Variation in growth and the influence of early growth in later life: a twin-sibling study

Frederiek Estourgie-van Burk

VRIJE UNIVERSITEIT

Variation in growth and the influence of early growth in later life: a twin-sibling study

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promotoren: prof.dr. D.I. Boomsma
prof.dr. H.A. Delemarre-van de Waal
prof.dr. W.P.F. Fetter

copromotor: dr. M. Bartels

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Chapter 1

General introduction

Background

Growth is a highly complex process of changes in shape, body composition and distribution of various tissues, which is influenced by biological, psychological and social factors. Different stages of growth have their specific characteristics, etiological factors, and consequences when disturbed or delayed. Physical and mental growth vary widely between individuals, both early in life (prenatal and postnatal) and later in life (during childhood and adolescence). In this thesis the etiology of individual differences during different stages of growth (prenatal, early postnatal, childhood, and adolescence) will be investigated in light of physical and mental development. The main focus is to what extent variation in growth can be explained by genetic and non-genetic ('environmental') factors.
This question is addressed by using a twin-sibling design, including monozygotic and dizygotic twins and their non-twin siblings. An important question when studying growth is whether results based on twin studies can be generalized to the population at large. Twins are born earlier and lighter than non-twins (Gielen et al., 2010). However, mean differences in parameters do not need to imply that the analysis of covariance structures (familial resemblance) is not valid. To empirically address this issue, siblings serve as optimal controls for studying growth in twins.

Growth

In daily practice growth of a child is mostly described in terms of height and weight. To obtain more information on body composition, body mass index (BMI), calculated as weight (kg) divided by height (m) squared, is often used. There are more measures which are used for the assessment of growth (body proportions) such as head circumference, sitting height and arm span.
To compare an individual value to the general population standard deviation scores (SDS) are used. SDS = $(X-X_1)/Sx$, where X is the child's measurement, X_1 is the mean value at the child's age in the general population, and Sx is the standard deviation at a given age in the general population. This score indicates how much the relevant measurement differs from the mean of the reference growth chart expressed by standard

deviation. SDS are a convenient way of comparing an individual or a specific group with the general population. In this thesis SDS are used to compare the data of twins and siblings with the Dutch general population. To correct for gestational age birth weight and length are standardized using Swedish reference data (Niklasson et al., 1991), since Dutch standards for preterm babies are not available.

Fetal growth

The growth rate of a child is highest in utero with a peak velocity in height and weight at approximately 20 and 34 postmenstrual weeks, respectively (Tanner, 1978). The intrauterine environment is of great importance for fetal growth (Bryan & Hindmarsh, 2006). Maternal factors such as size, age, nutrition, ethnicity, parity and smoking determine for a large part the size of the fetus. Also placental size and function play an essential role in fetal growth.
Maternal size regulates the size of the fetus in order to be successfully delivered. For example, a baby of a small mother and a tall father will be relatively small, but after birth it will show catch-up growth towards the genetic target height.
The endocrine regulation concerning fetal growth is complex and hormones, such as insulin, glucocorticoids and insulin-like growth factors (IGFs) are essential for normal growth and development in utero (Fowden & Forhead, 2009).
The growth of the fetus can be monitored with ultrasound, assessing crown-rump length or biparietal diameter in the first trimester and head circumference, abdominal circumference and femur length from week 10 of gestational age onwards (Verburg et al., 2008). In contrast to the prenatal period in which fetal size can only be adjusted from ultrasound images, size at birth can easily and reliably be assessed. At birth, weight is a routine measurement and length and head circumference should be assessed as well. Information on the influences of genes on size at birth comes from family, adoption and twin studies (Brooks et al., 1995; Hur et al., 2005; Levine et al., 1987; Livshits et al., 2000; Tanner, 1978; Van Baal & Boomsma, 1998; Van Dommelen et al., 2004a). As noted earlier, the intrauterine environment is particularly important, leaving a minor role for genetic effects. Variance in size at birth is, besides the length of gestation and the intrauterine environment, accounted for by small but significant

genetic influences. For example, Van Dommelen et al. (2004a) found that the variance in length and weight at birth is mostly explained by gestational age and environmental factors, genetic factors accounting for only 10 to 24% of the variance in length and weight (Table 1.1). Length at birth correlates poorly with adult height (0.3), but during the first years of life the correlation rises steeply (0.8 at age 2 years), reflecting maternal control of newborn size and the increasing importance of genes in childhood (Tanner, 1978).

Table 1.1. Standardized estimates of variance components for length and weight at birth in males and females (Van Dommelen et al., 2004a).

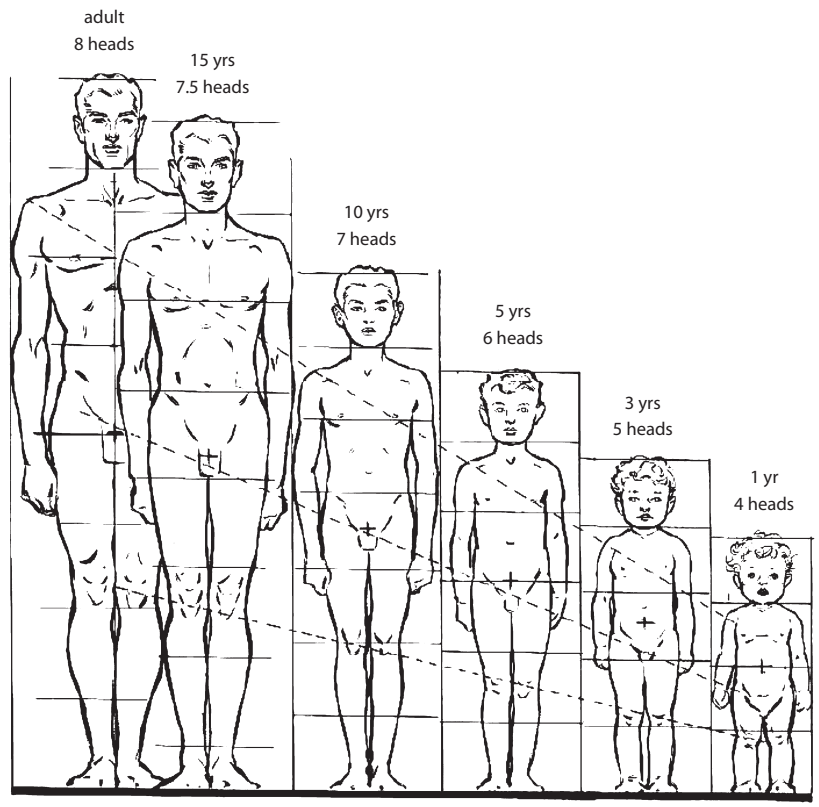
	Birth length	Birth weight
	males/females	males/females
Gestational age	39/38%	38/40%
Common environmental factors	27/30%	21/27%
Unique environmental factors	20/22%	17/20%
Genetic factors	15/10%	24/14%

Postnatal growth

During infancy the growth rate is still high but decreases to a lower stable velocity in childhood. During puberty a transient peak over 1-2 years, the so-called adolescent growth spurt, occurs (Tanner, 1978). The timing of the spurt varies from child to child, and is strictly genetically regulated (Silventoinen et al., 2008a). Therefore it is important to take pubertal development into account when studying growth. Growth in height ceases after puberty, girls attaining adult height at approximately age 18 years and boys around the age of 21 years (Fredriks et al., 2000a). Weight increases gradually during adult life. Body proportions change during growth (Figure 1.1), of which the size of the head is a good example; the length of the head is about $^{1}/_{4th}$ of total body length at birth decreasing to $^{1}/_{8th}$ in adulthood. When studying growth, it is of importance to consider body proportions (e.g. sitting height, arm span) as well, since intrauterine growth restriction or growth disorders may be related to body disproportions. As mentioned earlier, the influence of genetic effects on

growth increases dramatically in infancy, explaining nearly 60% of the variance in height and weight at the age of 2 years (Van Dommelen et al., 2004a). Evidence for the increasing importance of genetic factors also comes from family studies, which showed sibling correlations and parent-offspring correlations to increase during childhood (Biron et al., 1977; Byard et al., 1983a, 1983b; Maes et al., 1997). In adulthood, body size is still a highly heritable trait. Studies in adult twins showed that genetic factors account for 67 to 94% of the variance in height (Akerman & Fischbein, 1992; Fischbein, 1977; Phillips & Matheny, Jr., 1990; Schousboe et al., 2004; Silventoinen et al., 2003b) and for 64 to 90% of the variance in BMI (Akerman et al., 1992; Pietilainen et al., 2002; Plomin et al., 2001; Stunkard et al., 1986).

Figure 1.1. Body proportions during growth (Loomis, 1971).



Growth in twins

Information on causes of individual differences in fetal and postnatal growth is often obtained from twin studies. However, the use of the twin design to study growth, especially fetal and early postnatal growth, has been questioned due to the intrauterine growth restriction and smaller size at birth of twins. Even growth later in life could be different from that in singletons and thus may influence the results.

Twins experience intrauterine growth restriction starting early in the third trimester and are born after a shorter gestational age (mean 37 weeks) (Alexander et al., 1998; Glinianaia et al., 2000; Kiely, 1990; Liu & Blair, 2002; Min et al., 2000). The intrauterine growth restriction in multiple gestations is very likely due to poor early placental development (placental crowding of the uterus) resulting in lower placental weights from about 24 weeks onwards (Bleker et al., 1988). As a result twins are smaller at birth than singletons even after allowing for the shorter gestational age.

A study in Dutch twins showed that differences in body size between twins and infants from the general population decrease during infancy, but do not completely disappear despite correcting for gestational age. At the age of 2 years, twin height is 0.3 SD below the reference population, while the BMI is nearly at the level of the reference population (Van Dommelen et al., 2004b). Several studies have shown that these differences in body size disappeared during childhood, but in a few studies differences remained until adulthood (Buckler & Green, 2004; Luke et al., 1995; Moilanen & Rantakallio, 1989; Pietilainen et al., 1999; Wilson, 1979).

The Louisville Twin Study concluded that the effects of prenatal growth suppression on weight and height appeared to be fully dissipated by age 8 (Wilson, 1979), while a Finnish study amongst 17-year-old twins reported that twins were as tall as singletons, but that boy twins were still leaner (Pietilainen et al., 1999). In all of these studies growth of twins was compared with growth in unrelated singleton controls or to population standards. It has been demonstrated that mothers of dizygotic twins are taller and heavier than mothers of monozygotic twins (Hoekstra et al., 2010). Therefore, an optimal study design is to compare twins with their own siblings, who share, in addition to the same parents, also the family environment, such as socioeconomic status and diet. In this thesis

the body size of twins will be compared with that of their sibling and to population standards in order to find out whether differences in body size disappear during childhood or adolescence.

Influence of early growth in later life

Fetal origins hypothesis

A number of studies, initiated by Professor David Barker (1986) have demonstrated associations between lower birth weight and (risk factors for) disease in later life, such as hypertension, dyslipidemia and insulin resistance (Barker et al., 1990, 1993; Hales et al., 1991). The 'thrifty phenotype' or 'fetal programming' hypothesis from Barker's group postulates that intrauterine conditions may influence postnatal growth, development and disease in later life (Barker et al., 1993). Fetal undernutrition may lead to adaptations which permanently program body's structure and function, the so-called 're-programming'. An alternative explanation was put forward by Hattersley & Tooke (1999), proposing that the observed relations between intrauterine environment and disease in later life are not causal, but are mediated by genetic and environmental factors which influence both traits, the so-called thrifty genotype or fetal insulin hypothesis. Support for the latter explanation has recently been found in a study reporting on a genetic component which can partially explain the association between lower birth weight and type 2 diabetes (Freathy et al., 2010). Studies in adolescent twins suggested rather a combined influence of intrauterine and genetic factors on the association between birth weight and glucose metabolism, serum lipids and blood pressure (IJzerman et al, 2005).

Low birth weight has been associated with numerous somatic conditions such as insulin resistance, obesity and adrenal function (e.g. hyperandrogenism), but also neurodevelopmental, psychosocial and behavioral outcomes may be less favorable of children with low birth weight (Saenger et al., 2007).

Hormones such as dehydroepiandrosterone-sulfate (DHEAS) and insulin-like growth factor-I (IGF-I) have been suggested to play a role in the association between early growth and later-life chronic diseases possibly

by enhancing insulin resistance (Ibanez et al., 1999, 2009; Iniguez et al., 2006; Ong et al., 2004a, 2004b; Opdahl et al., 2008; Veening et al., 2004). In this thesis the twin design is used to study the possible role of these hormones in the association between early growth and adult diseases and to differentiate between genetic and environmental factors influencing this association.

Intrauterine growth restriction

Intrauterine growth restriction (IUGR) is commonly used to describe the pathological process that prevents the fetus to achieve its growth potential. IUGR may thus lead to newborn infants with a smaller size than expected for gestational age. IUGR is commonly classified as symmetrical versus asymmetrical (Wollmann, 1998). Asymmetrical IUGR develops when oxygen or substrate supply to the fetus is reduced during the last trimester of pregnancy due to a reduced functional capacity of the placenta. Skeletal growth and brain growth are less affected and these babies usually have a relatively normal head circumference and a slightly reduced length, but a significantly reduced weight. Asymmetrical IUGR is the most frequent type and is from the view of the baby of an extrinsic origin. Maternal conditions (e.g. preeclampsia, smoking) and placental factors (infarction, multiple pregnancy) are responsible for the asymmetrical IUGR. The symmetrical or intrinsic type of IUGR is present in early pregnancy, whereby the growth of the head, femur and abdomen is equally affected. A wide range of different causes like genetic diseases, fetal infections, congenital syndromes and toxic effects might create this type of growth restriction. The IUGR implied by the fetal origins hypothesis is of the asymmetrical type, which is thought to be a consequence of inadequate provision of nutrients and/or oxygen across the placenta. Most studies investigating the fetal origins hypothesis use birth weight as a marker of IUGR, because birth weight is readily available. Birth length and ponderal index (kg/m^3) are also commonly used features.

Catch-up growth

Catch-up growth is a growth velocity greater than the median for chronological age and gender (Saenger et al., 2007). Children with an asymmetrical type of IUGR show frequently catch-up growth after birth, in contrast to children with an IUGR of intrinsic origin. Catch-up growth is typically an early postnatal process that, in most infants, is completed by the age of 2 years. Early postnatal growth appears to play a critical role in the association between fetal growth and adult disease risks. Insulin sensitivity was reduced in children born small for gestational age, especially in children with catch-up growth (Soto et al., 2003; Veening et al., 2002). Furthermore, catch-up growth has been shown to be a risk factor to develop obesity (Monteiro & Victora, 2005), hypertension (Huxley et al., 2000), coronary heart disease (Eriksson et al., 1999) and type 2 diabetes (Forsen et al., 2000). A study in zebra finches demonstrated that the level of compensatory growth following a period of poor nutrition was associated with long-term negative consequences for cognitive function (Fisher et al., 2006). Therefore, one of our objectives was to find out whether catch-up growth in twins affects cognitive function in later life.
Catch-up growth can be expressed in terms of weight or height gain. In the fetal origins hypothesis compensatory weight growth plays an important role, rather than catch-up growth in height. In this thesis the definition (change in weight SDS between 0-2 years) proposed by Ong et al. (2000) is used, whereby a gain in SDS greater than 0.67 is considered as clinically significant catch-up growth, as 0.67 SDS represent the width of each percentile band on British growth charts. A recent study showed that the influence of additive genetic factors on the variation in postnatal weight gain, calculated as the change in weight SDS between birth and assessment at age 7-11 years, was high (80%) (Beardsall et al., 2009).

The current thesis

Sample

In the current thesis data from a group of twins and their non-twin siblings are analyzed to gain insight into the causes of individual differences in growth throughout childhood into adolescence. A longitudinal study into the development of cognition and behavioral problems was initiated in 1992 with the recruitment of 209 5-year-old twin pairs from the Netherlands Twin Register (Bartels et al., 2007; Boomsma et al., 2006). These twin pairs were followed up at the age of 7, 10 and 12 years, which resulted in three PhD theses (Bartels, 2003; Rietveld, 2003; Van Baal, 1997). At the age of 18 years these twin pairs were invited to participate for the fifth time, and asked to bring a sibling as well. Of the original sample 122 families participated (58%), so another 64 families had to be recruited to obtain a sufficient sample size. Subjects who continued to participate at age 18 years had higher mean verbal and nonverbal IQ scores at age 5 years compared with subjects who did no longer take part when they were 18 years old ($p<0.05$). There were no differences in birth weight, birth length or height and BMI at age 5 years between subjects who continued to participate at age 18 years and those who did no longer take part at age 18 years ($p>0.05$). For this fifth assessment the Department of Biological Psychology of the VU collaborated with the Department of Pediatric Endocrinology of the VU University Medical Center. A psychological study protocol including the follow-up study of behavioral problems and cognition was developed and used at the department of Biological Psychology (Hoekstra, 2007b). The physical development data, used in the current thesis, were collected at the Department of Pediatric Endocrinology.

Study protocol

When the twins were 18 years of age, they were invited with their siblings to come to the VU University in Amsterdam for the testing day. This fifth data collection took place between 24 November 2004 and 19 July 2006. In the morning I performed the medical protocol at the Department of Pediatric Endocrinology of the VU University Medical Center and in the afternoon Rosa Hoekstra carried out the psychological protocol at the

Department of Biological Psychology of the VU University. Information on the measures that were collected at the fifth measurement occasion can be found in Table 1.2 and in Appendix I.

Table 1.2. Overview of all measures assessed as part of the medical protocol in 18-year-old twins and their siblings.

Anthropometry	**Biological materials**
Height	Blood
Weight	Urine
Sitting height	Saliva
Arm span	Buccal swabs (DNA)
Head circumference	**Laboratory assessments**
Waist circumference	*Blood*
Hip circumference	Full blood count
Skinfold thickness	HbA1c
Pubertal development (Tanner stage)	Glucose
Breast development	Insulin
Pubic hair development	Lipid profile
Genital development	Triglyceride
Testis volume	CRP
	DHEAS
NHS growth data	IGF-I
Height	RNA expression (±challenged by LPS)
Weight	Lymphocyte isolation
Head circumference	*Saliva*
Questionnaires	Cortisol
Alcohol consumption	Testosterone
Pubertal development (DHBQ*)	**Cardiovascular function**
Menarche and details of menstrual cycle	Systolic and diastolic blood pressure
NTR Biobank	Heart rate
Maternal report about pre-, peri- and neonatal period of the twins and their sibling	Heart rate variability (VU-AMS)

* = Dutch Health and Behavior Questionnaire (Bartels et al., 2011)

Designs used

Monozygotic (MZ) twins are genetically identical, while dizygotic (DZ) twins and siblings share on average 50% of their segregating genes.
A higher observed similarity of MZ versus DZ twin pairs is an indication for genetic influences on the trait studied (Martin et al., 1997).
The twin design also enables the study of environmental factors, which are shared or non-shared by members of a twin pair. Shared or common environmental influences include for example socio-economic status and nutrition. Non-shared or unique environmental factors are the influences not shared by members of a twin pair, such as illness, but also include measurement error.
Similarity among twins and siblings can be expressed in correlations or covariances and the correlation matrix among family members for uni- or multivariate traits usually forms the starting point to test different models that aim to explain individual differences in the phenotype of interest. Structural equation modeling (SEM) is often used in genetic analyses to test different genetic models. In the final model that best fits the data, the total variation in the trait under study is decomposed into latent sources of genetic and shared or non-shared environmental variance. Based on this information it is possible to calculate the heritability of a trait as the genetic variance divided by the total phenotypic variance. In a design with multiple phenotypes, the covariance between the traits under study can be decomposed into genetic and environmental influences as well.
In the current study the design is extended by adding non-twin siblings. Adding these family members has several advantages. First, an increase in power to estimate the variance/covariance components is obtained (Posthuma & Boomsma, 2000). Second, non-twin siblings are the optimal controls for twins. In this thesis it is tested whether the mean and variance of body size and hormone levels differ between twins and siblings. Third, the design allows identification of twin-specific environmental influences, by testing whether the resemblance between twins equals that between (twin-)siblings by constraining the twin-sibling covariance to equal the DZ covariance.
Intra-pair analyses are an elegant way to study an association, as the co-twin provides the ideally matched control and no confounding factors

such as maternal height, parental social class or diet, have to be controlled for (Boomsma et al., 2005). For example, is the smallest twin also the one with the highest disease risk in later life? In addition, by differentiating between MZ pairs, who share 100% of their genotype, and DZ pairs, who share 50% of their genotype, the effects influencing the association may become clear. A larger association of intra-pair differences in DZ than in MZ twin pairs proves that the association is mediated by genetic factors.

Outline of this thesis

In Chapter 2 we use maternal reported height and weight data which are available from a large questionnaire study in 5-year-old twins. This chapter reports on the causes of individual differences in height, weight and BMI around the age of 5 years. In addition, we examine whether the results of twin studies can be expanded to the singleton population by comparing the data from twins with Dutch reference growth data and by looking at the twins' target height, which is derived from parental height.
Chapter 3 focuses on the question whether the differences in size at birth between twins and their non-twin siblings disappear in time and whether twins attain an average adult height and BMI compared with population standards.
In chapter 4 the genetic and environmental contributions to variation in human testis size are estimated in a group of 18-year-old twins and their non-twin brothers.
In chapter 5 the genetic influences on the variation in DHEAS, IGF-I and insulin levels in late adolescence are explored. In addition, the association between birth weight and DHEAS/IGF-I levels is studied taking catch-up growth and current body size into account. Furthermore, the association between insulin and DHEAS/IGF-I levels is analyzed.
Chapter 6 addresses the question whether catch-up growth has long-term negative consequences for cognitive function in a sample of twins at ages 12 and 18 years.
Chapter 7 presents the general discussion and future directions. This thesis ends with a summary in chapter 8.

Chapter 2

Body size in five-year-old twins: heritability and comparison to singleton standards

Estourgie-van Burk, G.F.
Bartels, M.
Van Beijsterveldt, C.E.M.
Delemarre-van de Waal, H.A.
Boomsma, D.I.
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Abstract

The aim of this study is to examine causes of individual differences in height, weight and BMI in 5-year-old children registered with the Netherlands Twin Register. In addition, we examine whether the results of twin studies can be expanded to the singleton population by comparing the data from twins to Dutch reference growth data and by looking at the twins' target height, which was derived from parental height.
For 2,996 five-year old twin pairs, information on height and weight and on parental height was available. Univariate and bivariate genetic analyses of height and weight and univariate analyses of BMI were conducted.
In order to compare the twins to the singleton population standard deviation scores (SDS) for height, BMI and target height were calculated based on Dutch reference growth charts for the general population from 1997. Genetic influences were an important source of variation in height, weight and BMI and the main source of covariation between height and weight. Additive genetic factors accounted for 69% and 66% of the individual differences in height in boys and girls, respectively. For weight, heritability estimates were 59% in boys and 78% in girls and for BMI 34% and 74%. The influence of common environment on height was 25% and 27%, on weight 24% and 10% and on BMI 44% and 12% in boys and girls. The bivariate model showed a large overlap between the genes influencing height and weight. Genes explain 78% (in boys) and 76% (in girls) of the covariance between weight and height. At the age of 5 years, female twins were as tall as singleton children, while male twins were shorter than singletons. For both boys and girls, however, mean height SDS was 0.6 SDS below the mean target height. All twins had lower BMI than singletons. Twins grow fairly well compared to singletons, but they grow below their target height. This may be due to the above-average height of twin parents.

