Early Origins of Adult Health Profile

Cardiovascular and Metabolic Profile and Gonadal Function in Early Adulthood

Gerthe F. Kerkhof

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Cover: This illustration, created in 1979 by Peter Meijer, inspired me during my childhood. It visualizes child development as a step by step process. One needs guidance and support of other people to grow and develop, while certain challenges in life can only be undertaken on an individual basis.
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Cardiovascular and Metabolic Profile and Gonadal Function in Early Adulthood

Vroege determinanten van cardiovasculair en metabool profiel en gonadale functie in jong volwassenen

Proefschrift

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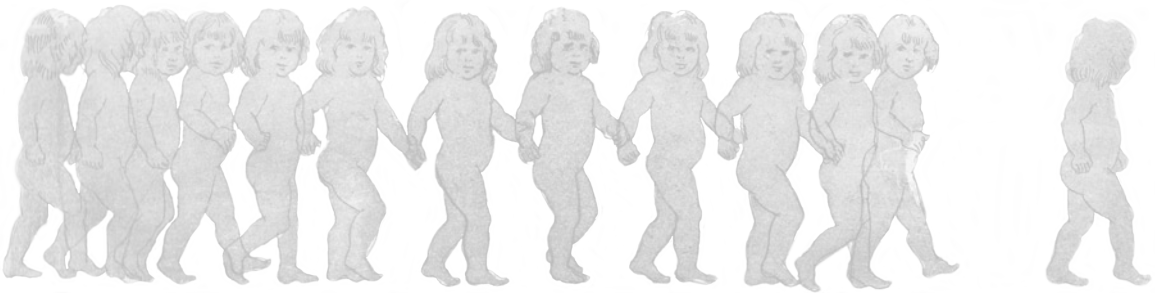
De ontdekking

Als je goed om
je heen kijkt
zie je dat alles
gekleurd is

K. Schippers

Contents

Chapter 1



General Introduction

Influence of low birth weight and childhood growth on later health

Several epidemiological studies showed an association between fetal growth restraint and/or subsequent catch-up growth, and the risk for cardiovascular events, type 2 diabetes, hypertension, adverse lipid profile, and disturbances in several other organ functions in later life (Figure 1).[1-25] The exact mechanism underlying these associations are yet unknown, but several hypotheses have been postulated over time.

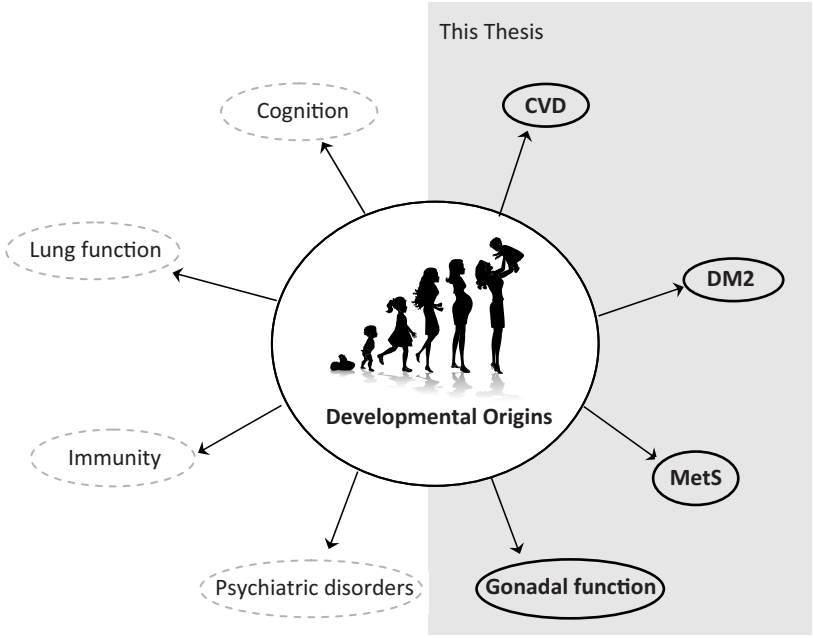


Figure 1. Disorders and functions that might be affected by early life factors such as intrauterine growth restraint and early life catch-up growth. This thesis describes early life determinants of cardiovascular disease (CVD), type 2 diabetes (DM2), metabolic syndrome (MetS), and gonadal function.

