligence, this is still presumptive evidence that the brain is fairly resistant to the effects of under-nutrition in utero.

The results from our study can assist obstetricians in decision process. Intrauterine growth restriction of the fetus due to placental dysfunction is

a major obstetric and neonatal problem. So far no effective therapy has been found to reverse the reduced blood flow of the placenta or to ameliorate it through nutritional mechanisms. Fetal growth restriction provides a major management dilemma to the obstetricians in deciding the optimal time to deliver healthy babies. Delivering babies at the first sign of growth restriction (risk the complications of prematurity), delivering as biophysical markers deteriorate (risk poor brain growth) or delivering at the last possible moment (risk fetal hypoxia due to acute compromise) remains major challenge. Based on the results of our study, birth weight discordance should not be considered as the only factor for contemplating an expedited preterm delivery. Other factors like abnormal umbilical artery Doppler measurements should be taken into account. Also, it is important to establish whether the lighter twin is growth restricted whenever severe discordance is suspected as discordance may not be a sign of growth restriction irrespective of gestational age. Conversely, whenever severe discordance is suspected, it is important to exclude appropriately grown twins. Previous studies support our conclusion. Birth weight discordance was associated significantly with preterm delivery because of unnecessary intervention that led to consequential neonatal morbidity because of prematurity (Hollier et al., 1999). A similar result was seen by Cooperstock et al who found that 16% of preterm births that were associated with a discordance level of 40% were attributable to the presence of a large-for-gestational age rather than to the presence of a growth restricted infant (Cooperstock et al., 2000). Talbot et al also suggest that birth weight discordance alone does not appear routinely to indicate preterm delivery of twins (Talbot et al., 1997).

Another possible clinical implication is the cut-off used for birth weight discordance. As we did not find that birth weight discordance of 25% or more significantly affected the general conceptual ability of the twins, we suggest to use a birth weight discordance value of 20% or more to identify twins that might benefit from intensive follow up with umbilical artery

Doppler flow measurements, rather than allowing the discordance to progress further.

The main message from this study to parents expecting monochorionic twin is that birth weight discordance does not severely affect the cognition of the lighter twin. The majority of lighter twins have a slightly lower general conceptual ability than their co-twins, though their general conceptual ability was within normal range for their age. They were in mainstream schools and doing well, and the differences are not large enough to result in major differences in academic achievement.

The results from our study can be extrapolated to wider singleton population. Our study design of intrapair control comparison and analysis using discordance provided additional insight into the impact of growth restriction on developmental outcome as compared to using birth weight per se in studies of singleton pregnancies.

5.6 Future research.

All these studies examining effects of growth restriction raise the hypothesis that infants who suffer growth restriction during the prenatal period are likely to be deprived of an optimal supply of nutritional substrates and therefore at risk of impaired neural and cognitive development. Development of strategies for tackling intrauterine growth restriction remains an important area for future focus. This is of global medical importance given the high prevalence of infants who fail to reach optimal birth weight.

Regarding monochorionic twins per se, there is sufficient evidence to suggest that the lighter twin of growth discordant twins is likely to have a lower cognitive score for their age but this is within the normal range of ability. Future research should concentrate on understanding why certain parts of brain are more susceptible than others to intrauterine growth restriction. This may help clinicians target management appropriately.

Regarding the unique cohort from this study, we plan to track these children into later childhood where we would plan further examination including metabolic outcomes. A larger grant would be submitted to complete this longer term follow up, and would look at markers such as lipid profiles, insulin resistance and epigenetic changes. A study in monochorionic twins will allow us to more precisely examine these effects as inter-twin comparisons will control for genetic and environmental influences. This would be a fantastic opportunity to examine specific issues in the field of “Developmental origins of health and disease”.

6. Summary

The twin situation marks a unique developmental risk that stems from a combination of biological factors which includes being a twin, possible prematurity and birth weight discordance and environmental factors. We used the twin model to determine the long term effects of intrauterine growth restriction. We found significant association between within pair differences in birth weight and general conceptual ability but worsening of birth weight discordance was not associated with worsening of general conceptual ability scores. Growth restriction was also associated with increased prevalence of behavioural problems in the lighter twin than the bigger twin as reported by both teachers and parents but this result was not statistically significant. We therefore concluded that growth restriction in utero was significantly associated with lower cognitive scores in later childhood confirming the long term cognitive effects of growth restriction.

7. Appendices

7.1 People involved in the study

Dr. Ravi S Swamy, Consultant in Neonatal Medicine (PI). Wrote the protocol and information leaflets, recruited participants, performed cognitive assessments, administered Quick Neurological Screening Test to and measured size variables for children at home, analysed data and presented in meetings.

Dr. Murthy Korada, Consultant Paediatrician: (administered Quick Neurological Screening Test to and measured size variables for children who came to hospital)

Dr. Nicholas D Embleton, Consultant Neonatologist (Supervisor, contributed to protocol formation, supervised data analysis and writing of thesis)

Prof. Helen McConachie, Professor of Child Clinical Psychology (Supervisor, contributed to protocol formation, supervised data analysis and writing of thesis)

Dr. Svetlana V Glinianaia, Senior Research Associate. (Contributed towards the study protocol formation)

Dr. Ruth Bell, Clinical Senior Lecturer in Public Health (Contributed towards the study protocol formation)

Dr. Judith M Rankin, Clinical Scientist (Contributed towards the study protocol formation)

Dr. Stephen Sturgiss, Consultant Obstetrician (Contributed towards the study protocol formation)

Dr. Martin Ward Platt, Consultant Neonatologist (Contributed towards the study protocol formation)

Dr. Jane Cookng and Kay Mann, Research group, (Contributed towards statistical analysis)

7.2 Abstracts presented

Abstract accepted for platform presentations at the “**1st World Congress on Twin Pregnancy**” April 16-18th, 2009, Venice, Italy & **Pediatric Academic Society meeting**, May 2-5th, 2009, Baltimore, USA.

7.3 Project documents

Ethics committee approval document

County Durham & Tees Valley 1 Research Ethics Committee

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19 November 2007

Dr. Ravi Swamy
Specialist Registrar in Paediatrics
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Ward 35 - Royal Victoria Infirmary
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Dear Dr. Swamy

Full title of study: Neuro-developmental outcome in twins with birth weight discordance
REC reference number: 07/H0905/88

Thank you for your letter of 16 November 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Committee held on 19 November 2007.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised. The school related information is not relevant as not attending schools.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA. There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Application	V:5.5	11 October 2007
Investigator CV		10 October 2007
Protocol	V:2.4	15 November 2007
Covering Letter		15 November 2007
Peer Review		15 November 2007
Statistician Comments		17 October 2007
Letter of invitation to participant	V:1	10 October 2007
GP/Consultant Information Sheets	V:1	10 October 2007
Participant Information Sheet	V:1.2	15 November 2007
Participant Consent Form	V:1.1	15 November 2007
Response to Request for Further Information		16 November 2007
Supervisor's C.V. - Dr N. Embleton		04 October 2007
Children's Information Leaflet	V.1	10 October 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from
<http://www.rdforum.nhs.uk/rdform.htm>.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

- a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
- b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

- c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

07/H0905/88

**Please quote this number on all
correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr John Drury
Chair

Email: carol.cheesebrough@stees.nhs.uk

Enclosures: Standard approval conditions

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8. Bibliography

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