Introduction

Growth during fetal life, childhood and adolescence is influenced by many factors. Size at birth depends, in addition to the length of gestation, on the intrauterine environment and on small but significant influence of genetic factors (Tanner, 1978). During infancy gestational age and fetal growth are still important factors of growth, but as their influence decreases, genetic factors become more important (Levine, et al., 1987; Van Dommelen et al., 2004). Genetic effects redirect growth towards the genetic target level in childhood. Height is a highly heritable trait. Van Dommelen et al. (2004a) studied the height and weight process in Dutch twins during the first 2.5 years of life. They found that the variance in length at birth is mostly explained by gestational age and common environmental factors. At birth genetic factors account for only 10 to 15% of the variance in length, increasing to 52-58% at the age of 2 years, when the influence of gestational age has almost disappeared. Evidence for the increasing importance of genetic factors also comes from a large longitudinal family study, which showed sibling correlations to increase from one (.4) to four years of age (.53) (Byard et al., 1983a). The Louisville twin study showed that at the age of 6 years genetic factors account for 94% of the variance in height. During puberty intra-pair similarity in height decreases, but increases again in adulthood, explaining 67% to 94% of the variation in height (Akerman & Fischbein, 1992; Fischbein, 1977; Phillips et al., 1990; Schousboe et al., 2004; Silventoinen et al., 2003b).
Variance in weight at birth is, like birth length, mainly explained by gestational age and common environmental factors (Hur et al., 2005; Livshits et al., 2000; Van Dommelen et al., 2004a). During infancy genes become increasingly important in determining weight, explaining nearly 60% of the variance at 2 years of age, while the influence of gestational age is reduced to practically none. At the age of 4-5 years genetic factors explain 61-74% of the variance in weight (corrected for height) (Koeppen-Schomerus et al., 2001; Wilson, 1979). In adolescence and adulthood, genes account for about 64 to 90% of the variation in body mass index (BMI) (Akerman & Fischbein, 1992; Pietilainen et al., 2002; Plomin et al., 2001; Stunkard et al., 1986). Most of these estimates come from twin studies. Family and adoption studies also show that weight and BMI are

heritable traits (Annest et al., 1983; Biron et al., 1977; Burns et al., 1989; Byard et al., 1983b; Treuth et al., 2001). Estimates from these studies range from 17 to 52%. The lower estimates from family studies may be due to the different ages of family members who are included in a family design. The correlation of length at birth with adult height is low. During the first years of life, the correlation rises steeply and by age 5 is of the order of .8 (Tanner, 1978). This would imply that 5-year-olds grow according to their genetic target level. Therefore, it is interesting to study whether genetic effects have become more evident at the age of 5 years and whether influences of environment have decreased compared to younger ages.
It may have become apparent that weight can be expressed in several ways; weight corrected for age; weight corrected for height; and BMI. In most studies, particularly in adolescents and adults, BMI is used because it provides more information on body composition than weight. In children aged 2 years and older, BMI has been recommended as a measure for overweight (Cole et al., 2000; Dietz & Robinson, 1998), and BMI reference charts have been published in several countries, among which The Netherlands (Fredriks et al., 2000b).
We will study the individual differences in height, weight and BMI around the age of 5 years in a univariate genetic analysis. In addition, weight and height will be studied using bivariate analyses in order to investigate whether there is an overlap in genetic effects between height and weight. We will investigate whether the results from this twin study can be generalized to the general population. It is well known that birth length and weight of twins is compromised due to a combination of intra-uterine growth restriction and shorter gestational age (Alexander et al., 1998; Glinianaia et al., 2000; Kiely, 1990; Min et al., 2000). Several studies have shown that these differences in body size between twins and singletons disappear during childhood, but in a few studies differences remain until in adulthood (Buckler & Green, 2004; Luke et al., 1995; Moilanen et al., 1989; Pietilainen et al., 1999; Wilson, 1979). A previous study in 2-year-old Dutch twins demonstrated that height was below the median of the reference population, while the BMI was nearly at the level of the reference population (Van Dommelen et al., 2004b). We will investigate the persistence of twin-singleton differences through early childhood in

relation to the Dutch reference growth charts (Fredriks et al., 2000a). Furthermore, we will study whether 5-year-old twins grow according to their target height, which will be calculated using parental height.

Methods

Procedure & subjects

All data were obtained from the Netherlands Twin Register (NTR) at the Vrije Universiteit in Amsterdam, the Netherlands (Boomsma et al., 1992). For this study data from twins from the birth cohorts 1986-1998 were used. Near the twins' fifth birthday, questionnaires were mailed to the families. Mothers were asked to report up to five different measurements of height and weight of the twins, starting from their third birthday onwards. Information on parental height and weight was collected by questionnaires at the time of registration of the twins. Of the Young NTR, 94% of the twins are registered by their parents within the first year of life. The initial sample was composed of 7051 twin pairs. For 3565 twin pairs at least one measurement between the age of 4.5 and 5.5 years was reported. Many mothers (n=3486) reported measurements beyond the age of 4.5-5.5 years. If height or weight data deviated 2.5 standard deviations from the mean value corrected for age using Dutch reference growth charts from 1997 (Fredriks et al., 2000a), data were checked for data-entry errors in the questionnaire. After exclusion owing to extreme values (n=137 twin pairs), the sample consisted of 3428 twin pairs. Four hundred and twenty-one twin pairs of non-Dutch parents (except if one parent was Dutch and the other West-European) were excluded. This is in line with the criteria used by the Dutch growth study (Fredriks et al., 2000a). Zygosity was determined for 955 same-sex twin pairs by DNA typing or blood group polymorphisms and for all other same-sex twins by questionnaire items on similarity. The agreement between zygosity assigned by the replies to the questions and zygosity determined by DNA markers / blood typing is around 93% (Rietveld et al., 2000). After exclusion owing to missing information on zygosity (n=11 twin pairs), there were 2984 twin pairs for height analysis and 2996 twin pairs for weight analysis. The final

sample for height analysis was composed of 471 MZM (monozygotic males), 512 DZM (dizygotic males), 550 MZF (monozygotic females), 480 DZF (dizygotic females), 491 DOSMF (dizygotic opposite sex male born first) and 480 DOSFM (dizygotic opposite sex female born first) twin pairs. For weight analysis, the final sample was composed of 478 MZM, 517 DZM, 561 MZF, 478 DZF, 499 DOSMF, and 463 DOSFM twin pairs. One hundred and thirty twin pairs were incomplete for height data and 118 twin pairs for weight data. Information on height was available for 2690 fathers and 2748 mothers, and information on weight for 2687 fathers and 2744 mothers.

Reliability of parent-reported height and weight was examined in a subsample of 94 twins, for which both maternal report as laboratory measured height and weight were available (Van Baal et al., 1996). Maximum time between measured and reported date was 3 months. The correlation between laboratory measured and parent-reported data was .96 for height and .92 for weight.

Hereafter, height, weight and BMI between 4.5 and 5.5 years of age will be referred to as just height, weight and BMI.

Genetic analyses

The descriptive analyses were performed by using SPSS-12 (SPSS Inc). All analyses were conducted using raw data. Effects of birth order, zygosity and sex on means and variances of height, weight and BMI were tested in univariate (saturated) models using the statistical package Mx (Neale, 1999). Age at measurement was included as a covariate. Twin correlations and the 95% confidence intervals were calculated for the six zygosity groups to get a first impression of the genetic and environmental influences on the variance in height, weight and BMI. We compared DZ same sex correlations to DOS correlations to explore whether the same genes and environmental factors play a role in males as in females. For height and weight, MZ and DZ cross-twin-cross-trait correlations were calculated using a bivariate script.

Genetic analyses were performed using structural equation modeling (SEM) implemented in the statistical package Mx (Neale, 1999). The total variation in height, weight and BMI was decomposed into sources of

additive genetic variance (A), common environmental variance (C) and unique environmental variance (E). A is due to additive genetic effects of different alleles, C is due to common environmental influences shared by members of a twin pair, and E is due to unique environmental influences not shared by members of a twin pair. E also includes measurement error and is therefore always included in the models. A full univariate ACE model was fitted to the height, weight and BMI data. The variance components A, C and E were estimated separately for males and females. We tested whether A, C and E for males and females could be constrained to be equal. Significance of the A and C component was tested by dropping the component from the model. Submodels were compared to the full ACE model using the likelihood ratio test. Genetic and environmental influences on height and weight were estimated using a full bivariate ACE model which decomposes the variance of each measured variable and the covariance between the measured variables into genetic and environmental sources. Significance of the individual path coefficients was tested by constraining paths to zero. Based on the twin correlations the full ACE model was tested against an AE model for height and weight and for males and females separately.

Twin-singleton comparison

Data from twins were compared to data from the general population using standard deviation scores (SDS). SDS for height and BMI were calculated with the software package Growth analyzer 3 (2004), using the Dutch reference growth charts for the general population from 1997 (Fredriks et al., 2000a, 2000b). SDS = $(X-X_1) / Sx$, where X is the twin's measurement, X_1 is the mean value at the child's age in the general population, and Sx is the standard deviation at a given age in the general population. These scores indicate the standard deviation the relevant measurement differs from the mean of the Dutch reference growth charts from 1997. SDS are used because they are a convenient way of comparing specific groups to the general population. Target height was calculated as the average of father's and mother's height plus 11 cm in boys and minus 2 cm in girls (Fredriks et al., 2000a). Target heights were also converted into SDS. In addition, height was corrected for target height (HcTH = height SDS

minus target height SDS). We also compared maternal and paternal height to reference standards, because twinning rates increase with increasing maternal height (Basso et al., 2004; Reddy et al., 2005). SDS of paternal and maternal height were calculated using the Dutch reference growth charts for the general population from 1980 (Roede & Van Wieringen, 1985). To assess whether twins differed from singletons in terms of height, target height, HcTH and BMI, mean SDS were constrained to zero in Mx. A one-sample t-test in SPSS was used to test whether paternal and maternal height SDS differed significantly from the mean of the general population, i.e. zero.

Results

Genetic analyses

Table 2.1 provides means for height, weight and BMI of first-born and second-born twins separately. Mean age of the sample was 5.14 years. First, we tested the effect of age, birth order, zygosity and sex on mean height, weight and BMI in the univariate saturated models. Age (years) affected height (cm) and weight (kg) significantly ($p<0.05$; $b=7.91$ and $b=2.45$ respectively), while no effect of age was found on BMI ($p=0.77$). Mean height, weight and BMI were significantly lower in second-born twins compared to first-born twins ($p<0.05$). Therefore, means were estimated separately for first-born and second-born twins in the genetic models. Dizygotic same sex twins (DZss) and DOS twins were comparable for mean height ($p=0.29$). MZ twins were significantly shorter than DZ twins ($p<0.05$). Small and significant, but inconsistent differences were shown for weight and BMI between MZ, DZ and DOS twins ($p<0.05$). Boys were significantly taller than girls ($p<0.05$). No sex effect was shown on mean weight and BMI ($p=0.18$ and $p=0.76$ respectively). The variances of height and BMI were not influenced by birth order, zygosity and sex. Regarding weight, we found an inconsistent pattern of effects of birth order, zygosity and sex on the variance, which resulted in a moderate fit of the saturated model compared to the univariate genetic model. The -2 log likelihood of the bivariate saturated model was 55525.878 with 11624 degrees

of freedom. We constrained the cross-trait correlations to be equal in twin 1 and twin 2 and the cross-twin-cross-trait correlations to be equal across twins. This did not worsen the statistical fit ($\Delta\chi^2 = 16.461$, $\Delta df = 12$, $p = 0.17$).

Table 2.1. Mean observed height (cm), weight (kg) and BMI (kg/m²) of first-born and second-born twins respectively.

	Males			Females		
	N*	Mean*	SD*	N*	Mean*	SD*
Height†	1433 / 1440	113.6 / 113.1	5.4 / 5.3	1481 / 1484*	112.9 / 112.6	5.4 / 5.4
Weight†	1456 / 1436	19.5 / 19.1	2.7 / 2.6	1475 / 1507	19.1 / 18.9	2.7 / 2.8
BMI†	1335 / 1322	15.1 / 14.9	1.4 / 1.5	1353 / 1377	15.0 / 14.8	1.5 / 1.6

N = number of individuals
SD = standard deviation
* = first-born / second-born
† = mean height, weight and BMI significantly different in first-born and second-born twins ($p<0.05$)

Table 2.2. Twin correlations for height, weight and BMI, cross-trait correlations (between height and weight within a twin) and cross-twin-cross-trait correlations (between height of one twin and weight of the co-twin) with 95% confidence intervals by zygosity group (age was used as a covariate).

	Height	Weight	BMI	Height-Weight	Cross-twin-cross-trait
MZM	.95 (.94-.96)	.83 (.80-.85)	.79 (.76-.82)	.66 (.62-.71)	.61 (.56-.66)
DZM	.57 (.51-.63)	.52 (.45-.58)	.62 (.56-.66)	.69 (.65-.72)	.34 (.27-.40)
MZF	.93 (.92-.94)	.90 (.88-.91)	.86 (.84-.87)	.69 (.65-.73)	.64 (.60-.69)
DZF	.62 (.56-.67)	.51 (.44-.58)	.53 (.47-.59)	.64 (.60-.69)	.36 (.29-.42)
DOSMF	.61 (.55-.67)	.52 (.45-.58)	.47 (.39-.53)	.69 (.64-.72)	.38 (.31-.44)
DOSFM	.57 (.51-.63)	.42 (.35-.50)	.47 (.39-.53)	.67 (.63-.71)	.34 (.27-.40)

Table 2.2 shows the twin correlations for height, weight and BMI. The higher MZ twin correlations versus DZ twin correlations indicate a large influence of genetic factors on all variables. However, the fact that the MZ correlations are less than twice the DZ correlations demonstrates influences of shared environment as well. Similarity in same sex and opposite sex twin correlations indicates influences of the same underlying set of genes for boys and girls. The correlations between height and weight within an individual are very similar in all zygosity groups.
The cross-twin-cross-trait correlations were calculated to explore the genetic and environmental influences on the observed association between height and weight. As can be seen in Table 2.2 the MZ cross-twin-cross-trait correlations are higher than the DZ cross correlations suggesting that the association between height and weight is at least partly due to genetic factors. However, the MZ cross correlations are not twice as high as the DZ cross correlations, which indicates influence of common environment as well.
Table 2.3 gives the results for the univariate genetic modeling. Univariate full ACE models with sex differences fitted the height, weight and BMI data best. Dropping additive genetic or common environmental factors to zero caused a significant worsening of fit ($p<0.01$).

Table 2.3. Univariate saturated and genetic model fitting results for height, weight and BMI.

	-2LL	df	χ^2	Δdf	c.t.m.	p	AIC
Height							
0. saturated	32724.365	5807					
1. full ACE	32728.554	5819	4.189	12	0	0.980	-19.811
2. no sex differences*	32740.721	5822	12.167	3	1	0.007	6.167
3. AE males	32791.666	5820	63.113	1	1	0.000	61.113
4. AE females	32784.753	5820	56.199	1	1	0.000	54.199
5. CE males	33193.924	5820	465.371	1	1	0.000	465.371
6. CE females	32738.316	5820	9.763	1	1	0.002	7.763
Weight							
0. saturated	25732.995	5765					
1. full ACE	25766.001	5777	33.006	12	0	0.001	9.006
2. no sex differences	25783.856	5780	17.855	3	1	0.000	11.855
3. AE males	25771.422	5778	5.421	1	1	0.020	3.421
4. AE females	25770.061	5778	4.060	1	1	0.044	2.060
5. CE males	25891.498	5778	125.497	1	1	0.000	123.497
6. CE females	25996.740	5778	230.739	1	1	0.000	228.739
BMI							
0. saturated	17704.983	5294					
1. full ACE	17725.814	5306	20.831	12	0	0.053	-3.169
2. no sex differences*	17758.222	5309	32.409	3	1	0.000	26.409
3. AE males	17772.438	5307	46.624	1	1	0.000	44.624
4. AE females	17757.995	5307	32.181	1	1	0.000	30.181
5. CE males	17765.377	5307	39.564	1	1	0.000	37.564
6. CE females	17892.344	5307	166.531	1	1	0.000	164.531

-2LL = -2 log likelihood
df = degrees of freedom
χ^2 = chi-square statistic
Δdf = difference in degrees of freedom
c.t.m. = compared to model
p = probability-value
AIC = Akaike's Information Criteria

A = additive genetic influences
C = common environmental influences
E = unique environmental influences
* = no sex differences in variance component estimates (sex differences in means allowed)

To gain insight into the overlap between height and weight, data were analyzed in a bivariate analysis, for which we used the full ACE model (Table 2.4). The variance components for males and females could not be constrained to be equal ($p<0.01$). Removing the shared environmental factors from the full ACE model significantly worsened the statistical fit for both height and weight in boys and in girls ($p<0.01$). Dropping A, C or E on the covariance between height and weight caused a significant loss of fit in both sexes ($p<0.01$), which indicates an overlap in genetic and environmental factors for height and weight.

Variance and covariance component estimates of the genetic models are provided in Table 2.5, based on the bivariate model (estimates from the univariate and bivariate models for height and weight were similar). Genetic factors explained 74% percent of the variance in BMI females and only 34% of the variance in BMI in males. Common environmental influences were more important in males, explaining 44% of the variance versus 12% in females. For weight, the estimates were more similar in boys and girls. Additive genetic factors accounted for 59% of the variance in boys and 78% in girls, while 24% of the variance in boys and 10% in girls was explained by the common environment. The estimates for height were nearly the same in boys and girls. Additive genetic effects explained 66 and 69% of the variance in height in boys and girls respectively, while the common environment accounted for 25 and 27%. The influence of the unique environment on the variance in height, weight and BMI varied from 5 to 22%. The bivariate model showed that the covariance between height and weight could be mainly explained by additive genetic factors (78 and 76%), while 14 and 16% of the covariance was explained by the common environment. The genetic correlation (r_g) was .84 in males and .70 in females, indicating a large overlap between the two sets of genes. The common environmental correlation (r_c) was .38 and .63 respectively and the unique environmental correlation (r_e) was .58 and .61 respectively.

Table 2.4. Bivariate saturated and genetic model fitting results for height and weight.

	-2LL	df	χ^2	Δdf	c.t.m.	p	AIC
Height-Weight							
0. saturated	55542.339	11636					
1. full ACE	55590.747	11666	48.408	30	0	0.018	-11.592
2. drop A on covariance males	55847.200	11667	256.452	1	1	0.000	254.452
3. drop A on covariance females	55771.887	11667	181.139	1	1	0.000	179.139
4. drop C on covariance males	55599.455	11667	8.707	1	1	0.000	6.707
5. drop C on covariance females	55599.007	11667	8.260	1	1	0.000	6.260
6. drop E on covariance males	55760.317	11667	169.570	1	1	0.000	169.570
7. drop E on covariance females	55822.996	11667	232.248	1	1	0.000	230.248

-2LL = -2 log likelyhood
df = degrees of freedom
χ^2 = chi-square statistic
Δdf = difference in degrees of freedom
c.t.m. = compared to model
p = probability-value
AIC = Akaike's Information Criteria
A = additive genetic influences
C = common environmental influences
E = unique environmental influences

Table 2.5. Standardized and unstandardized estimates of variance and covariance components for height and weight (bivariate model) and of variance components for BMI with 95% confidence intervals in brackets.

	Standardized			*Unstandardized*		
	V_A	V_C	V_E	V_A	V_C	V_E
Height						
M	.69 (.62-.77)	.25 (.18-.33)	.05 (.05-.06)	18.34	6.53	1.40
F	.66 (.56-.74)	.27 (.19-.36)	.07 (.06-.08)	17.35	7.24	1.88
Weight						
M	.59 (.49-.69)	.24 (.15-.33)	.17 (.15-.20)	3.94	1.63	1.16
F	.78 (.67-.85)	.10 (.04-.22)	.12 (.10-.13)	5.63	0.76	0.84
BMI						
M	.34 (.23-.46)	.44 (.33-.54)	.22 (.19-.25)	0.74	0.96	0.47
F	.74 (.67-.80)	.12 (.06-.19)	.14 (.12-.16)	1.68	0.28	0.32
Height-Weight	Cov_A	Cov_C	Cov_E	Cov_A	Cov_C	Cov_E
M	.78 (.69-.87)	.14 (.05-.23)	.08 (.07-.10)	7.14	1.25	0.73
F	.76 (.61-.87)	.16 (.05-.30)	.08 (.07-.10)	6.92	1.47	0.77

Height-Weight	r_g	r_c	r_e
M	.84	.38	.58
F	.70	.63	.61

VA = % of variance explained by additive genetic factors (heritability)
VC = % of variance explained by common environment
VE = % of variance explained by unique environment
Cov_A = % of covariance between height and weight explained by additive genetic factors (heritability)
Cov_C = % of covariance explained by common environment
Cov_E = % of covariance explained by unique environment
M = males
F = females
r_g = genetic correlation
r_c = common environmental correlation
r_e = unique environmental correlation

Twin-singleton comparison

Table 2.6 presents the mean SDS for height, target height, HcTH and BMI. Comparing twins to children from the general population at the age of 5, male twins were significantly shorter ($p<0.05$), while female twins were as tall as singletons ($p=0.072$). All twins had a significantly lower BMI than singletons at the age of 5 years. Mean SDS for maternal and paternal height were 0.26 and 0.10 respectively, implying that twin parents were significantly taller compared to the general population. This result leads to an above-average mean target height SDS for twins with values of 0.45 SDS for male twins and 0.58 SDS for female twins. No effect of zygosity was shown on mean target height SDS ($p=0.86$). Studying height SDS, twin girls were comparable to children from the general population, but when controlling for target height (HcTH) female twins were, like male twins, significantly shorter than singletons ($p<0.05$). Mean HcTH SDS did not differ between boys and girls ($p=0.071$). No zygosity effect was demonstrated on mean HcTH SDS ($p=0.29$).

Table 2.6. Mean standard deviation scores (SDS) for height, height corrected for target height (HcTH) and BMI of first-born and second-born twins respectively; mean SDS for target height.

	Males		**Females**	
	Mean*	**SD***	**Mean***	**SD***
SDS height†	-0.12 / -0.21‡	1.14 / 1.13	0.03 / -0.03	1.13 / 1.12
SDS HcTH†	-0.59 / -0.69‡	1.03 / 1.04	-0.57 / -0.62‡	1.05 / 1.04
SDS BMI†	-0.41 / -0.53‡	1.03 / 1.06	-0.44 / -0.56‡	1.11 / 1.22
SDS target height	0.45‡	0.73	0.58‡	0.79

SD = standard deviations
* = first-born / second-born
† = mean SDS significantly different in first-born and second-born twins ($p<0.05$)
‡ = mean SDS significantly different from 0 ($p<0.05$)

Discussion

The purpose of this study was to estimate the contribution of genetic and environmental influences to the variation in height, weight and BMI at age 5 years and to compare body size of twins to that of singletons. The genetic analyses showed that height, weight and BMI are highly genetic traits. When we compare the results at age 5 years to the results at age 2 years of a previous study in Dutch twins (Van Dommelen et al. 2004a), genetic influences have become more evident. This is in line with our expectation that genetic effects become more important, as children grow older.
For height, heritability increased from 58% to 69% in males and from 52% to 66% in females, from age 2 to 5. The heritability estimates for weight showed an increase from 58% to 78% in females, while the heritability in males remained the same (59%). Shared environmental influences on the individual differences in weight are larger in males than females, which is even more evident in the BMI analysis. The 95% confidence intervals for the weight estimates of boys and girls overlap, while there is no overlap between the intervals of BMI. We did not expect to find these sex differences, based on the hypothesis that infancy and childhood growth is similar in boys and girls (Karlberg, 1989). A large twin study in 4-year-olds found comparable heritability estimates (60%) for weight corrected for height in boys and girls (Koeppen-Schomerus et al., 2001).
The covariance between height and weight was mostly explained by genetic factors. The high genetic correlation between height and weight demonstrates that these two traits are mainly under control of the same additive genetic factors at the age of 5. Focusing on the nature of common environmental influences as another overlapping factor for the association between height and weight, nutrition, family environment (e.g. socio-economic status) and assortative mating are known to influence variance in height and weight (Silventoinen, 2003a, 2003c).
In this study a birth order effect was found on mean height, weight and BMI. First-born twins are slightly taller and heavier than second-born twins at the age of 5 years. Some studies reported a similar result, first-born twins being heavier at birth and at the age of 16 years, but in all these studies no explanation was given (Buckler & Green, 1994; Glinianaia et al., 2000; Pietilainen et al., 2002). It might be that the first-born twin is heavier and taller at birth due to better maternal-fetal nutrition, because its position is lower in the uterus. The differences in size at birth could be an explanation for the differences we noted at the age of 5 years.
Another goal of this study was to compare growth of twins with growth of general population children. Compared to singletons, twins are born substantially smaller with a mean weight deficit of 30% and height deficit of 17% (Wilson, 1979). During infancy differences in body size between twins and infants from the general population decrease, but do not completely disappear despite correcting for gestational age. At the age of 2 years, twin height is 0.3 SD below the reference population, while the BMI is nearly at the level of the reference population (Van Dommelen et al., 2004b). Some studies have shown that differences in body size between twins and singletons disappear at different ages in childhood, but in other studies differences remain until in adulthood (Andrew et al., 2001; Ljung et al., 1977; Moilanen et al., 1989; Pietilainen et al., 1999; Wilson, 1979). Wilson (1979) concluded that the prenatal growth suppression on weight and height had fully disappeared by the age 8 years compared to singleton standards. Two other studies showed that adolescent and adult twins are leaner than singletons, while height seemed to be comparable (Andrew et al., 2001; Pietilainen et al., 1999). Unfortunately, most of these twin studies have not included siblings or mid-parental or target height. One study included siblings and found that twins born appropriate for gestational age (birth weight > 10th percentile for singletons) are as tall as their siblings but lighter in childhood, while twins born small for gestational age (birth weight < 10th percentile for singletons) grow below their target height and singleton standards (Buckler & Buckler, 1987). They also showed mid-parental height to be 0.3 SD above-average, but the study consisted of small numbers and compared children of different ages. This study, comparing twins to singletons, showed that male twins are significantly shorter, while female twins have caught up in height (Table 2.6). We find more marked male twin-singleton differences and also greater shared environmental effects for boys than girls which may suggest perhaps lower generalizability of the findings to the general population for boys than for girls. When looking at height corrected for target height (HcTH), sex differences disappeared and both boys and girls grow below

their target height. Target height of twins is above-average, which can be partially explained by the fact that twin mothers are taller than women from the general population. This is in accordance with previous literature describing that twinning rates increase with increasing maternal height (Basso et al., 2004; Blickstein & Keith, 2005; Reddy et al., 2005). We also found paternal height to be above-average, though to a lesser extent. One explanation for this finding may be assortative mating (Silventoinen et al., 2003c). The above-average target height of twins implies that, although twin growth may be considered (nearly) normal compared to singleton standards, twin growth is restricted in respect to their target height. The growth restriction is unlikely to be of clinical importance, but it is an interesting finding, which needs more study in the future. To explore whether the differences in BMI and HcTH between twins and singletons are of genetic or environmental origin, a longitudinal design including siblings is needed.