1. Early life effects on cardiovascular disease and type 2 diabetes

The occurrence of cardiovascular disease (CVD) is high, and CVDs are the leading causes of death and disability in the world.[26-27] This emphasizes the need of investigating mechanisms involved in the development of CVD in order to seek prevention targets. The common soil hypothesis states that CVD and type 2 diabetes have common genetic and environmental antecedents, rather than atherosclerosis being a complication of diabetes.[28] That would imply that prevention targets of CVD and type 2 diabetes are similar. Several studies have indicated that the development of CVD

might start in childhood or even in infancy.[29] This points to the need for studying early life factors influencing development of CVD and type 2 diabetes risk, including blood pressure, intima media thickness, pulse wave velocity, inflammatory markers, lipid metabolism, body composition, and insulin sensitivity.

Fetal origin hypothesis

In the early 1990s Barker et al. were the first to formulate a hypothesis based on the inverse association found between birth weight and several adult diseases. They suggested that events during pregnancy leading to fetal malnutrition could result in permanent endocrine and metabolic changes in the fetus, called re-programming (Figure 2).[2,30] At first, the fetus would benefit from the adaptations, as it would remain alive during fetal life, but on the long-term, this re-programming would result in diseases in later adulthood.

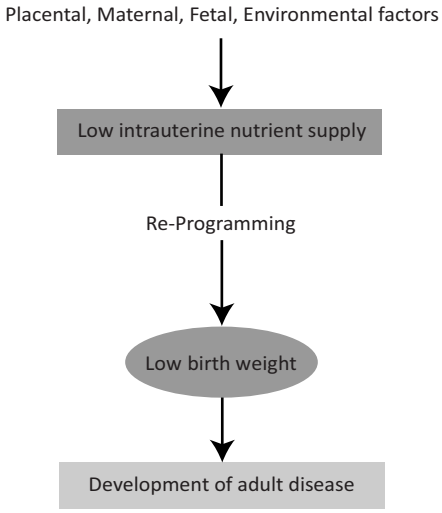


Figure 2. Representation of the fetal origin hypothesis. Adapted from Barker et al.[2, 4, 30]

Fetal insulin hypothesis

This hypothesis was generated in 1999 and states that the inverse association between birth weight and adult insulin resistance is principally genetically mediated (Figure 3).[31] Fetal genes involved in insulin resistance could result in low-insulin-mediated fetal growth and in insulin resistance leading to type 2 diabetes in later life.

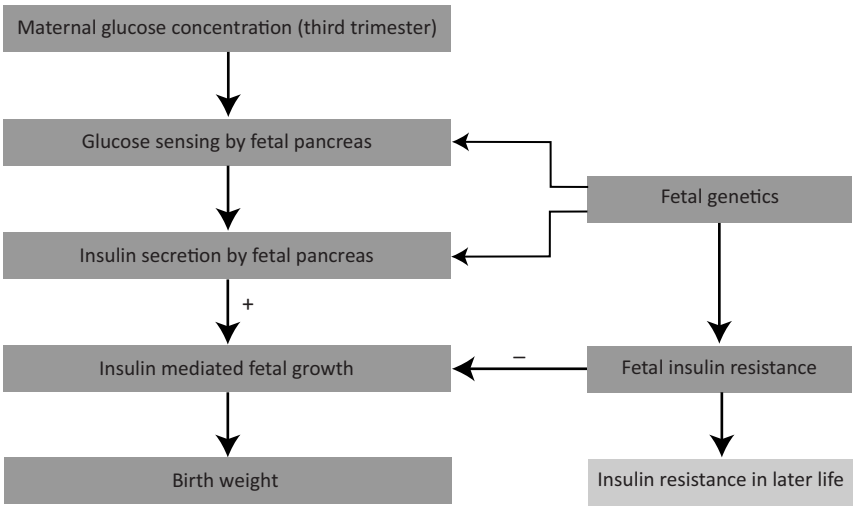


Figure 3. Simplified representation of the fetal insulin hypothesis. Adapted from Hattersley et al.[31]