Consistent with other studies, twins were significantly lighter than singletons (Buckler & Buckler, 1987; Ljung et al., 1977; Moilanen et al., 1989; Pietilainen et al., 1999; Wilson, 1979). Some studies reported these differences to disappear in childhood, while others showed these differences to remain in adulthood. Our results showed a decrease in BMI compared to the age of 2 (Van Dommelen et al., 2004b). In the light of the increase in BMI and obesity in young children (Hirasing et al., 2001), this is an interesting finding. It may be that the finding is specific to twins, who grow up under environmental conditions in which they always have someone to play with and thus may show increased activity levels, as compared to other children.

Chapter 3

Body size of twins compared with siblings and the general population: from birth to late adolescence

Estourgie-van Burk, G.F.
Bartels, M.
Boomsma, D.I.
Delemarre-van de Waal, H.A.
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Abstract

Objectives Twins are smaller at birth than singletons. We examined when these differences in body size disappear in time and whether twins attain normal final height and body mass index (BMI).

Study design Height, weight and BMI data of twins at ages 1, 4, and 18 years were compared with data from their non-twin siblings. Secondly, twin and sibling data were compared with population standards. In addition to height, weight and BMI, data on body proportions at age 18 years were analyzed.

Results At the age of 18 years twins were as tall as their siblings, but significantly leaner. Compared with children from the general population, adolescent twins attained the same height and BMI. Birth weight was shown to have a considerable effect on height in adolescent twins.

Conclusions Twins attained normal final height compared with siblings and children from the general population. As for BMzI, no differences were shown between 18-year-old twins and children from the general population, whereas the siblings of twins had increased BMI values.

Introduction

Multiple pregnancy rates have increased in many Western countries during the past decades, which is mainly attributable to the older maternal age and increasing number of infertility treatments (Blondel & Kaminski, 2002; Collins, 2007; Tandberg et al., 2007). It is generally known that twins are smaller at birth than singletons due to a combination of intrauterine growth restriction and shorter gestational age (Alexander et al., 1998; Glinianaia et al., 2000; Kiely, 1990; Min et al., 2000). The incidence of twin births in The Netherlands is 1.7% (Statistics Netherlands, 2008), which means that a considerable number of newborns have a reduced size at birth. Intrauterine growth restriction is not only associated with increased neonatal morbidity and mortality (McIntire et al., 1999), but also with an increased prevalence of developing adult diseases such as the metabolic syndrome and cardiovascular disease (Godfrey & Barker, 2000). Several studies have shown that the differences in body size between twins and singletons at birth disappeared during childhood, but in a few studies differences remained until adulthood (Buckler & Green, 2004; Luke et al., 1995; Moilanen et al., 1989; Pietilainen et al., 1999; Wilson, 1979). The Louisville Twin Study concluded that the effects of prenatal growth suppression on weight and height appeared to be fully dissipated by age 8 (Wilson, 1979), while a Finnish study amongst 17-year-old twins reported that twins were as tall as singletons, but that boy twins were still leaner (Pietilainen et al., 1999). We found in a previous study that at the age of 5 years female twins were as tall as singleton children, whilst male twins were still somewhat shorter than children from the general population. Furthermore, five-year-old twins had a lower body mass index (BMI) compared with the general population. In the current study, we examined whether these differences disappeared in time and whether twins attained normal final height and weight.

Therefore, we investigated the growth of twins by comparing them with two different groups. Firstly, data from twins were compared with data from their siblings, which were optimally matched controls as twins and siblings share part of their genetic background and environment. Secondly, twin and sibling data have been compared with population standards.

We furthermore expanded this comparison beyond the general measures of height, weight and BMI by adding specific measures on body proportions, such as sitting height, leg length and arm span. Short children tend to have relatively short legs and tall children relatively long legs (Yun et al., 1995). When studying height, it is of importance to take body proportions into account, as intrauterine growth restriction may be related to body disproportions.
We hypothesized that the differences in size at birth between twins, siblings and children from the general population would decrease during infancy and childhood and would have disappeared in adolescence.

Methods

Subjects

Twin families were recruited from the Netherlands Twin Register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam, the Netherlands (Boomsma et al., 1992, 2006). The study was approved by the Central Committee on Research Involving Human Subjects and the institutional review board of the VU University Medical Center of Amsterdam. Written informed consent was obtained from all participants and also from all parents of underage participants.
As part of a longitudinal project on physical and mental development 184 families of 18-year-old twin pairs and their siblings (N=98) participated in a test protocol (Bartels et al., 2002; Hoekstra et al., 2007a). Mean age at assessment was 18.14 years (SD 0.48) in the twin group and 18.78 years (SD 4.89) in the sibling group (youngest 7 years and oldest 35 years of age). We excluded data for several reasons: congenital anomalies (2 twins); severe growth restriction (1 sibling); non-Dutch parents (3 twin pairs); pregnancy or illness (weight data of 1 twin and 2 siblings); unavailability of weight and BMI reference values (28 siblings); unavailability of arm span reference values (7 twins and 23 siblings). Zygosity of same-sex twin pairs (145 pairs) was determined on the basis of DNA polymorphisms (140 pairs), blood group polymorphisms (4 pairs), or questionnaire items on similarity (1 pair (Rietveld et al., 2000)). The sample comprised 30 MZM

(monozygotic male), 33 DZM (dizygotic male), 44 MZF (monozygotic female), 38 DZF (dizygotic female) and 39 DOS (dizygotic opposite sex) twin pairs and 97 siblings (45% males and 55% females). There were 8 incomplete twin pairs.

Measures

Birth data Information on birth weight and length and gestational age was collected using questionnaires at the time of registration with the NTR, which was shortly after birth of the twins in the majority of the families.
Age 1 and 4 years Twins and siblings were asked to provide the report with growth data measured by the Dutch National Health Services (NHS) from birth to the age of 4 years. We copied the measurements from the NHS report. To obtain height and weight data of twins and their siblings around the age of 1 year, measurements between the age of 0.75 and 1.25 years closest to the age of 1.0 year were selected from the NHS report (mean age 1.00 years, SD 0.06). For height and weight data around the age of 4 years, we selected the NHS measurements between the age of 3.5 and 4.5 years closest to the age of 4.0 years (mean age 3.90 years, SD 0.18).
Age 18 years Around the age of 18 years twins and their siblings were invited to come to our outpatient clinic at the VU University Medical Center of Amsterdam, where height (cm) was determined to the nearest 0.1 cm and weight (kg) to the nearest 0.05 kg using a stadiometer and an electronic scale (SECA, Hanover, Md). Sitting height was measured by bringing the horizontal bar of the microtoise into the most superior midline of the head while the child was sitting in erect position on a special stool. Arching of the back was avoided as much as possible by applying upward pressure to the mastoid processes. Arm span was obtained by measuring the distance between the arms in stretched position using a plastic tape measure.
Pubertal development Stage of puberty was physically determined by the same trained researcher on the basis of secondary sexual characteristics using the stages of development devised by Tanner (1981).
Parental height Information on parental height was collected by measurement if present at the test protocol or by questionnaires. Height was available for all mothers and missing in 12 fathers.

Educational level Since socioeconomic status has shown to be a significant covariable of height and weight, it was taken into account in the linear regression analyses. To obtain a proxy for socioeconomic status of the family, the highest educational attainment level achieved within a family (by the father or mother of the twins) was selected. Information on educational level of the family was based on questionnaire data collected when the twins were 3, 7 and 10 years old. Educational level was classified into three categories: low (primary or lower secondary education), intermediate (higher secondary education) and high (college or university education). The educational level of the family was missing in 8 families.

Data calculations

Weight and length at birth were converted to standard deviation scores (SDS) with correction for gestational age using Swedish reference standards, because Dutch SDS reference values for birth weight and length are unavailable (Niklasson et al., 1991). Birth weight SDS was classified into tertiles in twins and siblings to study the relation with height and BMI SDS at age 1, 4 and 18 years.

BMI was calculated as weight (kg) divided by height (m) squared. Standard deviation scores (SDS) were calculated for height, weight and BMI at ages 1, 4 and 18 with the software package Growth analyser 3 (2004), using the Dutch reference growth charts for the general population from 1997 (Fredriks et al., 2000a, 2000b). Weight and BMI reference values were available up to the age of 21 years. Leg length was obtained by subtracting sitting height from height. SDS were calculated for sitting height and leg length using the Dutch age references from 1997 (Fredriks et al., 2005). Arm span was converted to SDS according to reference data from the Dutch Oosterwolde study (Gerver & Bruin, 2001). Arm span reference values were available up to the age of 18 years. We used the female reference data of 18-year-olds also for females older than 18.5 years, since it is very likely that they are fully grown. We excluded males older than 18.5 years for arm span SDS analysis (7 twins and 23 siblings), while it is known that male growth may continue up to the age of 21 years (Fredriks et al., 2000a).

Statistical analyses

Analyses were performed by using SPSS-15 (SPSS Inc, Chicago) or the structural equation modeling program Mx (Neale et al., 2006). An alpha level 0.05 was chosen for all tests. Univariate models in Mx were used to test for the effect of birth order on birth weight and length SDS and the effects of zygosity and sex on birth weight, birth length, height, weight, BMI, sitting height, leg length and arm span SDS. Mx was used to correct for the dependency among the dependent variables that is present in family data. Next, we tested whether twins differed from siblings in mean SDS. Furthermore, data from twins and siblings were compared with data from the general population using SDS. To this end, we tested whether mean SDS of birth weight, birth length, weight, height, BMI, sitting height, leg length and arm span in twins and siblings differed from the mean of the general population, i.e. zero. The effect of pubertal development on mean SDS at age 18 years was tested by including Tanner stage (genital development or breast development) as a covariate.

The following analyses were performed by using SPSS-15 (SPSS Inc, Chicago). A paired t-test was used to test whether mean gestational age differed between twin pairs and siblings. Linear regression analysis was conducted to analyze whether birth weight and birth length influenced height or BMI SDS at age 18 years in twins after adjustment for maternal height, paternal height and educational level. This was tested by mixed-model analyses of variance with birth weight SDS, birth length SDS, maternal height, paternal height and educational level as fixed factors and with family as a random factor to account for the within-family dependence of the dependent variable (height or BMI SDS at age 18 years).

Table 3.1. Mean standard deviation scores (SDS) for birth weight, birth length, height, weight, BMI, sitting height, leg length and arm span of twins and siblings.

	Twins		Siblings	
	N	Mean	N	Mean
SDS birth weight*	360	-0.87**	96	0.01
SDS birth length*	327	-0.42**	90	-0.01
SDS height at 1 yr*	270	-0.58**	81	-0.07
SDS weight at 1 yr*	270	-0.58**	81	0.04
SDS BMI at 1 yr*	270	-0.22**	81	0.16
SDS height at 4 yr	221	0.03	57	0.23
SDS weight at 4 yr	221	-0.14	57	0.09
SDS BMI at 4 yr	221	-0.22**	57	-0.08
SDS height at 18 yr	360	0.00	97	0.01
SDS weight at 18 yr	358	0.07	67	0.30**
SDS BMI at 18 yr*	358	0.08	67	0.33**
SDS sitting height at 18 yr	359	0.14**	96	0.06
SDS leg length at 18 yr	359	-0.06	96	-0.04
SDS arm span at 18 yr	353	-0.12	73	0.00

* Mean SDS twins significantly different from mean SDS siblings (p<0.05)
** Mean SDS significantly different from the general population, i.e. 0 (p<0.05)

Results

Table 3.1 presents the mean SDS for birth weight/length, height (age 1, 4, 18), weight (age 1, 4, 18) and BMI (age 1, 4, 18) of twins and siblings. SDS for sitting height, leg length and arm span around the age of 18 years are also shown in Table 3.1. Mean gestational age of the twin pairs was 36.9 weeks (SD 2.54), which was significantly lower (p<0.01) than that of the siblings (40.1 weeks; SD 1.66). Fifty-seven percent of the twin pairs and 97% of the siblings were born at term (gestational age 37 weeks or more). There were no differences in birth weight and birth length between first-born and second-born twins (p>0.05). No differences were shown in mean SDS between monozygotic and dizygotic twins, nor between

siblings of monozygotic twins and siblings of dizygotic twins for birth weight/length, height, weight, BMI, sitting height, leg length and arm span (p>0.05). Mean SDS were comparable for male and female twins and for male and female siblings (p>0.05).
At birth, twin weight and length SDS were significantly reduced compared with their siblings and children from the general population (p<0.001).
At the age of 1 year, twins were significantly shorter and had a significantly lower weight and BMI than their siblings and children from the reference population (p<0.01).
At the age of 4 years, twins and siblings were comparable for height, weight and BMI (p>0.05). Comparing twins with children from the general population, there was no difference in height (p=0.75) and weight (p=0.13), while the BMI of twins was still reduced (p=0.005). There were no significant differences in height, weight and BMI between 4-year-old siblings and children from the general population (p>0.05).
At the age of 18 years, twins were as tall as their siblings and children from the general population (p>0.05). Twins had an average weight and BMI (p>0.05), while siblings had a significantly increased weight and BMI in comparison with the general population (p<0.05). Regarding body proportions, twins were comparable with their siblings for sitting height, leg length and arm span SDS (p>0.05). In comparison with the reference population, sitting height was slightly increased in twins (p=0.020), while leg length was average (p=0.35). Body proportions of adolescent siblings (sitting height, leg length and arm span) were not different from the reference population (p>0.26).
Concerning pubertal development, all twins were in Tanner stage 4 (10%) or 5 (85%), as well as the majority of the siblings (stage 4: 13%, stage 5: 68%). Two percent of the siblings were in stage 1; 6% in stage 2; and 8% in stage 3. Tanner data were missing in 5% of the twins and 2% of the siblings. Pubertal development did not significantly affect mean SDS for height, weight or BMI and was not included as a covariate in the reported analyses.
Table 3.2 shows the mean SDS of the lowest, middle and highest birth weight SDS tertile. The higher the birth weight SDS, the taller and heavier twins were at age 1, 4 and 18 years (except for BMI SDS age 18). Classifying birth length SDS into tertiles in twins provided similar results (data not shown).

When birth weight SDS was classified into tertiles in siblings, no relation was seen between birth weight and height or BMI SDS at age 18 years (data not shown). In regression analysis (Table 3.3), after adjusting for educational level, birth weight SDS and parental height predicted twin height at age 18 years significantly ($p<0.05$). For every centimeter increase in maternal or paternal height, adolescent height SDS increased 0.06 or 0.05 respectively. Furthermore, for every increase in birth weight SDS of 1.0, height SDS at age 18 years increased 0.25. Birth length SDS did not contribute significantly to height SDS at age 18 years ($p=0.93$).

Table 3.2. Mean standard deviation scores (SDS) for birth weight, birth length, height and BMI of twins by birth weight SDS tertiles.

Birth weight SDS tertiles	Lowest tertile		Middle tertile		Highest tertile	
	N	Mean	N	Mean	N	Mean
SDS birth weight	120	-2.05	120	-0.75	120	0.19
SDS birth length	109	-1.35	107	-0.18	111	0.25
SDS height at 1 yr	89	-0.87	91	-0.61	90	-0.32
SDS BMI at 1 yr	89	-0.60	91	-0.25	90	0.20
SDS height at 4 yr	65	-0.43	74	-0.02	82	0.46
SDS BMI at 4 yr	65	-0.49	74	-0.38	82	0.13
SDS height at 18 yr	120	-0.36	120	0.08	120	0.29
SDS BMI at 18 yr	120	0.06	119	0.05	119	0.12

Lowest tertile = BW SDS ≤ -1.21
Middle tertile = -1.21 < BW SDS ≤ -0.38
Highest tertile = BW SDS > -0.38

Questionnaire sample

To verify the finding of the above-average BMI in siblings, we analyzed a sample of questionnaire data of 18-year-old twins and siblings (unrelated to the twin families mentioned above). When the twins were 18 years of age, the twins (mean age 18.79 years, SD 0.40; 35% males and 65% females) and their siblings (mean age 19.63 years, SD 3.83; 46% males and

54% females) filled out the Dutch Health and Behaviour Questionnaire (DHBQ), a large self-report questionnaire including questions about health, wellbeing, leisure activities and behavioural problems (Bartels et al., 2007). Subjects were asked to report recent height (cm) and weight (kg). After exclusion because of handicap (4 twins), unavailability of BMI reference values (78 siblings) or extreme values (BMI of 1 twin and height of 1 sibling), 800 twins and 171 siblings were eligible for height SDS analysis and 782 twins and 90 siblings for BMI SDS analysis. Twins (mean height SDS -0.01, mean BMI SDS -0.25) were significantly shorter and lighter than their siblings (mean height SDS 0.19, mean BMI SDS -0.02). Compared with the general population, twin children had the same height ($p=0.93$), but a lower BMI ($p<0.05$). Siblings were significantly taller than children from the general population ($p<0.01$), while their BMI was comparable ($p=0.84$).

Table 3.3. Regression analysis in twins with SDS height at age 18 years as dependent variable (adjusted for educational level).

	SDS height at 18 yr	
	Coefficient	P value
Maternal height (cm)	0.06	<0.001
Paternal height (cm)	0.05	<0.001
SDS birth length	-0.09	0.93
SDS birth weight	0.24	<0.001

Discussion

Our study showed that twins attained normal final height when compared with their non-twin siblings and when compared with children from the general population. At age 18 years, twins had an average BMI at age 18, while their adolescent siblings had increased BMI values. In line with our expectations, twin children had a smaller size at birth than their siblings and children from the general population. In accordance with previous literature birth length was less reduced than birth weight when compared

with singletons (Buckler & Green, 2004; Wilson, 1979). It is known from these studies that the fall off in birth length starts later in pregnancy and is of smaller magnitude than the fall off in birth weight. This may explain our findings that the deficit in height has disappeared amongst 4-year-old twins, while they were still somewhat underweight. Around the age of 18 years twins were as tall as their siblings and children from the general population, but were still leaner than their siblings. This is a remarkable finding, as twins and siblings share part of their genetic background and grew up in the same environment. The above-average BMI of the siblings may be in line with the increasing prevalence of overweight in children (Van den Hurk, 2007). The time interval between the reference values (Fredriks et al., 2000b) and our measurements was about 10 years and it is very likely that BMI age references will have increased in that time. Another possibility we investigated is that the above-average BMI of siblings could be explained by a higher BMI of the DZ siblings. Mothers of DZ twins are known to have a higher BMI than mothers of MZ twins and singletons (Basso et al., 2004; Hoekstra et al., 2010; Reddy et al., 2005). In our sample we did not find a difference in BMI between siblings of MZ and DZ twins, but this remains a question to be elucidated in larger studies. A study in Finnish adolescent twins reported that 17-year-old twin boys had reached the same height as singletons, but still had a lower BMI (Pietilainen et al., 1999). The same tendency, although not reaching significance, was seen in girls. They suggested that the catch-up growth from the lighter birth weight may not (yet) be completed in twin boys. Another study in 16-year-old twins showed that body size tracks from birth to adolescence (Pietilainen et al., 2001). Height at age 16 years was predicted by weight and length at birth and parents' height, whereas BMI was predicted by birth weight and parents' BMI. Looking at our data, twins in the lowest birth weight category were shorter, while children in the highest tertile were taller than children from the general population. Regression analysis showed that birth weight predicted height at age 18 years in twins. In contrast, no influence of birth weight was shown on BMI in 18-year-old twins. In siblings we saw no effect of birth weight on height or BMI SDS, which may be explained by the normal range of birth weight and the lower number of subjects available for analyses.

However, the difference in BMI between twins and siblings in our sample could not be explained by the lower birth weight of the twins. It could be speculated that twins have a lower food intake and/or a different energy balance. Experimental research in rats showed that food restriction during the early postnatal period, which is probably somewhat similar to undernutrition in human fetuses during the third trimester, programmed rats to remain small and lean in adult life with a lower food intake. These results indicate that the energy balance in rats can be programmed by early nutrition (Remmers et al., 2008a, 2008b). In line with these results is the finding that prepubertal children who were born small for gestational age that did not catch up, had a food intake below the recommended energy intake for their age (Boonstra et al., 2006). Regarding the energy balance, it was shown that infants born small for gestational age showed a greater total energy expenditure than appropriate for gestational age controls (Cauderay et al., 1988; Davies et al., 1996). Therefore, it would be interesting to study growth, food intake and the energy expenditure in twins, siblings and singleton controls in order to get more insight into the mechanisms that regulate body weight and thus can help us in the prevention of overweight.

Looking at body proportions, there were no significant differences between twins and siblings. Sitting height in adolescent twins was increased compared with the reference population, which was in line with the (non-significant) tendency of a shorter leg length and arm span. The finding of the slightly elevated sitting height in twins does not seem to be of much importance, as it is only an increase of 0.14 SDS from which no major conclusions can be drawn.

Because of the limited number of siblings in this study we also looked at data collected by questionnaire from an ongoing study in adolescent twins and siblings (Bartels et al., 2007). We were particularly interested whether there would also be a difference in BMI between adolescent twins and siblings. Indeed, siblings had a higher BMI than twins in the questionnaire sample, but in contrast to the test protocol sample, siblings were slightly taller than twins. It has to be noted, however, that although the sample size of the questionnaire study was larger, it concerned self-reported data in contrast to the test protocol data. Another limitation of

the questionnaire sample is the larger response rate among female twins. A preferable design for future studies into twin growth would be a large prospective cohort study including twins, siblings and matched singleton controls. Nevertheless, this cross-sectional study adds important information on the growth of twins, particularly by the inclusion of siblings.
In conclusion, we showed that twins attained normal final height compared with siblings and children from the general population. As for BMI, no differences were shown between 18-year-old twins and children from the general population, whereas the siblings of twins had increased BMI values. Birth weight was shown to have a considerable effect on height in adolescent twins.

Chapter 4

Heritability of testis size

Estourgie-van Burk, G.F.
Bartels, M.
Delemarre-van de Waal, H.A.
Boomsma, D.I.
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Abstract

Testis size is an important feature of male pubertal development.
The genetic and environmental contributions to variation in human testis size have hardly been studied. We estimated the heritability of human testicular size in a group of mono- and dizygotic twins and their non-twin brothers (145 twins and 20 brothers from 95 families). Participants were 18 years old on average and all had reached Tanner development stage 4 or higher.
Dizygotic twins and their siblings had a larger mean testis volume than monozygotic twins and their siblings. There was significant familial resemblance, with higher correlations in monozygotic twin pairs (0.59) than in dizygotic twin and sibling pairs (0.34). Heritability was estimated at 59% (95% CI = 37-75%), but a model that excluded genetic influences and attributed all familial resemblance to shared environment, fitted the data only marginally worse.
The finding of larger mean testis volume in dizygotic twins may be of interest for future research into the mechanisms underlying dizygotic twinning.

Introduction

Testis size is one of the hallmarks of male pubertal development.
The beginning of testicular enlargement is usually the first sign of puberty in boys. Therefore, the measurement of testis size is of great clinical importance when analyzing growth (disorders) in boys. Several studies have investigated testicular volume, which has resulted in the availability of nationwide reference values (Mul et al., 2001).
Testis size is related to testicular function. The number of Sertoli cells present determines both testis size and sperm output (Petersen & Soder, 2006) and thus is of importance for male fertility. However, little is known about the causes of individual differences in human testis volume. Ethnic differences in human testis size have been described (Short, 1984), testes being larger in Caucasian than in Asian men, which suggests that genetic influences play a role in the variation of human testis size. The pre- and perinatal period is known to be important for testicular development (Orth et al., 1988), implying a contribution of environmental factors to the variation in testis size as well. To gain more insight into the determinants of testis size, we will explore the genetic and environmental influences on the variation in testis size. One small pilot study in Australian twins investigated the genetic contribution to variability in human testicular function and size (Handelsman, 1997). A strong familial effect was seen, but a genetic component could not be confirmed due to the small number of participants (11 monozygotic (MZ) and 6 dizygotic (DZ) twin pairs). The aim of the current study is to estimate the genetic and environmental contribution to the variation in testis size in a sample of 18-year-old twins and their adolescent siblings.

Materials and methods

Subjects

All participants were contacted via the Netherlands Twin Register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam, the Netherlands (Boomsma et al., 1992, 2006). The current study sample is part of a longitudinal project on physical and mental

development and comprises 184 families of 18-year-old twin pairs and their siblings (Bartels et al., 2002). The initial sample was composed of 164 male twins and 46 of their male siblings. Data on testis size were available for 148 twins and 24 male siblings from 97 families. We excluded data for several reasons: orchidectomy (1 twin); traumatic injury (1 twin); torsio testis (1 twin). For analyses, we selected subjects in Tanner genital development stage 4 and above (145 twins and 20 siblings from 95 families). Mean age at assessment was 18.17 years (SD 0.21) in the twin group and 17.68 years (SD 3.09) in the sibling group. One twin pair had Surinam-Hindustan parents and 2 twin pairs had an Indonesian parent. Although ethnic variation in testis size has been described, we did not exclude these subjects as their testis size was within normal range compared with Dutch reference values (Mul et al., 2001).The final sample comprised 30 MZM (monozygotic male) and 32 DZM (dizygotic male) twin pairs, 33 DOSM (dizygotic opposite sex male) twins and 20 siblings (7 MZ siblings and 13 DZ siblings). The zygosity of the same-sex twin pairs (62 pairs) was determined by DNA analyses (59 pairs) or blood group polymorphisms (3 pairs).
This study was approved by the Central Committee on Research Involving Human Subjects. Written informed consent was obtained from all participants and also from all parents of underage participants.

Pubertal development

All participants filled out the Dutch Health and Behaviour Questionnaire (Bartels et al., 2007), a large self-report questionnaire including the pubertal development scale (Peterson et al., 1988). In addition, stage of puberty of all subjects was physically determined by the same trained researcher on the basis of secondary sexual characteristics using the stages of development devised by Tanner (Marshall & Tanner, 1970). Left and right testicular volume was measured using a Prader orchidometer, in which a series of testes models of volumes 2, 3, 4, 6, 8, 10, 12, 15, 20 and 25 ml were compared tactually with the actual testis (Zachmann et al., 1974). Total testis volume was calculated by taking the sum of left and right testis volume.

Data analysis

All analyses were carried out using structural equation modeling in the software package Mx (Neale et al., 2006). Saturated models in Mx were used to test for the effect of zygosity on mean and variance of total testis size and for differences between twins and siblings in mean and variance of total testis size. Twin and twin-sibling correlations for the 2 zygosity groups (MZ-DZ) were estimated. The twin-sibling correlation was constrained to equal the DZ correlation. All analyses were conducted using raw data. Age at measurement was included as a covariate.

Genetic modeling

Mx was also used to carry out genetic analyses. The variation in total testis size was decomposed into sources of additive genetic variance (A), common environmental variance (C) and unique environmental variance (E).
A is due to additive genetic effects of different alleles, C is due to common environmental influences shared by individuals from the same family, and E is due to unique (non-shared) environmental influences. E also includes measurement error and is always included in the models. Based on the twin and twin-sibling correlations an ACE model was fitted to the data. Significance of the A and C components were tested by dropping the component from the model. Submodels were compared with the full ACE model using the likelihood ratio test.

Table 4.1. Mean total testis size (ml) of twins and siblings from MZ (monozygotic) and DZ (dizygotic) families.

	Total testis size		
	N	**Mean**	**SD**
MZ twins + siblings	62	15.32	6.68
DZ twins + siblings	103	17.96	4.88

SD = standard deviation

Results

Table 4.1 provides mean testicular volume of MZ and DZ twins and their siblings derived from the saturated model. Information on pubertal stage is shown in table 4.2. Forty-one twins (28%) and 11 siblings (55%) reported that their testes were still growing. One twin reported not knowing whether his testes were still growing. The majority of twins and siblings were in the final pubertal stage (pubic hair stage 6 or genital development stage 5). All twin pairs were concordant for genital development stage.

Table 4.2. Pubertal development of twins and siblings.

	DHBQ			Tanner stages				
	Testes still growing?			*Genital development*		*Pubic hair development*		
	Yes	No	Do not know	4	5	4	5	6
	N	N	N	N	N	N	N	N
Twins	41	103	1	1	140	5	23	115
Siblings	11	9	0	6	13	7	2	10

DHBQ = Dutch Health and Behavior Questionnaire (Bartels et al., 2007)

Age (years) affected testis volume (ml) significantly (χ^2=16.41, df=1, p<0.01; b=1.54). There was a significant effect of zygosity on the mean and variance. Average testis volume and variance were larger in DZ than in MZ twins (mean: χ^2=4.10, df=1, p=0.04; variance: χ^2=4.89, df=1, p=0.03). There were no differences between twins and siblings within zygosity groups in mean (χ^2=1.14, df=2, p=0.57) or variance of testis size (χ^2=0.52, df=2, p=0.77). The twin correlation for testis volume was r=.59 (CI .34-.75) in MZ pairs and r=.34 (CI -.05-.59) in DZ twin and sibling pairs. The higher MZ twin correlations than DZ twin/sibling correlations indicate an influence of genetic factors on testes volume. However, since the MZ correlation is less than twice the DZ correlation influences of shared environment are to be expected.

Table 4.3. Genetic model fitting results for total testis size.

	-2LL	df	χ2	Δdf	c.t.m.	p	AIC
Total testis size							
1. Full model ACE	1016.30	159					
2. AE (no C)	1016.36	160	0.06	1	1	0.81	-1.94
3. CE (no A)	1018.38	160	2.08	1	1	0.15	0.08
4. E	1036.54	161	20.24	2	1	<0.01	16.24

-2LL = -2 log likelihood
df = degrees of freedom
χ^2 = chi-square statistic
Δdf = difference in degrees of freedom
c.t.m. = compared with model
p = probability-value
AIC = Akaike's Information Criterion
A = additive genetic influences
C = common environment
E = unique environment

Table 4.3 gives the results for the genetic modeling. Both the AE (p=0.81, AIC=-1.94) and CE model (p=0.15, AIC=0.08) (with age as a covariate) fitted the data well. Dropping both the additive genetic and common environmental factors to zero caused a significant worsening of fit (p<0.01), indicating significant familial resemblance for testis size. Variance component estimates are provided in Table 4.4, as well as the standardized estimates with the 95% confidence intervals. In the full ACE model 50% of the variation in total testis size is explained by genetic factors, while common environmental influences accounted for 9% of the individual differences. The confidence intervals around the standardized estimates for V_A and V_C show an overlap including zero, indicating a lack of statistical power to differentiate between genetic and common environmental influences.

Table 4.4. Standardized and unstandardized estimates of variance components for total testis size with 95% confidence intervals between brackets.

	Standardized			*Unstandardized*		
Total testis size	V_A	V_C	V_E	V_A	V_C	V_E
ACE	.50 (.00-75)	.09 (.00-.59)	.41 (.25-.66)	15.43	2.60	12.58
AE	.59 (.37-.75)	-	.41 (.25-.63)	18.07	-	12.35
CE	-	.48 (.27-.64)	.52 (.36-.73)	-	15.00	16.46

V_A = variance explained by additive genetic factors (heritability)
V_C = variance explained by common environment
V_E = variance explained by unique environment

Discussion

This study examined the genetic and environmental influences on testis size in late adolescence. Our data showed significant familial resemblance, with higher correlations in MZ twin pairs than in DZ twin and sibling pairs. Heritability of testis size was estimated at 59%. We were not able to differentiate between genetic and shared environmental effects due to a lack of statistical power. A model that excluded genetic influences and attributed all familial resemblance to shared environment fitted the data only marginally worse than a genetic model. Evidence for genetic influences on testis size comes from a study of inbred mouse strains (Chubb, 1992). It showed that there are genes that control testis size by regulating the number of Sertoli cells.

A remarkable finding is the larger testis volume in DZ twins. DZ twins had a significantly larger testis volume than MZ twins, both zygosity groups having a mean testis volume within the normal range (between the 50th and 90th percentile of Dutch reference values (Mul et al., 2001)). It has been hypothesized that a lower incidence of DZ twinning, within an ethnic population, may be correlated with lower average testis size in that population (Short, 1984). Asian populations have lower DZ twinning frequencies and smaller testis size compared with Caucasian populations.

Another indication for a possible relation between DZ twinning and testis size comes from a Belgian study (Fryns, 1986), which reported an increase in DZ twinning in the offspring of female carriers of the Fragile X. Large testes are one of the characteristic features of Fragile X mental retardation syndrome. These observations lead to the hypothesis that there may be genetic factors responsible for both DZ twinning and larger testis size. The follicle-stimulating hormone (FSH) may play a role in the association: mothers of DZ twins have shown increased FSH concentrations (Lambalk et al., 1998; Martin et al., 1984; Nylander, 1974), and smaller testes have been observed in men who lack a functional FSH receptor (Tapanainen et al., 1997). However, results from a study using a mouse model for the Fragile X syndrome (Slegtenhorst-Eegdeman et al., 1998), showed that macro-orchidism is caused by an increased rate of Sertoli cell proliferation in the embryonic and early postnatal period, which appeared not to be the result of a major change in FSH signal transduction. The larger testis size in DZ twins may be of interest in unraveling the mechanisms underlying DZ twinning.

Another explanation for the difference in mean testis volume between MZ and DZ twins could be that MZ twins generally experience more pre- and perinatal problems than DZ twins (Dube et al., 2002). The pre- and perinatal period is of great importance for testicular development, as Sertoli cells divide rapidly and extensively during fetal and early postnatal life (Orth et al., 1988). After adjusting for birth weight and gestational age we still found DZ testes to be significantly larger than MZ testes (data not shown).

A study of testis size in primates showed testis weight to increase with body weight (Harcourt et al., 1981). In our data, no significant correlation was demonstrated between body weight and testis size nor did the MZ-DZ differences in mean testis size disappear after adjusting for body weight (data not shown).

Since twins may not be representative of singletons, as they experience more often pre- and perinatal problems due to intrauterine growth restriction and prematurity, it is very important to include siblings as well. In our study no difference was shown in testis size between twins and siblings, although their number was small.

Testis size was measured using a Prader orchidometer, which has been reported to be less accurate than ultrasound measurements (Sakamoto et al., 2007a, 2007b). They showed that the orchidometer overestimated the testicular volume, especially in small testes. However, testicular volume estimated by Prader orchidometry correlated closely with the measurements by ultrasonography (r= 0.7 to 0.8).
Furthermore, all subjects in our study have been examined by the same trained researcher, excluding the possibility of inter-observer variability. However, this may have led to correlated measurement errors which are reflected in the C component in the genetic modeling (Bartels et al., 2007). Such a form of rater bias could explain part of the estimated common environmental influences on testis size. Unfortunately, we did not have the information to investigate the influence of rater bias on our results.
In summary, this study provides evidence that the variation in testis size is influenced by familial effects. Unfortunately, we were not able to differentiate between genetic and common environmental influences due to a lack of power. More knowledge of the genetic and environmental effects on the variation in testis size may help us in understanding testicular function and fertility problems. The finding of larger mean testis volume in DZ twins may be of interest for future research into the mechanisms underlying DZ twinning.

Chapter 5

Serum dehydroepiandrosterone-sulfate, insulin-like growth factor-I and insulin levels in late adolescence: the influence of genes, birth weight and postnatal weight gain

Estourgie-van Burk, G.F.
Bartels, M.
Boomsma, D.I.
Delemarre-van de Waal, H.A.
Submitted

Abstract

Objective: Low birth weight has been associated with higher childhood levels of dehydroepiandrosterone-sulfate (DHEAS) and insulin-like growth factor-I (IGF-I). It has been hypothesized that these hormones may contribute to links between reduced fetal growth and adult disease risks, possibly by enhancing insulin resistance. Our goal was to explore the genetic influences on the variation in DHEAS, IGF-I and insulin levels in adolescence and secondly, to study the association between birth weight and DHEAS/IGF-I levels taking catch-up growth and current body size into account. Furthermore, we studied the association between insulin and DHEAS/IGF-I levels.

Methods: Anthropometry and blood sample collection were performed in 184 pairs of 18-year-old mono- and dizygotic twins and their siblings (N=98).

Results: The variation in serum DHEAS, IGF-I and fasting insulin levels was largely explained by genetic factors (73%, 76% and 56% respectively). In linear regression analysis neither birth weight nor current body size predicted DHEAS and IGF-I levels significantly. In children who showed catch-up growth birth weight SDS predicted hormone levels significantly. DHEAS and IGF-I levels were not related to insulin levels.

Conclusions: Genetic influences on the variation in DHEAS, IGF-I and insulin levels are high in late adolescence. There was no significant influence of birth weight on hormone levels in this population. In subjects with catch-up growth birth weight was significantly inversely related to DHEAS and IGF-I levels. There was no association between insulin and DHEAS or IGF-I levels, leaving the mechanism whereby early growth is linked to disease in later life unclear.

Introduction

Low birth weight has been identified as a risk factor for disease in later life, such as type 2 diabetes and cardiovascular disease (Barker, 1995a). Dehydroepiandrosterone-sulfate (DHEAS) and insulin-like growth factor-I (IGF-I) have been suggested to play a role in the association between compromised intrauterine growth and adult disease risks (Ibanez et al., 1999, 2009; Iniguez et al., 2006; Ong et al., 2004b), possibly by enhancing central fat deposition and insulin resistance. Several studies have shown that lower birth weight is related to higher DHEAS levels in childhood and young adolescence (± 14 yr of age) (Ibanez et al., 1999; Opdahl et al., 2008; Ong et al., 2004b; Veening et al., 2004). At first, the association was only reported in small for gestational age (SGA) children, but later the inverse relationship between birth weight and DHEAS levels was also described throughout the range of normal birth weights (Ong et al., 2004b; Opdahl et al., 2008). However, there are also studies which did not find an association between birth weight and DHEAS (Boonstra et al., 2004; Dahlgren et al., 1998; Hernandez et al., 2006; Jaquet et al., 1999). Serum IGF-I levels have also been reported to be related to birth weight: children of lower birth weight have low IGF-I levels at birth, but higher IGF-I levels in childhood during catch-up growth (Fall et al., 1995; Garnett et al., 1999; Giudice et al., 1995; Ibanez et al., 2009; Iniguez et al., 2006; Ong et al., 2002). Postnatal growth and current body size play an important role in the relationship between birth weight and DHEAS/IGF-I levels in later life. In children of low birth weight, a higher gain in postnatal weight and larger current body size are independently related to higher DHEAS and IGF-I levels (Ong et al., 2002, 2004b). The higher IGF-I levels may have contributed to catch-up growth and normal stature, but are also associated with insulin resistance (De Zegher et al., 2002; Iniguez et al., 2006). Increased androgen levels, at least in females, are related to central fat deposition and reduced insulin sensitivity (Elbers et al., 1999; Ibanez et al., 2003; Nilsson et al., 1998). These two phenomena are thought to play a major role in the relation between early growth and later disease risks.

DHEAS is the most abundant circulating steroid hormone in the blood and is a classic marker for adrenarche. Approximately 60% of the variation

in serum DHEAS levels is explained by genetic factors according to a large study in adult twins (Nestler et al., 2002). However, to our knowledge no heritabilities of DHEAS in children and adolescents have been reported.
IGF-I has a major role in the regulation of human growth (D'Ercole, 1996). In utero and during infancy, growth and IGF-I levels are largely independent of growth hormone (GH) and closely related to nutrition and insulin secretion, while childhood growth is determined by GH secretion, but the GH/IGF-axis remains partly dependent on insulin and nutrition. More than half of the variation in circulating IGF-I is explained by genetic factors in children (Kao et al., 1994; Li et al., 2005). In adults, heritability estimates range from 38 to 63% (Harrela et al., 1996; Hong et al., 1996).
Insulin is a hormone that is central to regulating the energy and glucose metabolism in the body. Genetic factors account for 65% of the variation in fasting insulin levels in 9-year-old twins was (Beardsall et al., 2009) and for 20-41% in adults (Nestler et al., 2002; Snieder et al., 1999).
Our goal is quantify the causes of individual differences in serum DHEAS, IGF-I, and fasting insulin levels. We furthermore will test whether there is an association between birth weight and DHEAS/IGF-I in late adolescence, and investigate the role of postnatal growth and current body size. Finally, we examine the contribution of insulin to the link between early growth, higher DHEAS/IGF-I levels and disease in later life.

Subjects and Methods

Subjects

All participants were contacted via the Netherlands Twin Register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam, the Netherlands (Boomsma et al., 1992, 2006). The current study sample is part of a longitudinal project on physical and mental development and comprises 184 families of 18-year-old twin pairs and their siblings (Bartels et al., 2002). The initial sample was composed of 368 twins (mean age 18.14 years, SD=0.48) and 98 siblings (mean age 18.78 years, SD=4.89). Three families participated with two twin pairs. There were 6 incomplete twin pairs. Zygosity of the same-sex twins was

established by DNA analyses (143 pairs), blood group polymorphisms (4 pairs), or questionnaire items (Rietveld et al., 2000) on similarity (1 pair). The twin sample consisted of 32 MZM (monozygotic male), 34 DZM (dizygotic male), 44 MZF (monozygotic female), 38 DZF (dizygotic female) and 39 DOS (dizygotic opposite sex) twin pairs.
Blood withdrawal was performed in 347 twins and 81 siblings. Data were excluded for several reasons: congenital anomalies (2 twins); severe growth restriction (1 sibling); pregnancy (2 siblings). The final sample consisted of 345 twins and 78 siblings. Serum IGF-I is known to increase during childhood until it reaches a peak in puberty (14.5 yr of age in girls and 15.5 yr in boys), where after the levels decrease to low adult values (Juul et al., 1994). Because of this age effect we selected female subjects older than 14.5 yr of age and boys older than 15.5 yr for the IGF-I analyses (342 twins and 60 siblings). Glucose and insulin data of non-fasting subjects were excluded (44 twins and 9 siblings). The exact numbers of DHEAS, IGF-I, glucose and insulin samples can be found in Table 5.1.
This study was approved by the Central Committee on Research Involving Human Subjects. Written informed consent was obtained from all participants and also from all parents of underage participants.

Blood samples

Participants were invited to come to our outpatient clinic in the morning, where a venous blood sample was taken after overnight fasting (mean time 1035h). Serum samples were centrifuged (3000 rpm) during 10 minutes at room temperature and stored at -20°C until assay. Serum DHEAS was measured by solid phase competitive chemiluminescent enzyme immunoassay (IMMULITE 2500, Siemens, USA). Intra- and inter-assay coefficients of variation (CVs) were 7 and 9%, respectively. Serum IGF-I was measured by immunometric assay (IMMULITE 2500, Siemens, USA). Intra- and inter-assay CVs were 5 and 5%, respectively. Glucose and insulin concentrations were measured in heparin plasma (see for details: (Willemsen et al., 2010)). Glucose concentrations were assessed using the Vitros 250 Glucose assay (Johnson&Johnson, Rochester, USA). The intra-batch CV was lower than 2% and the inter-batch CV was lower than 4%. Insulin measurements were performed using the Immulite 1000

Insulin Method (Siemens Medical Solutions, Breda, NL). Intra- and inter-assay CVs were lower than 6.5% and 6% respectively. The cross-reactivity with proinsulin was 8.5% and the sensitivity was 2 μIU/ml.

Body size

Information on birth weight and gestational age was collected using questionnaires at the time of registration with the NTR, which was shortly after birth of the twins in the majority of the families. Weight data at age 2 were obtained from the report with growth data measured by the Dutch National Health Service's (NHS) or, if not available, from questionnaires collected around the twins' second birthday. We selected the measurement between the age of 1.5 and 2.5 years closest to the age of 2.0 years.
For the present study the adolescents were seen at the outpatient clinic where height (cm) was determined to the nearest 0.1cm and weight (kg) to the nearest 0.05kg using a stadiometer and an electronic scale (SECA, Hanover, Md). Stage of puberty of all subjects was assessed by the same physician according to the criteria of Tanner (1981).

Calculations

Birth weight was converted to standard deviation scores (SDS) using Swedish reference standards, because Dutch SDS reference values for birth weight are unavailable (Niklasson et al., 1991). Weight data at age 2 were standardized dependent on sex and age using the Dutch reference growth charts for the general population from 1997 (Fredriks et al., 2000a). Postnatal weight gain was calculated as the standard deviation score (SDS) for weight at age 2 minus the SDS for weight at birth; a gain in weight SDS greater than 0.67 was considered as clinically significant catch-up growth (Ong et al., 2000). Body mass index (BMI) at age 18 yr was calculated as weight (kg) divided by height (m) squared. Standard deviation scores (SDS) were calculated for height and BMI at age 18 yr with the software package Growth analyser 3 (2004), using the Dutch reference growth charts for the general population from 1997 (Fredriks et al., 2000a, 2000b). BMI reference values were available up to the age of 21 years (unavailable for 23 siblings).

Statistics

Descriptive statistics were calculated using untransformed data in SPSS-15 (SPSS Inc, Chicago). All statistical analyses were performed on square root-transformed DHEAS and IGF-I concentrations and log-transformed insulin levels, because these transformations gave the best approximation to a normal distribution of the data. The following analyses were carried out using structural equation modeling in the software package Mx (Neale et al., 2006) because of the dependency among the dependent variables that is present in family data. First, we tested the effects of covariates to be accounted for in the genetic analyses using univariate saturated models. For all hormones the following covariates were tested: age; BMI SDS; and time of blood withdrawal. For DHEAS and IGF-I the effect of fasting state (yes/no) was tested. For DHEAS we also tested the effect of pubic hair development. For IGF-I the covariates Tanner stage and height SDS were analyzed. Then, we tested for the effect of zygosity and sex on the mean and variance of DHEAS, IGF-I and insulin levels, and for differences between twins and siblings in means and variances. Furthermore, we tested whether the twin-sibling covariance could be constrained to equal the DZ covariance and whether the covariance differed between males and females. Twin and twin-sibling correlations for the two groups (MZ-DZ) were estimated. The variation in DHEAS, IGF-I and insulin levels was decomposed into sources of genetic variance (G) and unique environmental variance (E). We tested for the significance of G by dropping the component from the model. The submodel was compared with the full GE model using the likelihood ratio test.
To study the association between birth weight SDS and hormone levels (DHEAS/IGF-I/insulin), cross-trait correlations (between 2 variables within a subject) and cross-twin/sibling-cross-trait correlations (between a variable of one twin and another variable of the co-twin/sibling) were estimated. Furthermore, we tested whether the cross-twin/sibling-cross-trait correlations differed between the two zygosity groups (MZ-DZ). To study the effect of catch-up growth (change in weight SDS 0-2 yr greater than 0.67) on the association between birth weight SDS and hormone levels we estimated the cross-trait correlations and cross-twin/sibling-cross-trait correlations in a subsample of 146 twins and 11 siblings who showed

catch-up growth. To investigate whether insulin levels are related to DHEAS or IGF-I levels, cross-trait correlations and cross-twin/sibling-cross-trait correlations were calculated in the entire sample and in the subsample with catch-up growth.

Linear regression analysis was conducted to analyze whether birth weight and current body size influenced hormone levels (DHEAS/IGF-I/insulin). This was tested by mixed-model analyses of variance in SPSS-15 (SPSS Inc, Chicago) with sex, age, pubic hair development (DHEAS), birth weight SDS, BMI SDS (DHEAS, insulin), height SDS (IGF-I) as fixed factors and with family as a random factor to account for the within-family dependence of the dependent variable (DHEAS, IGF-I or insulin levels). An alpha level of 0.05 was used for the bivariate saturated analyses and mixed-model procedure. Because of the multiple tests, we used an alpha level of 0.01 for the univariate Mx analyses.

Table 5.1. Observed means of twins (first-born/second-born) and siblings by sex.

	Twins				Siblings			
	Males		Females		Males		Females	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Birth weight SDS	82	-0.88 (0.97)	91	-0.84 (0.99)	32	0.15 (1.01)	40	0.12 (0.93)
	78	-0.91 (1.13)	92	-0.90 (1.12)				
Change in weight SDS 0-2 yr	67	0.77 (1.17)	81	0.71 (0.90)	22	-0.19 (0.89)	28	0.07 (0.87)
	68	0.48 (1.18)	79	0.69 (1.04)				
Age (yr)	82	18.14 (0.38)	92	18.20 (0.32)	36	18.67 (4.19)	42	19.39 (4.67)
	79	18.10 (0.69)	92	18.17 (0.21)				
Height SDS	82	-0.12 (1.09)	92	0.14 (0.94)	36	-0.20 (0.97)	42	0.11 (0.87)
	79	-0.02 (0.96)	92	-0.03 (0.94)				
BMI SDS	82	0.07 (1.04)	91	0.13 (1.10)	26	0.27 (1.29)	29	0.39 (0.99)
	79	0.01 (1.10)	92	0.10 (1.03)				
DHEAS (µmol/L)	82	7.62 (2.72)	92	5.90 (2.93)	36	5.67 (2.89)	42	4.70 (2.24)
	79	8.32 (3.18)	92	5.45 (2.69)				
IGF-I (nmol/L)	81	36.86 (9.21)	92	35.75 (8.70)	24	34.38 (9.89)	36	32.94 (13.71)
	77	36.53 (9.49)	92	35.07 (8.59)				
Glucose (mg/dL)	73	97.27 (9.06)	82	91.60 (8.13)	30	95.52 (9.12)	39	94.06 (6.53)
	63	98.34 (8.78)	83	91.37 (8.88)				
Insulin (µU/mL)	72	7.63 (3.56)	83	9.56 (3.72)	30	7.89 (3.48)	39	10.25 (4.74)
	63	8.44 (4.31)	82	9.03 (3.32)				

SD = standard deviation

Results

Table 5.1 shows observed mean birth weight SDS, change in weight SDS between birth and 2 yr, body size at age 18 yr and hormone levels at age 18 yr of twins and siblings by sex.

Univariate analyses

Mean square root-transformed DHEAS levels (µmol/L) were significantly affected by pubic hair development (b=-0.05; χ^2_1=40.29, p<0.01). The effect of the interaction between age and pubic hair development, tested with a dummy variable (age*pubic hair development), was significant (b=0.02; χ^2_1=36.23, p<0.01). Pubic hair development increased with increasing age, as expected. Mean square root-transformed IGF-I levels (nmol/L) were significantly affected by age (years) (b=-0.19; p<0.01). In subsequent analyses we allowed for these significant covariates. Subjects who did not fast were also included in the final analyses, as there were no differences in mean DHEAS and IGF-I levels between fasting and non-fasting subjects (χ^2_1=0.78, p=0.38 and χ^2_1=2.24, p=0.13 respectively). None of the covariates influenced mean log-transformed insulin levels significantly (p>0.01). Zygosity did not affect the mean levels of DHEAS, IGF-I and insulin (p>0.01). There was a significant effect of sex on mean DHEAS, males having higher DHEAS levels than females (χ^2_4=17.25, p<0.01). No significant sex difference in mean IGF-I was found (χ^2_4=9.61, p=0.05). Males had significantly lower mean insulin levels than females (χ^2_4=16.95, p<0.01). The variance of DHEAS, IGF-I and insulin did not differ between males and females (p>0.01). The means and variances of DHEAS, IGF-I and insulin levels were equal in twins and siblings (p>0.01). Constraining the twin-sibling covariance to equal the DZ covariance did not worsen the fit of the models of DHEAS, IGF-I and insulin (p>0.01). No sex effect was found on the covariance of DHEAS, IGF-I and insulin (p>0.01). The correlation for DHEAS was r=.73 (CI .62-.81) in MZ pairs and r=.33 (CI .19-.46) in DZ twin and sibling pairs; for IGF-I r=.80 (CI .70-.86) and r=.16 (CI .02-.30); and for insulin r=.59 (CI .43-.71) and r=.20 (CI .04-.35) respectively. These correlations suggest a large influence of genetic factors on the variation in DHEAS, IGF-I and insulin.

Table 5.2. Genetic model fitting results for DHEAS, IGF-I and insulin (best-fitting models are shown bold faced) and standardized estimates of variance components for DHEAS, IGF-I and insulin with 95% confidence intervals between brackets.

	-2LL	df	χ2	Δdf	c.t.m.	p	AIC
DHEAS							
1. GE	**540.90**	**413**					
2. E	614.24	414	73.34	1	1	<0.01	69.34
IGF-I							
1. GE	**845.86**	**395**					
2. E	895.70	396	49.84	1	1	<0.01	45.84
Insulin							
1. GE	**-221.77**	**364**					
2. E	-187.92	365	33.85	1	1	<0.01	31.85

	Standardized variance components	
	G	**E**
DHEAS	.73 (.61-.81)	.27 (.19-.39)
IGF-I	.76 (.61-.85)	.24 (.15-.39)
Insulin	.56 (.40-.69)	.44 (.31-.60)

-2LL = -2 log likelihood
df = degrees of freedom
χ^2 = chi-square statistic
Δdf = difference in degrees of freedom
c.t.m. = compared with model
p = probability-value
AIC = Akaike's Information Criterion
G = genetic influences
E = unique environment
G = variance explained by genetic factors (heritability)
E = variance explained by unique environment

Table 5.2 gives the results for the genetic modeling. Dropping the genetic factors to zero caused a significant worsening of fit (χ^2_1=73.34, p<0.01), indicating significant genetic influences on DHEAS. The same held true for IGF-I (χ^2_1=49.84, p<0.01) and insulin (χ^2_1=33.85, p<0.01). The standardized variance component estimates with the 95% confidence intervals are provided in Table 5.2. Genetic effects explained 73% of the variation in DHEAS, while unique environmental influences accounted for the remaining 27% of the individual differences. The proportion of variance in IGF-I explained by genetic factors was 76% and by unique environmental factors 24%. For insulin these estimates were 56% and 44% respectively.

Influence of birth weight, postnatal weight gain and current body size

The cross-trait correlations and cross-twin/sibling-cross-trait correlations between DHEAS/IGF-I/insulin and birth weight SDS were not significantly different from 0 (p>0.05). The cross-trait correlation between birth weight SDS and DHEAS was r=-.05 (CI: -.16-.06); between birth weight SDS and IGF-I r=-.02 (CI: -.13-.09); and between birth weight SDS and insulin r=-.03 (CI: -.14-.08). The cross-trait correlations and cross-twin/sibling-cross-trait correlations between DHEAS/IGF-I and insulin were approximately zero (p>0.05), both in the entire sample and in the sample with catch-up growth.

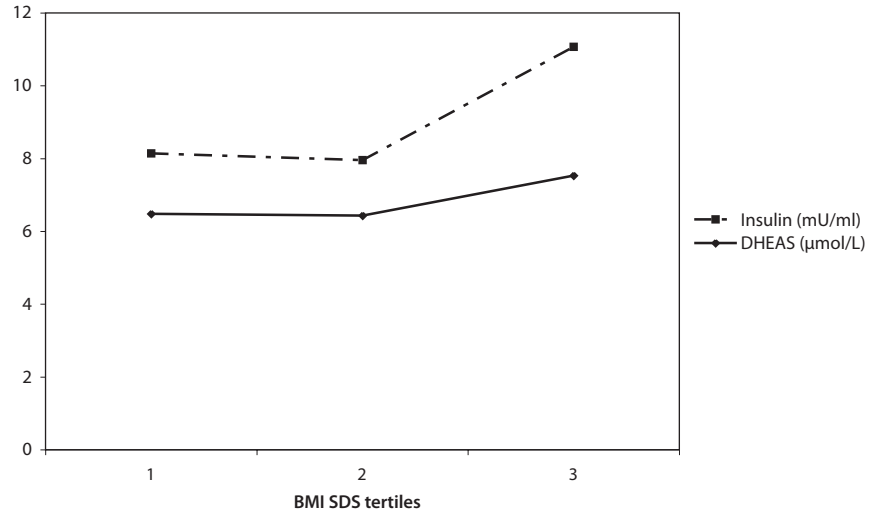

Figure 5.1. Mean DHEAS and insulin levels in subjects of low birth weight (lowest birth weight tertile; n=149) by tertiles of BMI SDS.

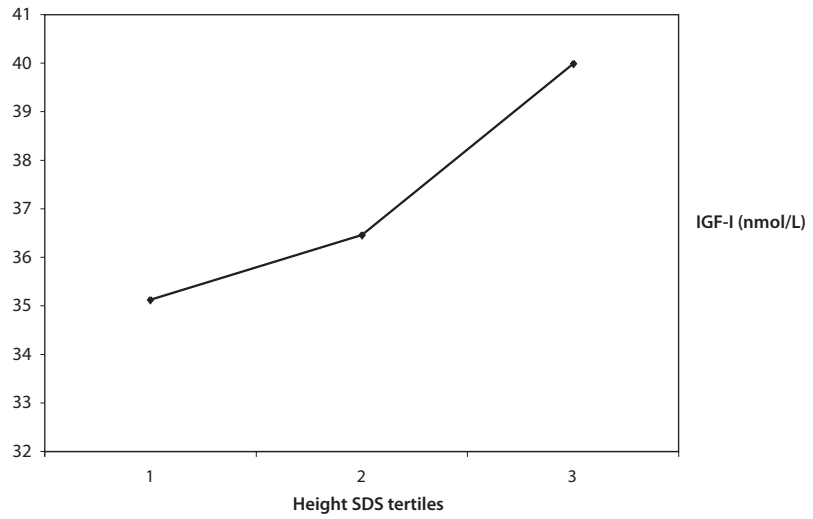

Figure 5.2. Mean IGF-I levels in subjects of low birth weight (lowest birth weight tertile; n=149) by tertiles of height SDS.

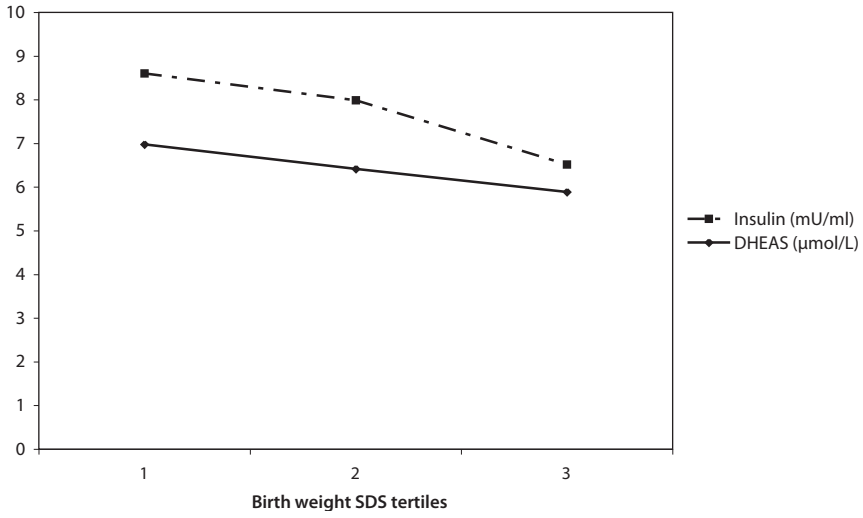

Figure 5.3. Mean DHEAS and insulin levels in subjects with catch-up growth (n=147) by tertiles of birth weight SDS.

Figure 5.4. Mean IGF-I levels in subjects with catch-up growth (n=147) by tertiles of birth weight SDS.

Figures 1 and 2 show that subjects of low birth weight (lowest birth weight tertile) who become heaviest or tallest at age 18 yr tend to have slightly higher mean hormone levels. Considering twins and siblings with catch-up growth, subjects within the lowest birth weight tertile had the highest mean DHEAS and IGF-I levels (Figure 3 and 4). All figures represent the entire sample, while the figures showed similar trends when analyzing twins and siblings apart.

In regression analysis (Table 5.3) birth weight did not affect hormone levels significantly ($p>0.05$). However, in children who showed catch-up growth birth weight SDS predicted DHEAS and IGF-I levels significantly (DHEAS, $p<0.01$; IGF-I, $p=0.03$; Table 5.3). For every increase in birth weight SDS of 1.0, the square root-transformed DHEAS level decreased 0.10 µmol/l and the square root-transformed IGF-I level decreased 0.14 nmol/l.

Regarding body size, BMI SDS predicted insulin levels significantly in the entire sample ($\beta= 0.04$; $p<0.01$) and height SDS affected IGF-I levels significantly in the catch-up growth sample ($\beta= 0.16$, $p=0.04$; Table 5.3).

Table 5.3. Regression coefficients between DHEAS, IGF-I or insulin levels and SDS for birth weight and current body size.

	DHEAS [a]		IGF-I [b]		Insulin [b]	
	All	**CUG**	**All**	**CUG**	**All**	**CUG**
Birth weight SDS	-0.03 (NS)	-0.10 (p<0.01)	-0.02 (NS)	-0.14 (p=0.03)	-0.002 (NS)	-0.02 (NS)
BMI SDS	0.03 (NS)	0.06 (NS)	-	-	0.04 (p<0.01)	0.03 (p=0.04)
Height SDS	-	-	0.07 (NS)	0.16 (p=0.04)	-	-

[a] = adjusted for sex, age and pubic hair development
[b] = adjusted for sex and age
CUG = catch-up growth
NS = not significant

Discussion

This is the first study to report heritability estimates of serum DHEAS, IGF-I and insulin levels in late adolescence. A significant contribution of genetic effects to serum DHEAS levels was found, explaining 73% of the variation. The remaining proportion of the variance was accounted for by nonshared environmental influences. The fact that the within-pair correlations for dizygotic same-sex and opposite-sex pairs were similar suggested the same source of interindividual variation in males and females. This is in line with the findings of a large Australian twin study (Nestler et al., 2002), which reported a heritability of about 60% in adults. Individual differences in serum IGF-I levels were mainly controlled by genetic factors in late adolescence with a heritability of 76%. This is about as high as findings at birth (cord blood) and in childhood (Kao et al., 1994; Verhaeghe et al., 1996). A study in female pubertal twin pairs (age 11.45 ± 0.18 yr) reported a heritability of 59%, while 18% of the variance could be attributed to age (Li et al., 2005). The proportion of variance explained by nonshared environmental effects was similar to ours (23%). Hong et al. (1996) showed that genetic influences on the variation in IGF-I remain important in later life with a heritability estimate of 63% in middle-aged and elderly twins. Regarding fasting insulin levels, our estimate of heritability was 56%, which is in between the estimate reported in younger children (65%) and those in adults (20-41%) (Beardsall et al., 2009; Nestler et al., 2002; Snieder et al., 1999).

Twins had small but significant higher mean DHEAS and IGF-I levels than their siblings. We hypothesized that this may be due to the smaller size at birth and the subsequent catch-up growth of twins. Twins are born with a significantly lower birth weight, but attain normal final height and weight compared with singletons (Estourgie-van Burk et al., 2010).

We found no relation between birth weight and adolescent hormone levels, nor when we considered twins only (data not shown). However, when we looked at a subsample of children who showed catch-up growth, the children of lower birth weight had significantly higher DHEAS and IGF-I levels. Therefore, rather than birth weight, postnatal growth appears to be essential for the association between birth weight and hormone levels in later life. This is in line with previous studies (Beardsall et al., 2009; Ong et al., 2002, 2004b) and may be outspoken in twins (Beardsall et al., 2009). In a sample of 9-year-old twins postnatal weight gain was associated with childhood risk factors for adult metabolic disease, such as blood pressure and insulin levels. Insulin levels, however, were not related to birth weight in our sample, nor in the subsample with catch-up growth. We did observe that subjects of low birth weight and highest BMI had the highest mean fasting insulin levels (Fig. 1). It has been reported before that size at birth and particularly current body size are important determinants of insulin resistance (Bavdekar et al., 1999; Ong et al., 2004a; Whincup et al., 1997; Yarbrough et al., 1998).

Our findings support the hypothesis that intrauterine and early postnatal growth affect DHEAS and IGF-I levels, which may contribute to links between early growth and disease in later life. SGA newborns have low circulating IGF-I levels at birth (Giudice et al., 1995; Iniguez et al., 2006), but conversely they have higher IGF-I levels than appropriate for gestational age (AGA) children at age 3 yr despite similar heights and weights (Iniguez et al., 2006). The higher IGF-I levels may have contributed to catch-up growth and normal stature, but are also associated with insulin resistance (De Zegher et al., 2002; Iniguez et al., 2006). The peripheral insulin resistance result in higher circulating insulin levels, which may cause increased IGF-I levels as well because of a direct stimulatory effect of insulin on liver IGF-I production (Bereket et al., 1995). The combination of low birth weight and rapid weight gain in early postnatal life predict higher DHEAS levels in childhood (Ong et al., 2004b). Higher androgen levels, at least in females, are related to central fat deposition and reduced insulin sensitivity (Elbers et al., 1999; Ibanez et al., 2003; Nilsson et al., 1998). These two phenomena are thought to play a major role in the relation between early growth and later disease risks. To study whether increased DHEAS or IGF-I levels may explain this relation by enhancing insulin resistance, we calculated the bivariate correlations between insulin and DHEAS or IGF-I. No association was found between DHEAS and insulin, or between IGF-I and insulin levels. Performing the analyses in the subsample with catch-up growth did not change the results. Previous studies in adult twins and young women did not show a relation between DHEAS and insulin levels either (Jaquet et al., 1999; Nestler et al., 2002).

In childhood IGF-I levels are related to insulin levels, but in adulthood the relation is presumably more complex (Jones & Clemmons, 1995; Sandhu et al., 2002; Thissen et al., 1994). Based on our results it can be questioned whether DHEAS and IGF-I play a role in the association between early growth and adult metabolic disease by enhancing insulin resistance. Recently, a genetic component has been described which can partially explain the association between lower birth weight and type 2 diabetes (Freathy et al., 2010). Future studies may identify more genes underlying the association between early growth and adult disease risks.

Another question to be elucidated is whether increased DHEAS levels may have adverse consequences, while studies in adults suggested that DHEAS has rather positive effects on health (Allolio & Arlt, 2002).

Thus, the mechanism whereby DHEAS and/or IGF-I levels are linked to the association between early growth and disease in later life remains unclear and is presumably complex. However, it is important to realize that children of lower birth weight who are heavier than average have an increased risk of insulin resistance. One should be cautioned and encouraged to decrease these disease risk factors.

It has to be noted that our study population included twins and their siblings. Although intrauterine growth in twins may be different from that in singletons (Doyle et al., 1999), the associations of size at birth with cardiovascular risk factors in twins suggest that birth weight in twins is relevant for the development of cardiovascular disease in twins as well (IJzerman et al., 2000; Iliadou et al., 2004; Dwyer et al., 1999). So twin studies may help us in revealing the mechanisms underlying the association between early growth and disease in later life. Moreover, the possibility to gain insight into the factors (genetic or environmental) which mediate the association between size at birth and later outcome variables makes twins and their siblings an interesting study population.

In conclusion, we showed that the genetic influences on the variation in DHEAS, IGF-I and insulin levels are high in late adolescence. There was no significant influence of birth weight on hormone levels in the study population. However, in subjects with catch-up growth birth weight was significantly inversely related to DHEAS and IGF-I levels, but not to insulin levels. No relation was shown between insulin and DHEAS or

IGF-I levels, leaving the mechanism whereby increased DHEAS and IGF-I levels are linked to disease in later life unclear.

Chapter 6

A twin study of cognitive costs of low birth weight and catch-up growth

Estourgie-van Burk, G.F.
Bartels, M.
Hoekstra, R.A.
Polderman, T.J.
Delemarre-van de Waal, H.A.
Boomsma, D.I.
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Abstract

Objectives Recently, the level of compensatory growth following a period of poor nutrition was found to be associated with long-term negative consequences for cognitive function in zebra finches. In humans, postnatal catch-up growth has been related to diseases in later life. Our aim was to investigate if the association between catch-up growth and cognitive performance is also seen in humans.

Study design Catch-up growth was defined as the change in weight standard deviation scores during the first 2 years of life. Cognitive performance was assessed with psychometric IQ tests, administered at ages 12 and 18 years. Data were collected in twin pairs and analyses were carried out within pairs.

Results A significant negative association between catch-up growth and IQ is found at both ages 12 and 18.

Conclusions A larger gain in weight during the first 2 years of life is associated with a lower IQ. However, catch-up growth is correlated with birth weight and this correlation may explain part of the association.

Introduction

Several studies have demonstrated a positive association between weight at birth and childhood IQ (Boomsma et al., 2001; Breslau et al., 1994; Christensen et al., 2006; Matte et al., 2001; Richards et al., 2001). We showed in a twin study that part of the positive association between birth weight and childhood IQ may be mediated by genetic effects (Boomsma et al., 2001). Now we are interested if there is any relation between catch-up growth and cognition, as a recent animal study reported that in zebra finches the level of compensatory growth following a period of poor nutrition was associated with long-term negative consequences for cognitive function (Fisher et al., 2006). During the early post-hatching period, same-sex sibling pairs of zebra-finches were exposed to 2 different nutritional environments: a normal diet or a low-quality diet. After 20 days normal nutrition was restored in the deficit member of a pair. The degree of growth depression, the degree of compensatory growth when normal nutrition was restored, and subsequent learning performance in adulthood were linked to each other. Within pairs the size of compensatory growth was negatively related to learning speed, while the hypo-caloric diet itself and the amount of early growth depression were not related to learning speed. In humans, early postnatal catch-up growth and excessive childhood weight gain have been related to obesity and an increased risk of adult cardiovascular disease and type 2 diabetes (Ong et al., 2000, 2006; Singhal et al., 2004). A remaining question is whether catch-up growth also influences cognitive function. In this paper we describe the role of catch-up growth during the first 2 years of life on IQ scores at ages 12 and 18 years. Between the third trimester of pregnancy and the age of 2 years there is a so-called critical spurt in brain growth (Dobbing, 1981). Using human sibling pairs (twins) we aimed to replicate the experimental design in zebra finches in humans. Same sex twin pairs provide an ideally matched sibling pair, since they share not only pre- and post natal maternal and family factors, but also sex and age. In addition, monozygotic (MZ) pairs share 100% and dizygotic (DZ) pairs 50% of their genotype. We looked within pairs if intra-pair differences in catch-up growth correlate with intra-pair differences in IQ. We hypothesize a negative relationship between change in weight during the first 2 years of

life and IQ scores at age 12 and 18 years. Two groups of twin pairs took part: the first group (N = 203 same sex pairs) had IQ measured at ages 12 and 18 years; the second group (N= 149 same sex pairs) had IQ measured at age 12. For both groups data on gestational age, weight at birth and weight at age 2 years were available.

Methods

Participants

Twin pairs were recruited through the Netherlands Twin Register (NTR) (Bartels et al., 2002; Boomsma et al., 1992, 2002). There were 2 groups of pairs. The first group takes part in a longitudinal project on physical and mental development. Cognitive ability was assessed at the ages of 12.0 years (SD 0.1) and 18.0 years (SD 0.2). Data from opposite-sex dizygotic twin pairs were excluded. In the first group, 203 same-sex pairs took part. The second group of twin pairs participated in a study of cognition and attention. The age of the second sample was 12.4 years (SD 0.2). There were 149 same-sex twin pairs in the second group. Zygosity was determined on the basis of DNA polymorphisms or blood group polymorphisms in 98.6 % of the twin pairs and for the other twin pairs by questionnaire items on similarity (Hoekstra et al., 2007a; Polderman et al., 2006). The agreement between zygosity assigned by the replies to the questions and zygosity determined by DNA markers / blood typing is around 93% (Rietveld et al., 2000).

Intelligence Tests

In the first group, IQ data were available for 154 same-sex complete twin pairs at age 12 and for 140 pairs at age 18 years (overlap is 91 pairs). At age 12, the twins completed the entire WISC-R, Dutch version (Van Haasen et al., 1986). At age 18 the Wechsler Adult Intelligence Scale (WAIS-III), Dutch version, was used (Wechsler, 2000). Eleven out of the 14 subtests were administered. From these 11 subtests 4 index scores were derived. Full-scale IQ was derived as the composite score of these 4 index scores. For further details on the assessment procedure in this group, see (Bartels

et al., 2002) and (Hoekstra et al., 2007a). In the second group, IQ data were available for 149 complete same-sex twin pairs. Six subtests from the WISC-R, Dutch version were employed (Van Haasen et al., 1986). Standardized scores of this shortened form of the WISC correlate 0.94 with standardized IQ scores based on all subtests of the WISC-R (Sattler, 1982, 1992). For further details on the procedure of this assessment see (Polderman et al., 2006).

Growth Data

Catch-up growth was calculated as the standard deviation score (SDS) for weight at two years of age minus the SDS for weight at birth (Ong et al., 2000). For example, if 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. Information on birth weight and gestational age was collected using questionnaires at the time of registration of the twins. Weight data at age 2 were obtained from the Dutch National Health Services. We selected the measurement between the age of 1.5 and 2.5 years closest to the age of 2.0 years. Weight data at age 2 were standardized dependent on sex and age using the Dutch reference growth charts for the general population from 1997 (Fredriks et al., 2000a). SDS weight were calculated with the software package Growth analyser 3 (2004). For weight at birth we used the reference data of Niklasson et al. (1991) with correction for gestational age. If birth weight SDS or weight SDS deviated 2.5 standard deviations from 0, data were checked for data-entry errors.

From the first sample we excluded 1 pair due to extreme values and 53 pairs because of incomplete weight data. From the second sample 19 twin pairs were excluded because of incomplete data. Finally, from the first sample 109 pairs aged 12 years and 116 pairs aged 18 years (overlap 76 pairs) were available for analysis, and from the second sample 130 pairs The first sample consisted of 55 monozygotic and 54 dizygotic twin pairs at the age of 12 years, and 60 monozygotic and 56 dizygotic twin pairs at the age of 18 years. The second sample was composed of 82 monozygotic and 48 dizygotic twin pairs.

Data Analysis

SPSS-12 was used to perform the descriptive analyses and the paired *t* test (SPSS Inc). The paired *t* test was used to compare twins with the lowest catch-up growth from each pair with their cotwins with the highest catch-up growth. If catch-up growth was equal within a twin pair, the twin pair was excluded for the *t* test analyses. To avoid birth order effect to interfere with our analyses, twin 1-twin 2 status within a pair was randomly assigned using SPSS. Intra-pair differences for catch-up growth and IQ were computed by subtracting the scores of twin 1 from those of twin 2. Correlations and their 95% confidence intervals were estimated between the intra-pair differences in catch-up growth or birth weight SDS and IQ using the statistical package Mx (Neale, 1999).

Results

Mean gestational age of all twins from both samples was 36.9 weeks (SD 2.4) with a minimum of 29 weeks and a maximum of 41 weeks. 60% of the twin pairs were born at term (gestational age 37 weeks or more). For each twin pair the twin with the highest and the lowest catch-up growth was identified. For these groups, Table 6.1 provides means and standard deviations for catch-up growth, birth weight, birth weight standard deviation score (SDS), weight SDS and IQ. One pair from the second sample had to be excluded for these analyses because the catch-up growth of both twins was equal. Twins with the lowest catch-up growth within a pair were significantly heavier at birth. At age 2 they had a significantly lower catch-up growth than their co-twins who had lower birth weight. Table 6.2 shows significant negative correlations between intra-pair differences in catch-up growth and IQ at age 12 (both samples) and 18. Thus, within pairs, a larger gain in weight during the first 2 years of life is associated with a lower IQ. Within twin pair differences in birth weight SDS were significantly and positively associated with differences in IQ at ages 12 and 18 years (Table 6.2), confirming earlier observations that the twin who is heavier at birth has higher IQ scores later in life. Birth weight SDS and catch-up growth were significantly negatively correlated in both samples and the correlation did not differ between the two samples

(r = -0.71; χ^2_1, p=0.48). A paired t- test showed that co-twins with the lowest birth weight were significantly lighter at age 2. They had a significantly higher catch-up growth and lower IQ scores at age 12 years than their co-twins with the highest birth weight (p<0.01).

Table 6.1. Means and standard deviations for catch-up growth, birth weight (gram), birth weight standard deviation scores (SDS), SDS for weight at age 2 and Full Scale IQ (IQ) for twins with the lowest and the highest catch-up growth.

	Sample	N	Lowest catch-up growth	Highest catch-up growth	p
Catch-up growth	I	149	0.42 ± 1.05	1.01 ± 1.17	<0.001
	II	129*	0.39 ± 1.02	1.09 ± 1.06	<0.001
Birth weight	I	149	2594 ± 472	2398 ± 478	<0.001
	II	129*	2657± 503	2424 ± 530	<0.001
Birth weight SDS	I	149	-0.68 ± 0.92	-1.18 ± 0.99	<0.001
	II	129*	-0.58 ± 0.88	-1.18 ± 0.98	<0.001
SDS weight age 2	I	149	-0.26 ± 0.95	-0.17 ± 1.05	0.12
	II	129*	-0.19 ± 1.00	-0.09 ± 1.00	0.098
IQ 12	I	109	101.3 ± 12.7	100.5 ± 12.3	0.41
	II	129*	101.6 ± 15.3	98.4 ± 13.4	0.002
IQ 18	I	116	102.1 ± 8.9	101.8 ± 8.8	0.67

* = One pair had to be excluded because the catch-up growth within a pair was equal.

Table 6.2. Pearson correlations (with 95% confidence intervals between brackets) between intra-pair differences in catch-up growth / birth weight SDS and intra-pair differences in IQ.

	Sample	N		Correlation in all pairs	p	N		Correlation in pairs with GA ≥ 37 weeks	p
IQ age 12	I	109	ΔCUG-ΔIQ	-0.21 (-.37 - -.04)	χ^2_1=3.51 p=.02	64	ΔCUG-ΔIQ	-0.32 (-.50- -.11)	χ^2_1=8.24 p=.004
			ΔBW-ΔIQ	0.24 (.06-.40)	χ^2_1=6.728 p=.009		ΔBW-ΔIQ	0.36 (.14-.54)	χ^2_1=9.73 p=.002
	II	130	ΔCUG-ΔIQ	-0.28 (-.43 - -.12)	χ^2_1=10.82 p=.001	81	ΔCUG-ΔIQ	-0.23 (-.41 - -.02)	χ^2_1=7.24 p=.007
			ΔBW-ΔIQ	0.25 (.09-.40)	χ^2_1=7.82 p=.005		ΔBW-ΔIQ	0.24 (.03-.42)	χ^2_1=5.05 p=.025
IQ age 18	I	116	ΔCUG-ΔIQ	-0.22 (-.38 - -.05)	χ^2_1=6.41 p=.01	68	ΔCUG-ΔIQ	-0.29 (-.47 - -.08)	χ^2_1=4.74 p=.029
			ΔBW-ΔIQ	0.24 (.07-.40)	χ^2_1=8.96 p=.003		ΔBW-ΔIQ	0.26 (.06-.45)	χ^2_1=6.25 p=.001

ΔCUG-ΔIQ = intra-pair differences in catch-up growth and intra-pair differences in IQ
ΔBW-ΔIQ = intra-pair differences in birth weight SDS and intra-pair differences in IQ
GA = gestational age

Excluding twin pairs with a gestational age shorter than 37 weeks from the analysis showed no systematic effect on the association between catch-up growth and IQ (Table 6.2). Confidence intervals of both correlations show a large overlap. Therefore, prematurity appeared not to be of major importance in the association between catch-up growth and IQ.
The correlations between intra-pair differences in catch-up growth and IQ were similar in MZ and DZ pairs suggesting that this association may not be mediated by genetic effects (data not shown).

Discussion

This is one of the first studies to investigate the relation between catch-up growth and cognitive ability in humans. Like the 'zebra finch study' we employed a family design with pairs of siblings (twins) in order to study the association between catch-up growth and IQ. Intra-pair analyses are an elegant way for this type of research, as the co-twin provides the ideally matched control and no confounding factors such as maternal height and IQ, parental social class or diet, have to be controlled for.
Our results showed that within pairs there was an inverse association between catch-up growth and IQ at the age of 12 and 18 years. The twin showing more catch-up growth in weight during the first 2 years of life had lower IQ scores both at ages 12 and 18 years. The correlations were modest with values between -0.2 and -0.3. The association was not due to an effect of prematurity, since excluding prematurely born twins gave similar results.
Our study, however, illustrates the difficulty in differentiating the effects of birth weight from the effects of catch-up growth. Generally, children with a lower birth weight show more catch-up growth. An interesting question is whether the relation between catch-up growth and IQ can be explained by birth weight or by a combination of birth weight and catch-up growth. Fisher et al. (2006) were able to manipulate the nutritional environment and induce compensatory growth in zebra finches. They could separate the effects of growth during nutritional deficit from the effects of compensatory growth, because these two effects were not significantly correlated. We tried to distinguish the effects associated with birth weight from those of catch-up growth by looking in a subgroup of the 12-year olds twins, namely those with comparable birth weight (difference in BW <= 165 gr; n=83 pairs). The association between intra-pair differences in catch-up growth and IQ was negative (*r*=-0.18), but did not reach statistical significance (p=0.10). However, this finding is in line with our theory that catch-up growth may have a negative effect on cognitive development. Unfortunately, the subgroup was small and these analyses require larger samples. A design with a large number of twins of comparable birth weight discordant for catch-up growth is needed to elucidate the role of catch-up growth and separate it from the effect of birth weight.

A limitation because of ethical reasons is that catch-up growth cannot be induced in humans by creating a nutritional deficit and comparing these individuals with others receiving a normal diet. Therefore research using animal models can help us to clarify the role of catch-up growth. There are several studies that related nutrient-enriched diet to better cognitive outcome (Lucas et al., 1998; O'Connor et al., 2001; Sachdev et al., 2005; Wharton et al., 2004), but these studies looked at the association between diet and cognitive function rather than compensatory growth and cognitive development. A study in full-term babies small for gestational age compared the effect of standard formula and enriched formula on neurodevelopment (Morley et al., 2004). Children who were fed with enriched formula had greater gains in length and head circumference, but had poorer neurodevelopmental outcome at 9 months of age. This was especially marked in girls, showing the greatest compensatory growth. These findings support our results that catch-up growth may have negative effects on cognitive abilities. However, the developmental disadvantage at 9 months was not seen at the age of 18 months.

Adequate caloric intake is needed for proper brain growth and cognitive development, particularly in preterm infants requiring higher caloric intake. However, promoting nutrient-enriched diets to induce catch-up growth should be done with reluctance, since catch-up growth has not only been associated with obesity and disease in later life, but may also have a negative effect on cognitive development. The mechanism by which increased physical growth rates may adversely affect cognitive function is still unclear. Compensatory growth may require a lot of energy, which could come at the expense of neural development. Another possibility is that a period of catch-up growth induces a kind of stress, which may impair hippocampal function. The hippocampus is important for processes of learning and memory. Stress is known to alter hippocampal plasticity and memory in a negative way (Kim et al., 2006). Recently, early calorie intake was related to caudate volumes and IQ (Isaacs et al., 2008). The high-nutrient group had larger caudate volumes and higher verbal IQ scores. The caudate nucleus could therefore be an area of interest in explaining the association between compensatory growth and neurodevelopmental outcome.

At long term more insight in these mechanisms may result in new guidelines about feeding and control of weight during infancy.

Chapter 7

General discussion

This thesis focuses on growth and the influence of early growth in later life. In this chapter the findings are discussed and future perspectives are considered.
This thesis is the result of the collaboration between the Department of Biological Psychology of the VU University and the Department of Pediatric Endocrinology of the VU University Medical Center. As part of a longitudinal study into the development of cognition and behavioral problems, data were collected in 18-year-old twins and their non-twin siblings, who were recruited from the Netherland Twin Register (NTR). We also analyzed questionnaire data from a large sample of 5-year-old twins (chapter 2) and from a longitudinal sample of 12-year-old twins (chapter 6).

Growth

Variation in growth

During infancy the influence of genetic effects on the variation in height and weight increases enormously. Van Dommelen et al. (2004a) studied height and weight in a large sample of Dutch twins during the first 2.5 years of life and showed the influence of genes to increase from 10-24% at birth to 52-59% at age 2 years and the influence of gestational age to practically disappear. In the present thesis height and weight at the age of 5 years were studied in a large sample of Dutch twins.. From age 2 to 5 years the heritability estimates of height increased further to 69% in boys and 66% in girls. This is in line with the expectation, since height at age 5 years correlates well with adult height (Tanner, 1978). Adult height is videlicet a highly heritable trait with estimates of heritability ranging from 67 to 94% (Akerman & Fischbein, 1992; Fischbein, 1977; Phillips et al., 1990; Schousboe et al., 2004; Silventoinen et al., 2003b). The differences in heritability estimates may be due to differences in age of study population, sample size or statistical methods used. With the development of new genetic techniques there is a search for the genes that can explain the variation in height. To date genome-wide association (GWA) studies have discovered at least 180 loci, that are associated with height in the population,

but these account for a relatively small percentage of the variation in height (Gudbjartsson et al., 2008; Lango et al., 2010; Lettre et al., 2008; Visscher, 2008; Weedon et al., 2008). However, a recent publication showed that a larger proportion (45%) of the heritability for human height could be explained by common single-nucleotide polymorphisms (SNPs) (Yang et al., 2010), and that the other part of the variation is likely explained by genetic variants not included on the current SNP chips. Height is a simple measure, but its genetic architecture is likely to be characterized by the additive effects of a (very) large number of loci. Increased knowledge of the genetic and environmental factors influencing individual differences in height will hopefully help us in the diagnosis, treatment and eventually prevention of growth disorders.
Clinically even more interesting and important than height are weight and body mass index (BMI) and their causes of individual differences. The worldwide pandemic of obesity suggests an important role for environmental influences on weight, but genetic factors are also of great importance explaining more than half of the variation in weight. At the age of 5 years, genetic effects accounted for 59% of the variation in weight in boys and for 78% in girls. In adolescence and adulthood, genes accounted for about 45 to 90% of the variation in BMI (Akerman & Fischbein, 1992; Pietilainen et al., 2002; Plomin et al., 2001; Schousboe et al., 2003; Stunkard et al., 1986). Thus weight or BMI is a very heritable trait, but this does not imply that one cannot help being overweight. Because of the limited variation in availability of and access to (high calorie) food in current (western) society, genes are the main source of variation in body weight. The regulation of body weight is highly complex and has a polygenic basis, of which several variants have been detected, but the greater part remains to be elucidated. Recently, Elks et al. (2010) showed that weight gain and growth even in the first few weeks after birth may be the beginning of a pathway of greater adult obesity risk. They studied eight common genetic variants which are associated with adult obesity and childhood BMI to derive an obesity-risk-allele score. This combined obesity-risk-allele score was associated with higher rates of weight and length gain particularly in early infancy. However, only a relatively small proportion (1.7%) of the variation in BMI at nine years of age was

explained by the obesity-risk-allele score. Hopefully, it will be possible in the future to identify the children who are at increased risk of developing overweight and to develop an effective intervention.

Chapter 2 describes a high genetic correlation between height and weight at the age of 5 years implying that these two traits are mainly under control of the same additive genetic factors. This is in concordance with the results from a very large study in young adult men showing that the phenotypic and genotypic correlation between height and weight was fairly high in young adulthood (Silventoinen et al., 2008b). This is another illustration of the complexity of the genetic mechanisms underlying individual differences in body size.

A further body size feature that was studied is testis volume (chapter 4), which is related to testicular function and thus of importance for male fertility (Petersen et al., 2006). Little was known about the causes of individual differences in testis volume, but this study in male twins and siblings showed significant familial resemblance. Heritability was estimated at 59%, but a model that excluded genetic influences and attributed all familial resemblance to shared environment, fitted the data only marginally worse. However, it is likely that genetic effects play a role, considering the existence of ethnic differences with testes being larger in Caucasian than in Asian men (Short, 1984) and the finding of genes in inbred mouse strains controlling testis size by regulating the number of Sertoli cells (Chubb, 1992). Dizygotic twins and their brothers appeared to have a larger mean testis volume than monozygotic twins. This difference in testis volume could not be explained by body weight or pre/perinatal problems, but it has been suggested that this may be related to dizygotic twinning (Fryns, 1986; Short, 1984). Observations of testis volume in different ethnic populations and the fragile X mental retardation syndrome suggest genetic factors responsible for both dizygotic twinning and larger testis size. The larger mean testis volume in dizygotic twins is an interesting finding which needs to be replicated and may be of interest for future research into the mechanisms underlying dizygotic twinning and fertility, as this phenotype may be the expression of 'twinning' genes in men.

Comparison of twins with singletons

Twins are smaller at birth than singletons due to a combination of intra-uterine growth restriction and shorter gestational age (Alexander et al., 1998; Glinianaia et al., 2000; Kiely, 1990; Min et al., 2000). Our goal was to examine when these differences in body size disappear during childhood and adolescence. Therefore, we investigated twin growth in relation to singleton standards and to non-twin siblings. First we looked at a large dataset collected by questionnaires and discovered that 5-year-old twins were particularly leaner than children from the general population (chapter 2). Female twins were as tall as singleton children, while male twins were slightly shorter than singletons. At age 5, both male and female twins grew below their target height, which may be due to the above-average height of twin parents. Thus, we concluded that twin growth could be best analyzed in relation to their singleton siblings who have the same parents (and target height) and grew up in the same environment. Therefore the longitudinal sample was used which showed no differences in height between the 18-year-old twins and their siblings. At the age of 18 years twins had the same height and BMI as children from the general population. Additional analyses revealed that both twins and siblings grew below their target height, so our finding in 5-year-old twins was not twin-specific. The deviation (mean -0.4 SDS) was within the normal range and thus has little or no clinical importance, but it is still remarkable. Thus the height of both adolescent twins and siblings did not differ from the mean of the general population (0 SDS), while the parents of twins were generally taller. The average height of twins and siblings may be due to the phenomenon of regression to the mean, which was first described by Francis Galton in 1886. Galton measured height of adult children and their parents and noted that when the average height of the parents was greater than the mean of the population, the children tended to be shorter than their parents. Likewise, when the mean height of the parents was shorter than the population mean, the children tended to be taller than their parents. The fact that twins caught up and attained same adult height as singletons implies a rapid height and weight gain during infancy (average 0.7 SDS). One has to be aware that catch-up growth has been associated with a number of adverse outcomes (see below: Influence of early growth in later life).

Another remarkable finding was that the 18-year-old twins were leaner than their siblings. The lower BMI in twins could not be explained by their lower birth weight or effects of zygosity. It could be hypothesized that twins have less appetite or a different energy balance than their singleton siblings. There is growing evidence that the fetal and perinatal nutritional environment may have impact on the function of the appetite regulating neural network and therefore the way in which an individual regulates its feeding behavior, energy balance and, ultimately body weight throughout later life (Muhlhausler, 2007; Remmers et al., 2008a, 2008b). It could also be hypothesized that twins grow up under environmental conditions in which they always have someone to play with and thus may show increased activity levels, as compared with other children. There is no literature about this topic in children, but studies in adolescent and adult twins indicate that increased activity levels are not observed after childhood. There are no differences in sedentary behavior or exercise behavior between twins and siblings in adolescence (Van der Aa, N., personal communication) and adulthood (De Moor, M.H.M., personal communication). For future research it would be interesting to study whether the differences in BMI between twins and siblings persist in later life taking possible differences in (feeding) behavior, energy balance and physical activity into account.

Concerning generalizability, one has to be careful in extrapolating results from twin growth data to the general population because of the differences that we found in mean BMI between twins and siblings. However, the covariance of BMI between adolescent twins and siblings could be constrained to equal the covariance between dizygotic twins, implying that the causes of variation do not differ between twins and siblings.

The new opportunities based on GWA studies will make it possible to test SNPs, discovered in general population samples, in twin populations.

Influence of early growth in later life

Twins experience intrauterine growth restriction (IUGR) and are consequently born with a lower birth weight than (non-twin) siblings and singletons, followed by a period of compensatory growth. Both conditions, lower birth weight and compensatory growth, have been associated with adverse outcome in later life.

In chapter 3 the influence of size at birth on height and BMI in late adolescence was investigated. The decreased BMI values in twins could not be explained by their lower birth weight. However, height at age 18 years was significantly positively associated with birth weight in twins. Twins who were heavier at birth, were generally taller in adolescence, which is in correspondence with the findings from a study that tracked body size from birth to late adolescence (Pietilainen et al., 2001).

Chapter 5 describes the influence of genes, birth weight and catch-up growth on serum dehydroepiandrosterone-sulfate (DHEAS), insulin-like growth factor-I (IGF-I) and fasting insulin levels. It has been hypothesized that DHEAS and IGF-I may contribute to links between reduced fetal growth and adult disease risks, possibly by enhancing insulin resistance (Ibanez et al., 2009; Iniguez et al., 2006; Ong et al., 2004b; Veening et al., 2004). Genetic influences on the variation in DHEAS, IGF-I and insulin levels were high in late adolescence, but there was no significant influence of birth weight on hormone levels in the entire sample. However, in subjects with catch-up growth birth weight was significantly inversely related to DHEAS and IGF-I levels. Thus within the group of catch-up growth subjects of low birth weight had the highest DHEAS and IGF-I levels. It concerned only a small effect and its clinical significance is uncertain, while there is no association between insulin and DHEAS or IGF-I levels, leaving the mechanism whereby early growth is linked to disease in later life unclear.

Chapter 6 looked at the influence of early growth on cognitive function in later life. Birth weight is known to be positively related to cognitive function (Boomsma et al., 2001; Breslau et al., 1994; Christensen et al., 2006; Matte et al., 2001; Richards et al., 2001), but the relation between catch-up growth and cognitive function has not been studied in twins before. Like the study in zebra finches (Fisher et al., 2006) postnatal weight gain was

negatively associated with cognitive performance in twins at age 12 and 18 years. Thus a larger gain in weight during the first 2 years of life was associated with a lower IQ in adolescence. However, catch-up growth was highly correlated with birth weight and this correlation may explain part of the association. Because it is difficult to differentiate between the effects of birth weight and catch-up growth, interpretation of these results awaits further studies in human populations. Catch-up growth is, in contrast, associated with better cognitive outcome in children born small for gestational age (SGA) when compared with SGA children without catch-up growth (Fattal-Valevski et al., 2009; Geva et al., 2006; Lundgren et al., 2001). It has to be noted that the IUGR is generally more severe in SGA children than in twins and may have a different origin (see below). It could be hypothesized that a certain compensatory growth is needed to obtain adequate brain volume and function, but there may be a critical point after which catch-up growth has negative consequences as well. A design including mono- and dizygotic twins concordant for birth weight and discordant for catch-up growth would be helpful to gain more insight into the potential effects of postnatal catch-up growth.

In the last 25 years after the introduction of the 'Barker hypothesis', research in this field has been very productive. Many associations between early growth and disease (risks) in later life have become clear, but still very little is understood about the underlying mechanisms. It is clear that both genetic and environmental factors play a role, but to date only a few genetic variants have been identified. Recently, a large study showed that loci near *CCNL1* and at *ADCY5* are associated with birth weight (Freathy et al., 2010). The subjects carrying four of these alleles are, on average, 113 gram lighter at birth than the persons with zero or one alleles. The *ADCY5* locus is also known to have pleiotropic effects on glucose regulation and type 2 diabetes in adulthood, suggesting that the widely described association between lower birth weight and subsequent type 2 diabetes also has a genetic component. This is a remarkable and important finding in line with the thrifty genotype theory (Hattersley & Tooke, 1999), but it explains only a small part of the association between fetal growth and adult diseases. The concept of developmental plasticity (the ability of an organism to develop in various ways depending on the particular

environment or setting (Bateson et al., 2004)) increases the understanding of how early growth may result in a greater individual's risk on adverse outcome in later life (Gluckman & Hanson, 2008). Developmental plasticity can be described as a set of mechanisms that can adjust the evolutionarily determined genetic potential of the organism to be better adapted to its environment, taking into account information from early life of that particular individual. The processes of developmental plasticity act through changes in anatomical and functional development that are mediated directly or indirectly by epigenetic processes. Epigenetics in the current context refers to those mechanisms that lead to long-term changes in gene expression through chemical modification to or alterations in the packaging of DNA such that the capacity for transcriptional regulation is altered (Goldberg et al., 2007). These developmental pathways do not directly cause a disease such as type 2 diabetes but rather alter the risk of an individual developing a disease later in life. At least two classes of developmental pathways are involved. The first pathway can be cued by prenatal undernutrition or stress that leads the organism to adapt to better survive in an environment of scarcity. However, when an environment of food abundance is present, the alterations predispose to diseases such as obesity and cardiovascular disease. Secondly, fetal or infant overnutrition can also affect later development in a manner that can be manifested as obesity and disease. Such overnutrition may have its origin in maternal diabetes, maternal obesity or infant overfeeding (Boney et al., 2005; Harder et al., 2005; Hillier et al., 2007).

The concept of developmental plasticity illustrates the complexity of the contribution of genetic and environmental factors, which is unique in every individual, finally resulting in a disease risk or adverse outcome in that particular individual.

It has to be emphasized that though catch-up growth is related to adverse outcome in later life, a certain level of compensatory growth is needed to attain normal adult height (Karlberg & Albertsson-Wikland, 1995). Moreover, in children born SGA good catch-up growth is associated with better outcome at later ages with respect to IQ and cognition (Fattal-Valevski et al., 2009b; Geva et al., 2006; Lundgren et al., 2001). It is important to differentiate between catch-up growth in height or weight,

while particularly great postnatal weight gain has been associated with adverse outcome in later life.

Methodological considerations

Twins and the fetal origins hypothesis

Barker excluded twins from the fetal origins hypothesis since twins have different patterns of fetal growth than singletons (Barker, 1995b). Barker argued that twins are a heterogeneous group of proportionately and disproportionately small babies, referring to a relatively small study measuring biparietal diameter in twins (Leveno et al., 1979). Several studies have shown that twins experience significant IUGR starting early in the third trimester from at least 30 weeks of gestation (Alexander et al., 1998; Bleker et al., 1988; Glinianaia et al., 2000; Kiely, 1990; Min et al., 2000; Taylor et al., 1998; Wilson, 1974). At birth, weight is more compromised than length and head circumference (Buckler & Green, 2004; Wilson, 1974) which is called disproportionately or asymmetric growth restriction. In our sample weight was also more compromised than length at birth (chapter 3). Unfortunately, data on head circumference at birth were not available. The timing (third trimester) and type of IUGR (asymmetric) in twins is not that different from IUGR in SGA singletons for which the hypothesis has been developed. However, there are two possible differences in IUGR between twins and singletons, which are of importance for the interpretation of our results.

First, the pathophysiology. In multiple pregnancies there is a clear cause for IUGR. Due to maternal utero-placental constraints twins grow below their potential (Bleker et al., 1988). In singletons, IUGR of the asymmetric type is generally based on placental insufficiency or dysfunction with inflammatory processes leading to changes in the placenta on the metabolic level and by secretion of hormones and growth factors to the maternal and fetal circulation (Fowden et al., 2009; Wollmann, 1998). These processes may induce developmental alterations which lead subsequently to a greater risk of adverse outcome in later life.

Second, twins generally have a reduced size at birth, but often do not fulfill

the criteria of SGA (growth below -2 SDS, -2.5 SDS or the 10th percentile). For example, the mean twins' birth weight in our sample was -0.9 SDS. So twins do experience some IUGR, but it is not that severe as for children born SGA.

Despite the differences in intrauterine growth between twins and singletons the associations between birth weight and adult disease risks, such as blood pressure, are remarkably similar in twins and siblings (Huxley et al., 2000; IJzerman et al., 2000). Thus intrauterine growth is relevant for the development of adult disease (risks) in twins and differences in birth weight in twins can be used as a model for differences in birth weight in singletons. It is important to realize that there are no differences between twins and the general population with regard to all-cause mortality or cardiovascular mortality (Christensen et al., 2001; Vagero & Leon, 1994). Hence, the IUGR experienced by twins does not result in any fetal programming of cardiovascular diseases. There is still an important role for twins to play in the testing of the fetal origins hypothesis, namely within (monozygotic) twin pairs who are for example discordant for birth weight. An important test is whether the twin with the lower birth weight is at increased risk for later adult diseases such as type 2 diabetes (Iliadou et al., 2004). Moreover, the classical twin design has the advantage of providing information on the genetic and environmental factors underlying the associations between early growth and later health outcome.

Questionnaire data

In this thesis both measured data and data assessed by maternal report (survey studies) were used. Due to instrument imprecision and human inconsistencies measurements are not free of error. Because the reliability of reported data is often questioned, correlations were estimated between measured and reported data. In chapter 3 data were used from a large questionnaire sample collected near the twins' fifth birthday. In a subsample of 94 twins the correlation between laboratory measured and parent-reported data was .96 for height and .92 for weight. Furthermore the correlation was calculated between reported and measured height and weight of twins and siblings who participated in the medical protocol and filled out a questionnaire. The Pearson correlation was .98 for both height

and weight ($p<0.05$) (n=435 and 421 respectively).
These correlations show that the reliability of reported height and weight was high and thus can safely be used for studies.

Clinical implications and future perspectives

The incidence of twin births is 1.7% in The Netherlands (Statistics Netherlands, 2008), which means that a considerable number of newborns have a reduced size at birth. This study gives more insight into the growth of twins, which is relevant for the daily practice when monitoring growth of twins. The finding that adolescent twins were thinner than their siblings needs further to be elucidated. If twins remain thinner in adulthood this may be of interest for future research into (the prevention of) obesity.
Another interesting aspect of growth is the higher mean testicular volume of dizygotic male twins and their siblings. It has been suggested that there are genetic factors responsible for both dizygotic twinning and larger testis size which requires further investigation, as testicular size may be the phenotypic expression of 'twinning' genes in men.
Regarding the long-term consequences of IUGR in twins there was no relationship between birth weight and serum DHEAS, IGF-I and fasting insulin levels in late adolescence. As expected, BMI predicted fasting insulin levels significantly. The children of low birth weight with catch-up growth and the highest BMI had the highest fasting insulin levels. It is known from literature that these children have an increased risk of developing diseases such as type 2 diabetes and cardiovascular disease. These children should be monitored carefully from early age on to prevent overweight and to support a healthy lifestyle.
Serum DHEAS and IGF-I levels were not associated with fasting insulin levels and thus may not contribute to links between early growth and disease in later life via the pathway of enhancing insulin resistance.
The mechanism by which early growth is linked to diseases in later life needs to be unraveled. To this end twin studies could be helpful by providing information about the factors (genetic vs. environmental) underlying these associations.
Finally, this study demonstrated that a larger gain in weight during the

first 2 years of life is associated with a lower cognitive performance. Catch-up growth is strongly negatively correlated with birth weight which may partially explain the association. To date there is not enough evidence to discourage catch-up growth (for weight) because of cognitive function. For medical reasons (metabolic consequences) there is yet enough evidence to monitor postnatal weight gain carefully and to intervene when developing overweight, particularly in high-risk children like preterm and/or SGA born individuals. Future studies should investigate the possible negative consequences of catch-up growth on cognitive function in later life.

Chapter 8

Summary

Samenvatting

This thesis describes the etiology of individual differences in growth and the influence of early (i.e. intrauterine and postnatal) growth on physical and mental outcome in later life. To this end a sample of 18-year-old twins and their siblings were invited to participate in medical and psychological testing. Anthropometry and IQ tests were performed and blood samples were collected.

The *first chapter* of this thesis served as an introduction into the variation in growth in fetal and postnatal life and its possible effects on developmental outcome in later life. This chapter also described the study protocol and different designs used in this thesis and the longitudinal sample that was initially recruited at the age of 5 years.

C*hapter 2* examined the causes of individual differences in height, weight and BMI in 5-year-old twins using data from a large questionnaire sample. In addition, the data from twins were compared with Dutch reference growth data and the twins' target height, derived from parental height, was investigated. As expected, genetic influences were an important source of variation in height, weight and BMI and the main source of covariation between height and weight. At the age of 5 years, female twins were as tall as singleton children, while male twins were shorter than singletons. All twins had a lower BMI than singletons. Twins grow fairly well compared with singletons, but they grow below their target height, which may be due to the above-average height of their parents.

In *chapter 3* growth data of twins were compared with data from their non-twin siblings and secondly, twin and sibling data were compared with population standards. Data from the longitudinal sample were analyzed. Twins attained normal adult height compared with siblings and children from the general population. Birth weight was shown to have a considerable effect on height in adolescent twins. As for BMI, no differences were shown between 18-year-old twins and children from the general population, whereas the siblings of twins had increased BMI values.

Chapter 4 describes the study of the heritability of testis size of the 18-year-old male twins and their non-twin brothers from the longitudinal sample. There was significant familial resemblance, with an estimated heritability of 59%, but a model that excluded genetic influences and attributed all familial resemblance to shared environment, fitted the data only marginally worse. Dizygotic twins and their brothers had a larger mean testis volume than monozygotic twins and their brothers which may be of interest for future research into the mechanisms underlying dizygotic twinning, as testicular size may the phenotypic expression of 'twinning' genes in men.

In *chapter 5* the influence of genes and early growth on hormone levels in late adolescence were studied using the longitudinal sample of twins and their siblings. Low birth weight has been associated with higher childhood levels of dehydroepiandrosterone-sulfate (DHEAS) and insulin-like growth factor-I (IGF-I). It has been hypothesized that these hormones may contribute to links between reduced fetal growth and adult disease risks, possibly by enhancing insulin resistance. Genetic influences on the variation in DHEAS, IGF-I and fasting insulin levels were high in late adolescence. However, there was no significant influence of birth weight on hormone levels in the study population, but in subjects with catch-up growth birth weight was significantly inversely related to DHEAS and IGF-I levels. There was no association between insulin and DHEAS or IGF-I levels, leaving the mechanism whereby early growth is linked to disease in later life unclear.

In *chapter 6* we explored whether postnatal catch-up growth was associated with long-term negative consequences for cognitive function as was suggested by Fisher et al. in 2006 with a study in zebra finches. Indeed, a greater gain in weight during the first 2 years of life was associated with lower IQ scores at ages 12 and 18 years. However, catch-up growth was correlated with birth weight and this correlation may have explained part of the association. In conclusion, the findings in the present thesis show that genes were the most important source of variation in growth and hormonal parameters. Concerning the reduced size of twins at birth, twins showed almost complete catch-up growth compared with non-twin siblings and children from the general population. Twins attained normal adult height, but twins were still somewhat leaner at the age of 18 years than their non-twin siblings. There was no association between birth weight and the hormonal parameters that were tested in late adolescence. Regarding mental development, we found that catch-up growth might be associated with a slightly impaired cognitive function.

Based on these results we conclude that findings from twin studies into growth and hormonal parameters that we studied in this thesis can be generalized to the general population, particularly when analyzing covariance structures (familial resemblance).

Samenvatting

Groei is een zeer complex proces van veranderingen in vorm, lichaamssamenstelling en verdeling van verschillende soorten weefsels. Dit proces wordt beïnvloed door biologische, psychologische en sociale factoren.
Er zijn grote individuele verschillen in lichamelijke en geestelijke ontwikkeling, zowel vroeg in het leven (tijdens de zwangerschap en op de zuigelingenleeftijd) als later in het leven (kinderleeftijd en adolescentie).
In dit proefschrift wordt ingegaan op de oorzaken van individuele verschillen in fysieke groei en cognitieve ontwikkeling tijdens verschillende fases van groei (zwangerschap, zuigelingenleeftijd, kinderleeftijd en adolescentie). Daarbij richt dit proefschrift zich op de vraag in hoeverre variatie in groei verklaard kan worden door genetische en niet-genetische ('omgevings') factoren.
Dit soort onderzoeksvragen kan met behulp van tweeling- en familieonderzoek bestudeerd worden. Eeneiige tweelingen zijn genetisch (vrijwel) identiek, terwijl twee-eiige tweelingen gemiddeld de helft van hun genetisch materiaal delen, net als gewone broers en zussen. Als eeneiige tweelingen van elkaar verschillen kan dit worden veroorzaakt door omgevingsinvloeden (naast bijv. epigenetische invloeden). Verschillen tussen twee-eiige tweelingen kunnen zowel door omgevingsinvloeden als door verschillen in genetische aanleg worden veroorzaakt. Dit geldt ook voor gewone broers en zussen, maar zij zijn daarnaast ook nog op een ander moment geboren en opgegroeid. Door het vergelijken van de gelijkenis van eeneiige tweelingen en twee-eiige tweelingen en hun niet-meerling broers en zussen kan worden onderzocht in hoeverre genetische en omgevingsinvloeden van belang zijn bij het verklaren van verschillen tussen mensen in bijvoorbeeld lengte of gewicht. De omgevingsfactoren kunnen worden onderscheiden in twee soorten invloeden. De gedeelde factoren zijn de invloeden die voor een ieder binnen het gezin hetzelfde zijn en doen gezinsleden meer op elkaar lijken. Daarnaast zijn er omgevingsinvloeden die voor ieder gezinslid uniek zijn, en ervoor zorgen dat gezinsleden van elkaar verschillen.
Een andere belangrijke onderzoeksvraag wanneer men groei bestudeert in tweelingen, is of resultaten van tweelingenstudies gegeneraliseerd kunnen worden naar de algemene bevolking. Tweelingen worden vaker

na een kortere zwangerschapsduur en met een lager gewicht geboren dan eenlingen. Om mogelijke verschillen tussen tweelingen en eenlingen nader te onderzoeken werd ook een niet-meerling broer of zus van de tweeling uitgenodigd deel te nemen aan onze studie. Niet-meerling broers of zussen zijn het meest geschikt om tweelingen mee te vergelijken, omdat zij in hetzelfde gezin opgroeien en gemiddeld de helft van hun genetisch materiaal delen.
Voor dit onderzoek werd een groep van 18-jaar-oude tweelingen en een broer of zus uitgenodigd deel te nemen aan medische en psychologische tests. De procedures omvatten anthropometrische metingen, intelligentie en cognitie tests en bloedafname.
In het *eerste hoofdstuk* van dit proefschrift worden verschillende aspecten van groei voor en na de geboorte toegelicht. Tevens worden de begrippen intrauteriene groeirestrictie en inhaalgroei besproken en de mogelijke gevolgen daarvan op de lange termijn. Gecompromitteerde foetale groei en compensatoire groei na de geboorte kunnen namelijk nadelige effecten hebben op latere leeftijd, zoals een verhoogde kans op hart- en vaatziekten of diabetes. Verder worden de studiepopulatie, studieprotocol en statistische methoden die worden gebruikt in dit proefschrift beschreven.
In *hoofdstuk 2* worden de oorzaken van individuele verschillen in lengte, gewicht en body mass index (BMI) op de leeftijd van 5 jaar onderzocht. Hiervoor werd gebruikt gemaakt van gegevens van een groot aantal 5-jaar-oude tweelingen, van wie de ouders meededen aan vragenlijst-onderzoek. Ook wordt gekeken hoe 5-jaar-oude tweelingen groeien ten opzichte van leeftijdsgenoten (m.b.v. referentiewaarden van de Nederlandse bevolking) en ten opzichte van hun streeflengte (berekend op basis van lengte van vader en moeder). Zoals verwacht zijn genetische invloeden de belangrijkste bron van variatie in lengte, gewicht en BMI en de voornaamste bron van covariantie tussen lengte en gewicht. Op de leeftijd van 5 jaar zijn tweelingmeisjes net zo lang als eenlingmeisjes, maar tweelingjongens zijn kleiner dan eenlingjongens. Alle tweelingen hebben een lagere BMI dan eenlingen. Hieruit kan geconcludeerd worden dat tweelingen goed groeien ten opzichte van niet-meerlingen, maar dat zij wel onder hun streeflengte groeien, wat mogelijk verklaard kan worden door de bovengemiddelde lengte van ouders van tweelingen.

In *hoofdstuk 3* worden de groeidata van tweelingen vergeleken met die van hun niet-meerling broer of zus en vervolgens met referentiewaarden van de Nederlandse bevolking. Tweelingen bereiken een normale eindlengte in vergelijking met hun broers/zussen en leeftijdsgenoten (18-jarigen uit de Nederlandse bevolking). Wat betreft BMI verschillen 18-jaar-oude tweelingen niet van hun leeftijdsgenoten, terwijl de broers/zussen van tweelingen gemiddeld een hogere BMI hebben.
Hoofdstuk 4 beschrijft een van de weinige studies naar erfelijkheid van testisvolume in 18-jaar-oude tweelingen en hun niet-meerling broers. Er is een significante familiegelijkenis met een geschatte erfelijkheid van 59%. Echter, een model dat alle familiegelijkenis toeschrijft aan gedeelde omgeving en genetische invloeden uitsluit paste de data slechts iets minder goed. Twee-eiige tweelingen en hun niet-meerling broers hebben gemiddeld een groter testisvolume dan eeneiige tweelingen en hun niet-meerling broers, wat mogelijk interessant is voor toekomstig onderzoek naar de mechanismen die een rol spelen bij het krijgen van twee-eiige tweelingen.
In *hoofdstuk 5* worden de invloed van genetische factoren, geboortegewicht en inhaalgroei op hormoonwaarden op jong volwassen leeftijd bestudeerd. Uit de literatuur is bekend dat een laag geboortegewicht geassocieerd is met een eerdere start van de productie van bijnierandrogenen als dehydroepiandrosterone-sulfate (DHEAS), maar ook met hogere serumwaarden van DHEAS en insulin-like growth factor-I (IGF-I) op de kinderleeftijd. Er zijn aanwijzingen dat deze hormonen een rol spelen bij de associatie tussen intrauteriene groei en ziekte op latere leeftijd, bijvoorbeeld door het bevorderen van insulineresistentie. De invloed van genetische factoren op de variatie in serum DHEAS, IGF-I en nuchtere insulinewaarden is groot op de leeftijd van 18 jaar. Er werd geen significant verband aangetoond tussen geboortegewicht en hormoonwaarden. Echter, in de subgroep met personen die inhaalgroei hebben vertoond, is een lager geboortegewicht wel significant geassocieerd met hogere serum DHEAS en IGF-I waarden. Wij vonden geen verband tussen insuline enerzijds en DHEAS of IGF-I anderzijds. Aangezien inhaalgroei gepaard gaat met een hoger risico op complicaties zouden deze factoren toch een rol kunnen spelen in het ontstaan hiervan.

In *hoofdstuk 6* wordt nagegaan of inhaalgroei na de geboorte nadelige gevolgen heeft voor de cognitieve functie op de lange termijn, zoals bijvoorbeeld eerder in een studie met zebravinken werd aangetoond. Een grotere gewichtstoename in de eerste twee jaren van het leven is inderdaad geassocieerd met lagere IQ-scores op de leeftijd van 12 en 18 jaar. Echter, inhaalgroei hangt nauw samen met geboortegewicht, wat mogelijk bovenstaande correlatie grotendeels verklaart.
In hoofdstuk 7 worden de resultaten uit de eerdere hoofdstukken in een groter verband geplaatst en bediscussieerd.
De bevindingen van dit proefschrift samenvattend kunnen we concluderen dat genen de voornaamste bron van variatie in de door ons bestudeerde groei- en hormonale parameters zijn. Wat betreft de groeiachterstand van tweelingen bij de geboorte, hebben tweelingen bijna volledige inhaalgroei laten zien ten opzichte van hun niet-meerling broers/zussen en leeftijdsgenoten. Tweelingen bereiken een normale eindlengte, maar zij waren wel wat dunner op de leeftijd van 18 jaar dan hun niet-meerling broers/zussen. Er werd geen verband aangetoond tussen geboortegewicht en de hormoonwaarden die wij gemeten hebben op jong volwassen leeftijd. Inhaalgroei na de geboorte zou mogelijk geassocieerd kunnen zijn met een iets verminderd cognitief functioneren. Op basis van deze resultaten kan geconcludeerd worden dat uitkomsten van tweelingstudies naar groei- en hormonale parameters, voor zover bestudeerd in dit proefschrift, gegeneraliseerd kunnen worden naar de algemene bevolking.

Appendices

Appendix I

Sample characteristics and data collection

The medical protocol was carried out in 184 families of 18-year-old twin pairs and their siblings (N=98). There were three families with two twin pairs and there were six incomplete twin pairs of which only one of the twins participated. Two families participated only in the psychological protocol.

Mean age at assessment was 18.14 years (SD 0.48) in the twin group and 18.78 years (SD 4.89) in the sibling group (youngest sib was 7 years and oldest sibs was 35 years). Zygosity of the same-sex twin pairs (148 pairs) was determined on the basis of DNA polymorphisms (145 pairs), blood group polymorphisms (2 pairs), or questionnaire items on similarity (1 pair (Rietveld et al., 2000)). The sample comprised 32 MZM (monozygotic male), 34 DZM (dizygotic male), 44 MZF (monozygotic female), 38 DZF (dizygotic female) and 39 DOS (dizygotic opposite sex) twin pairs and 97 siblings (45% males and 55% females).
The complete program of the testing day and the approximate starting times of the different tests are provided in Table I.1. The measures and procedures are described below.

Anthropometry

The medical protocol was carried out at the outpatient clinic of the VU University Medical Center. **Height** (cm) was determined to the nearest 0.1cm and **weight** (kg) to the nearest 0.05 kg using a stadiometer and an electronic scale (SECA, Hanover, Md). **Sitting height** was measured by bringing the horizontal bar of the microtoise into the most superior midline of the head while the child was sitting in erect position on a special stool. Arching of the back was avoided as much as possible by applying upward pressure to the mastoid processes. **Arm span** was obtained by measuring the distance between the arms in stretched position using a plastic tape measure. **Head circumference** was measured with a

plastic tape measure over the occiput and just above the eyebrows and ears. **Waist circumference** was measured at the level of the umbilicus after full expiration and hip circumference at the level of the greater trochanter, both with the use of a flexible tape measuring to 0.1 cm accuracy. Four **skinfold-thickness** measures were taken three times by me on the non-dominant side of the body using a calibrated Harpenden skinfold calliper at the biceps, triceps, subscapular and suprailiacal regions.
Parental height and weight were measured if present at the testing day (59 mothers and 39 fathers).

Pubertal development

Stage of puberty was physically determined by the principle investigator on the basis of secondary sexual characteristics using the stages of development devised by Tanner (1981). Left and right testicular volume was measured using a Prader orchidometer, in which a series of testes models of volumes 2, 3, 4, 6, 8, 10, 12, 15, 20 and 25 ml were compared tactually with the actual testis (Zachmann et al., 1974).

Cardiovascular function

The Vrije Universiteit Ambulatory Monitoring System (VU-AMS) is a device to ambulatory record electrocardiogram and impedance cardiogram (De Geus et al., 1995; Willemsen et al., 1996). The testing day started by attaching the VU-AMS to the body of each participant. Heart rate, systolic and diastolic blood pressure were assessed four times during the testing day using a Spacelabs 90207 ambulatory blood pressure monitor (Redmont, Washington, USA).

Biological materials & Laboratory assessments

Blood Venous blood samples were taken from twins and their siblings between 10.00 and 11.00 a.m. (mean time 10.35 a.m.) after overnight fasting. A total of 9 blood tubes were collected from all participants for DNA, lymphocytes, RNA (basal and after LPS challenge), plasma, and serum. The order of sample draw was 1 x serum, 1 x 4,5 ml CTAD, 1 x ACD, 2 x 7 ml heparin, 2 x 7 ml EDTA, and 2 x 2 ml EDTA. To prevent clotting all tubes were inverted gently immediately after collection. The two 2 ml EDTA

tubes were used for immediate measurement of hematology parameters and HbA1C by the laboratory at the VU University Medical Center.
The haematology parameters assessed were the following: hemoglobin (mmol/l), hematocrit, platelets (x 10^9/l), erythrocytes (x 10^{12}/l), mean cellular volume (fl), mean cell hemoglobin (amol/cel), mean cell hemoglobin concentration (mmol/l), white blood cells (x 10^9/l) with a differentiation by the computer.
The serum tube was centrifuged for 10 minutes at 3000 rpm at 18° C, after which serum plasma was harvested from the buffy coat and red blood cells, aliquoted (0.5 ml), snap-frozen in dry ice, and stored at -20° C (1x0.5 ml at -70° C) until assay. Serum dehydroepiandrosterone-sulfate (DHEAS) was measured by solid phase competitive chemiluminescent enzyme immunoassay (IMMULITE 2500, Siemens, USA) and serum insulin-like growth factor-I (IGF-I) by immunometric assay (IMMULITE 2500, Siemens, USA) in the Endocrinological Laboratory of the VU University Medical Center (see for details: chapter 5).
One heparin tube, the two 7 ml EDTA tubes and the CTAD tube were stored in melting ice until being processed at the laboratory. After centrifugation of the tubes for 20 minutes at 3000 rpm at 4°C, the heparin, citrated and EDTA plasma was obtained and divided into subsamples of 0.5 ml, snap-frozen and stored at -20°C. The EDTA buffy coats were snap-frozen in dry ice, and stored at -20° C for later DNA extraction.
Glucose and insulin concentrations were measured in heparin plasma by the Gaubius Laboratory TNO-Quality of Life (Biomedical Research, Leiden, NL). Glucose concentrations were assessed using the Vitros 250 Glucose assay (Johnson&Johnson, Rochester, USA) and insulin measurements were performed using the Immulite 1000 Insulin Method (Siemens Medical Solutions, Breda, NL). Other measurements that were performed in heparin plasma by the same laboratory for the NTR biobank were the following: lipid profile; triglyceride; and C-reactive protein (see for further details: (Willemsen et al., 2010)).
About 15 minutes after collection 2.5 ml blood from the second heparin tube was added to a PAX tube with code A. After adding 50 microliter of lipopolysaccharide (LPS) to the same heparin tube, the tube was inverted gently and stored at 37° C during 5 hours. After these 5 hours, 2.5 ml of

the challenged heparin blood was added to a PAX tube with code B. The PAX-A tube was then, after 5 hours at room temperature, inverted gently and stored at -20° C. After leaving the PAX-B tube 1.5 hour at room temperature, the tube was inverted gently and subsequently stored at -20° C for later RNA extraction for expression studies.
Once to twice a week the ACD tubes were processed in a sterile flow cabinet. For subsequent lymphocyte isolation, 15 ml Ficoll was added to a sterile tube and centrifuged for 30 seconds at 1000 x *g* at room temperature. Then, 10 ml phosphate-buffered saline (PBS) and the ACD tube were added to the Ficoll and centrifuged for 15 minutes at 800x *g* at room temperature. The middle layer containing the leucocytes was transferred into a new tube and 10 ml phosphate-buffered saline (PBS) was added. The solution was centrifuged 10 minutes at 250x *g* at room temperature. This washing step was repeated after discarding the supernatant. The lymphocyte pellet was then taken up into 4 ml RPMI: 20% Foetal Calf Serum (FCS), 10% and dimethyl sulfoxide (DMSO). Finally, pellets were frozen and stored overnight at -80° C and then transferred to liquid nitrogen for long-term storage.
Urine The urine sample collected by the participant in the morning after getting up was stored in melting ice until processing. In the laboratory 30 ml urine was directly stored at 20^0 C and approximately 20 ml urine was transferred to two 10 ml vacutainers, which were centrifuged for 20 min at 3000 rpm at 4° C. The urine without the debris pellet was then decanted in two 10 ml plastic tubes, snap-frozen and stored at -20^0 C.
Saliva collection devices (for assessment of cortisol and testosterone levels) were sent to the twin families by mail prior to the testing day. The participants were instructed to collect their samples on the same day as their siblings and to do so on two week days, to try to restrict the awakening time and time of sampling. Each participant was asked to write down the exact sampling time in a 'saliva diary' and to note exceptional events interfering with the daily routine. Moreover, their awakening times on the sampling days and on the two days prior to the day of sampling were noted. Subjects were instructed not to brush their teeth and not to eat or drink in the 30 minutes preceding the saliva collection. Saliva samples were stored in a refrigerator at the twin family's home and brought to the testing day. In the laboratory, samples were stored at -20^0 C until the immunoassay analyses. For the assessment of cortisol levels, subjects were asked to collect saliva at five time points on one day using the salivette, a polyester saliva collection device (Sarstedt AG&Co., Nümbrecht, Germany). The first sample was taken in the morning just before getting up, the second, third and fourth sample were taken 15, 30 and 45 minutes respectively after the first sample. The fifth sample was taken just before lunch. At this last time point, the participants were asked to collect an additional saliva sample by passive drool for the analysis of testosterone levels. The participants were asked to follow this procedure twice in one week resulting in ten (2x5) cortisol samples and two testosterone samples.
Buccal swabs To obtain buccal swabs for DNA isolation all participants (twins, sibling and parents) were mailed a sample collection kit containing a tube with cotton buds, tubes with collection buffer and a sampling protocol. They were asked to refrain from eating or drinking one hour prior to collection. Participants took mouth swabs themselves following the sampling protocol which instructed on how to gently rub the cotton swab tips along the inside of the mouth. After rubbing, the mouth swabs were placed in a Falcon tube, containing 0.5 ml of STE buffer (100 mM NaCl, 10 mM Tris and 10 mM EDTA) with proteinase K (0.1 mg/ml) and sodium dodecyl sulfate (0.5%) per mouth swab. In most participants, 16 mouth swabs were collected by conducting 4 separate swabs twice per day during two days. The sample tubes were brought to the testing day. Upon arrival, swabs were stored at room temperature in the laboratory until DNA extraction.

NHS growth data

Twins and their siblings were asked to bring the report with growth data measured by the Dutch National Health Services (NHS) from birth to the age of 4 years ('Groene Boekje'). The measurements were copied from the NHS report.

Questionnaire data

All participants were asked to fill out at home the Dutch Health and Behavior Questionnaire (DHBQ), an extensive questionnaire encompassing the Youth Self Report (Achenbach & Rescorla, 2001; Verhulst et al., 1997) to assess behavioral and emotional problems and questions concerning pubertal status; physical health; grade in school; sports participation; leisure activities; peer and self smoking behavior and drug use; eating problems; self esteem; life events; religiosity; happiness; life satisfaction; family situation; and family functioning (Bartels et al., 2011).

Twins and their siblings were asked to assess their pubertal development at home with an extended version of the self-report Tanner questionnaires (Marshall & Tanner, 1969, 1970). Girls were asked whether they had experienced their menarche and to rate their stage of breast and pubic hair development. Boys were asked about their genital and pubic hair development and testis size (4 categories). The different stages of puberty were illustrated by photographs.

The mothers of the twins and siblings were sent a questionnaire about the pre-, peri- and neonatal period of the twins and their sibling.

Interview

During the medical test protocol participants received a brief interview (see Appedix II). They were asked about their alcohol consumption and about their pubertal development using standardized questions from the DHBQ (Bartels et al., 2011). The girls were asked about their age of menarche and about the frequency and regularity of their periods. Furthermore, the interview used by the NTR Biobank project was carried out (Willemsen et al., 2010). The participants were asked about time of getting up that morning, the fasting status and physical exercise just prior to the visit. In addition we asked for the use and kind of medication or oral contraceptives, the number of days since the first day of last menstruation, in case of oral contraceptives the number of days since starting last pill pack, physical health, smoking behavior and illness (its kind and timing: less than 1 week ago, less than 1 month ago, or more than 1 month ago). The forms used as part of the medical protocol are provided in Appendix II, III and IV.

Psychological protocol

After the physical assessment in the morning the twins and their sibling attended after lunch the psychological protocol performed by dr. Rosa Hoekstra. She studied the development of cognition, behavioral problems and autistic traits. All participants of 16 years of age or above completed 11 subtests of the Wechsler Adult Intelligence Scale (WAIS-III), Dutch version (Wechsler, 1997). Verbal and nonverbal intelligence scores were calculated as the mean subtest score on the 6 verbal, respectively the 5 nonverbal subtests. The participants younger than 16 years completed the Dutch version of the full Wechsler Intelligence Scale for Children-Third edition (WISC-III; (Wechsler, 2002)). Verbal and nonverbal IQ scores were measured as the standardized score on 5 verbal subtests, and 5 nonverbal subtests. Both in the WAIS-III and the WISC-III, the scores were standardized for the appropriate age group, based on a population sample of same-aged subjects in the Netherlands. Standardization norms were the same across the sexes.

Additional verbal tasks included the California Verbal Learning Test (CLVT; (Mulder et al., 1996)) to measure verbal learning and memory, and a test of verbal letter fluency and category fluency.

For the study into autistic traits the twins and their siblings filled out the Autism-Spectrum Quotient (AQ; (Baron-Cohen et al., 2001)). Problem behavior was assessed using the Youth Self Report (Achenbach et al., 2001; Verhulst et al., 1997) which is part of the DHBQ (filled out by twins and siblings at home).

See for further detail on measures assessed as part of the psychological protocol Table I.1 and the thesis of Rosa Hoekstra (Hoekstra, 2007b).

Table I.1. Medical protocol and approximate starting time of the different tests.

Program testing day	starting time
Start medical protocol at VU University Medical Center	10.00 am
Attach electrodes & VU-AMS device	10.05
Blood pressure	10.30
Blood withdrawal	10.30
Food & drinks	10.40
Physical examination (simultaneously the other participants wait in the corridor and fill out the AQ)	10.45
Wait in the corridor, fill out the AQ (while the other participants are being examined)	11.10
Blood pressure	11.45
Photocopy of both hands	11.50
Lunch break	12.00
Start psychological protocol at VU psychology test laboratory	
Collection questionnaires, saliva measures and DNA samples	12.30
Blood pressure	12.40
Finger print scan	12.40
Wechsler IQ	13.00
Tea break	14.30
Stroop	14.45
Verbal Fluency	14.50
Box marking test	14.55
CLVT part 1	15.58
Reading the mind in the eyes	15.15
Blood pressure	15.25
Π inspection time task	15.28
CLVT part 2	15.35
n-back task	15.40
Corsi block tapping task	16.00
End test protocol	16.10
Participants leave VU University	± 16.20

AQ = Autism-Spectrum Quotient
CLVT = California Verbal Learning Test

Appendix II

Fill-out form medical protocol twins and siblings

ONDERZOEK DOOR ARTS
Onderzoek kind

VU medisch centrum

Naam Familie: .....
Naam kind: .....
Registratienummer NTR: ..... **TNO-nummer:**.....
Naam onderzoeker: ..... **Datum:** ☐☐-☐☐-200 ☐

Algemene informatie

Geboortedatum: ☐☐ ☐☐ 19 ☐☐ **Geslacht:** ☐ 0. Jongen ☐ 1. Meisje

Plaats in het gezin:
☐ 1. tweeling
☐ a. eerste kind
☐ b. tweede kind
☐ 2. sibling

Anamnese

Op hoeveel dagen, van de afgelopen 4 weken, heb je alcohol gedronken (wijn, bier, sterke drank)?
☐☐ dagen

Heb je een pacemaker? ☐ 1. nee ☐ 2. ja

Heb je orthopaedisch plaatmateriaal in je lichaam? ☐ 1. nee ☐ 2. ja

Mobiele telefoon uit?

Groeigegevens Groene Boekje

Datum (dag, maand jaar)	gewicht	lengte	hoofdomtrek
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm
_____-_____-_____	_____ gram	_____ cm	_____ cm

- Ziekenhuis geboren:
- Amenorrhoeduur:
- Apgar scores: 1' 5' 10'

Cyclusanamnese (meisjes)

Menarchedatum: ☐☐-☐☐- ☐☐☐☐

Orale anticonceptie:
☐ 1. nee
☐ 2. ja, preparaat:..... sinds:.....

Menses regulair:
☐ 1. ja, namelijk elke ☐☐ dagen
☐☐ weken

☐ 2. nee, interval variërend van ☐☐ dagen tot ☐☐ dagen
☐☐ dagen tot ☐☐ weken
☐☐ weken tot ☐☐ weken
☐☐ weken tot ☐☐ maanden
☐☐ maanden tot ☐☐ maanden

Duur menses: gemiddeld ☐☐ dagen

Dysmenorrhoe:
☐ 1. nee
☐ 2. ja
evt medicatie.....

1ste dag laatste menstruatie: ☐☐-☐☐-200 ☐

Zwangerschap?:
☐ 1. nee
☐ 2. ja

Puberteitsvragen Dutch Health and Behavior Questionnaire (DHBQ) Mei 2004

1. Wat zou je zeggen over je lichaamslengte?
   - ☐1 ik ben nog niet snel aan het groeien
   - ☐2 ik ben net snel aan het groeien
   - ☐3 ik ben al enige tijd snel aan het groeien
   - ☐4 de groeispurt is voorbij

2. Wat zou je zeggen over je schaambeharing?
   - ☐1 er zijn nog geen zwarte krulharen
   - ☐2 eerste zwarte haren zijn er
   - ☐3 de haren vormen een driehoeksvorm
   - ☐4 het schaamhaar neemt niet verder toe

3. Wat zou je zeggen over je okselbeharing?
   - ☐1 er zijn nog geen haren
   - ☐2 de eerste haren zijn er
   - ☐3 er is duidelijk okselhaar aanwezig
   - ☐4 het okselhaar neemt niet verder toe

4. Heb je last van overbeharing?
   - ☐1 nee
   - ☐2 ja

5. Gebruik je deodorant?
   - ☐1 nee
   - ☐2 ja, soms
   - ☐3 ja, elke dag

6. Heb je last van puistjes?
   - ☐1 nee
   - ☐2 ja

Alléén voor meisjes

7. Zijn je borsten aan het groeien?
   - ☐1 nee, er is nog geen groei
   - ☐2 ja, net begonnen met groeien, het voelt pijnlijk aan
   - ☐3 ja, maar het zijn nog kleine borsten
   - ☐4 nee, het groeien is al afgelopen

8. Ben je ooit ongesteld geweest? ☐1 nee ☐2 ja

9. Zo ja, op welke leeftijd was je voor het eerst ongesteld? …….jaar en ……..maanden

10. Zo ja, ben je regelmatig ongesteld?
    - ☐1 erg regelmatig, de dag is voorspelbaar
    - ☐2 redelijk regelmatig, meestal binnen drie dagen rond de verwachte dag
    - ☐3 onregelmatig, het is onvoorspelbaar

11. Hoe vaak ben je ongesteld
    - ☐1 vaker dan 1 keer per maand
    - ☐2 iedere 28 dagen (1 keer per maand)
    - ☐3 minder dan 1 keer per maand
    - ☐4 minder dan 1 keer in de twee maanden

12. Zo ja, heb je, sinds je ongesteld bent, ooit 3 menstruaties achter elkaar gemist?
    - ☐1 nee
    - ☐2 ja

Alléén voor Jongens

12. Is je stem aan het veranderen (lager worden en/of overslaan)?
    - ☐1 nee, nog niet begonnen met veranderen
    - ☐2 ja, net begonnen met veranderen
    - ☐3 ja, duidelijk aan het veranderen
    - ☐4 nee, de veranderingen zijn al afgelopen

13. Zijn je teelballen aan het groeien?
    - ☐1 nee
    - ☐2 ja

14. Moet je je al scheren?
    - ☐1 nee
    - ☐2 ja, soms
    - ☐3 ja, een aantal keer per week, namelijk…….keer

Tanner stadia:

♀		♂		
Mamma	**Pubisbeharing**	**Genitalia**	**Pubisbeharing**	**Testesvolume**
M1	P1	G1	P1	L: ..... ml
M2	P2	G2	P2	R: ..... ml
M3	P3	G3	P3	
M4	P4	G4	P4	
M5	P5	G5	P5	
	P6		P6	

Fotocopie beide handen op A4: ☐

Fotocopie treinkaartje: ☐

Antropometrie volgens Dauncy [1]

Lichaamslengte: ..... □□□,□ cm

Gewicht: ..... □□,□□ kg

Zithoogte: ..... □□□,□ cm

Hoogte zitbankje: ..... □□,□ cm

Hoofdomtrek: ..... □□,□ cm

Tailleomtrek: ..... □□,□ cm

Heupomtrek: ..... □□,□ cm

Spanwijdte: ..... □□□,□ cm

Handvoorkeur: rechts / links

Bloeddruk: □□□ - □□□ HA: □□□ na binnenkomst

(niet-dominante kant) □□□ - □□□ HA: □□□ na einde ochtenddeel

□□□ - □□□ HA: □□□ na binnenkomst

□□□ - □□□ HA: □□□ in pauze

Afstand ICG-electrodes

Borst □□,□ cm

Rug □□,□ cm

Huidplooien niet-dominante kant:

◊ **Biceps regio** □□,□ en □□,□ en □□,□

◊ **Triceps regio** □□,□ en □□,□ en □□,□

◊ **Subscapulaire regio** □□,□ en □□,□ en □□,□

◊ **Supra-iliacale regio** □□,□ en □□,□ en □□,□

[1] M.J. Dauncy, Arch. Dis. Child. 1977; 52: 233-227.

Appendix III

Fill-out form medical protocol parents

ONDERZOEK KINDERARTS
Onderzoek ouders

VU medisch centrum

Naam Familie: .....
Meisjesnaam moeder: .....
Registratienummer NTR: .....

Naam onderzoeker: ..... **Datum:** □□ □□ 200□

Vader

Geboortedatum: □□ □□ 19□□

Lichaamslengte: ..... □□□,□ cm

Gewicht: ..... □□,□□ kg

Handvoorkeur: rechts / links

Bloeddruk niet-dominante kant: □□□ - □□□ **HA:** □□□

□□□ - □□□ **HA:** □□□

Bloeddrukregulerende medicatie: ja/nee

Moeder

Geboortedatum: □□ □□ 19□□

Lichaamslengte: ..... □□□,□ cm

Gewicht: ..... □□,□□ kg

Handvoorkeur: rechts / links

Bloeddruk niet-dominante kant: □□□ - □□□ **HA:** □□□

□□□ - □□□ **HA:** □□□

Bloeddrukregulerende medicatie: ja/nee

Appendix IV

NTR Biobank interview and registration form

NTR-ID:

TNO nr: 20.

REGISTRATIEFORMULIER

VU – BIOBANKING (Project ROS: Hoekstra / Estourgie)

ALGEMENE GEGEVENS

Datum bloedafname		Tijd van opstaan	
Roepnaam		Normale tijd van opstaan	
Initialen		Ontbijt/ evt extreem avondeten	Nee/Ja, nl:
Geb.datum		Medicatie(vast en/of incidenteel in laatste week)	Nee/Ja:
Lengte		Datum 1e dag laatste en volgende menstruatie	
Gewicht (ondergoed)		Dag pil - strip / vrije week	
Middel/heupomvang		Pil soort	

Hoe was in de afgelopen week je **lichamelijke** gezondheid?	1. slecht 2. matig 3. redelijk 4. goed 5. uitstekend	Specificeer:
Laatste keer ziek?	1. verkouden (ZONDER koorts) 2. griep (MET koorts) 3. ontsteking, anders dan 1. 4. anders, namelijk	Wanneer: 1. < 1 week geleden 2. > 1 week geleden 3. > 1 maand geleden
Heb je misschien een chronische aandoening?	1. Ja 2. Nee	Specificeer:
Rook je?	1. Ja 2. Nee, wel in het verleden 3. Nooit	1a: hoe lang rook je al? 1b: hoeveel sigaretten/ shagjes per dag? 2a: hoelang is dat geleden? 2b: hoeveel jaren heb je gerookt? 2c: hoeveel rookte je per dag?
Gerookt binnen laatste uur voor bloedafname	1. ja 2. nee 3. nvt	
Lichamelijk ingespannen binnen laatste uur voor bloedafname	1. nee 2. matig 3. veel	Specificeer: fietsen / naar poli lopen auto / trein / bus / anders, nl:

Notities

NTR-ID:

TNO nr: 20.

Bloedafname

Tijd start bloedafname :

Vacutainer	Aantal	Afgenomen	Toelichting	Opslag
EDTA (7 ml)	2		N cupjes =	
Heparine (7 ml)	2		N cupjes =	
CTAD (4.5 ml)	1		N cupjes =	
Serum (7 ml)	1		N cupjes =	serum- spijt-
ACD (6 ml)	1		Datum leuko-isolatie:	
EDTA (2 ml)	2			
Urine (10 ml)	2		N buizen = ochtend: ja / nee	
Urine (endo)	1		ochtend: ja / nee	
Speeksel (testosteron)	2			

Bloedverwerking

Tijd start RNA-challenge proef : box:

Tijd aankomst TNO : n.v.t.

Tijd stoppen RNA-challenge proef : Tijd PAX-B -20°C: nacht:

		HEMOLYSE					
Vacutainer	Aantal	< 0.025	0.025 – 0.05	0.05 – 0.1	0.1 – 0.2	> 0.2	Bijzonderheden
EDTA (7 ml)	2						
Heparine (7 ml)	2						
Serum (7 ml)	1						
CTAD (4.5 ml)	1						
Urine	2						

Opmerkingen:

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List of abbreviations

A	additive genetic variance
AGA	appropriate for gestational age
b	regression coefficient
BMI	body mass index
C	common environmental variance
CI	confidence interval
CUG	catch-up growth
CV	coefficient of variation
df	degrees of freedom
Δdf	difference in degrees of freedom
DHEAS	dehydroepiandrosterone-sulfate
DHBQ	Dutch Health and Behavior Questionnaire
DNA	desoxyribo nucleic acid
DOS	dizygotic opposite sex
DOSFM	dizygotic opposite sex female born first
DOSMF	dizygotic opposite sex male born first
DZ	dizygotic
DZF	dizygotic female
DZM	dizygotic male
DZss	dizygotic same sex
E	unique environmental variance
FSH	follicle-stimulating hormone
G	genetic variance
GA	gestational age
GH	growth hormone
HcTH	height corrected for target height
IGF-I	insulin-like growth factor-I
IGFs	insulin-like growth factors
IQ	intelligence quotient
IUGR	intrauterine growth restriction
MZ	monozygotic
MZF	monozygotic female
MZM	monozygotic male
N	number of individuals
NHS	National Health Services
NS	not significant
NTR	Netherlands Twin Register
p	probability-value
SD	standard deviation
SDS	standard deviation score
SEM	structural equation modeling
SGA	small for gestational age
WAIS-III	Wechsler Adult Intelligence Scale
WISC-R	Wechsler Intelligence Scale for Children
$\chi 2$	chi-square statistic

Dankwoord

Mijn derde bevalling is nabij...

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dr. P. van Dommelen
prof.dr. J.B. van Goudoever
prof.dr. H.N. Lafeber
prof.dr. C.D.A. Stehouwer
prof.dr. J.M. Wit

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