Growth acceleration hypothesis

In 2004 Singhal and Lucas postulated the hypothesis that not low birth weight per se, but growth acceleration during childhood is responsible for the increased risk for adult diseases in later life.[32] Children are genetically determined to grow to their growth potential. Thus, children born after fetal growth restriction, being below their genetic growth potential at birth, will experience postnatal catch-up growth. According to the hypothesis, this postnatal catch-up growth, which might be stimulated by nutrient-enriched diets, will lead to the development of diseases in later life (Figure 4).

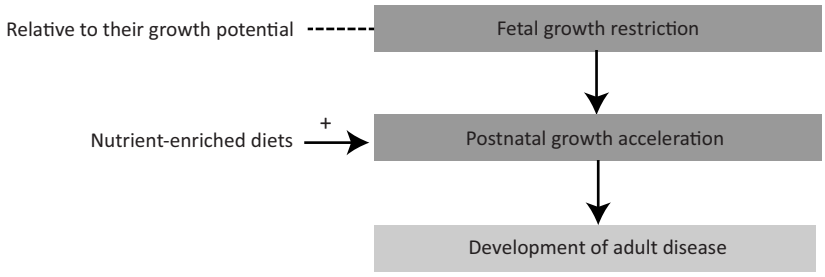


Figure 4. Representation of the growth acceleration hypothesis. Adapted from Singhal et al.[32]

Fat accumulation hypothesis

In the PROGRAM-study, started in 2002, our research group showed that fat accumulation during childhood was related to reduced insulin sensitivity,[33] an increase in serum levels of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDLc), apolipoprotein B[34] and acylation stimulation protein (ASP),[35] and systolic and diastolic blood pressure in early adulthood, independent of size at birth. Therefore, Leunissen et al. specified the hypothesis of Singhal and Lucas, by postulating that increased fat accumulation during childhood, independent of birth size, results in an increased risk for development of CVD and type 2 diabetes (Figure 5).[33,36]

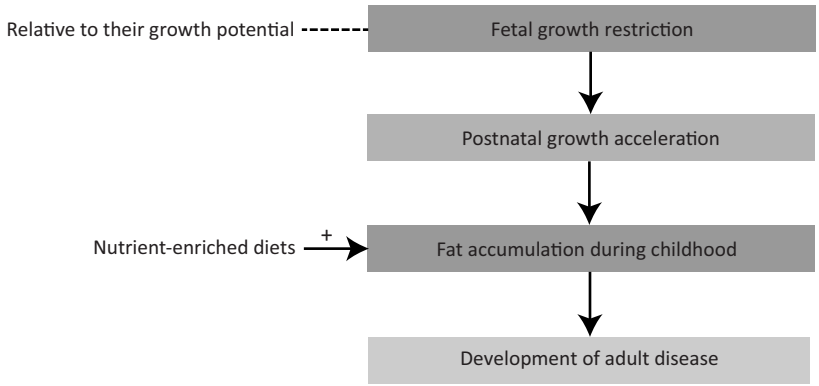


Figure 5. Fat accumulation model. Adapted from Leunissen et al.[33, 36]

Preterm birth and risk for cardiovascular disease

Small size at birth can be caused by poor fetal growth and/or preterm birth. Nowadays, 5-13% of all newborns in developed countries are being born preterm.[37] Because of advances in neonatal intensive care, survival of preterm infants has improved, with most children reaching adulthood. However, young adults born preterm might be at increased risk for developing CVD[22,38] and have increased cardiovascular mortality.[39] Preterm birth has been associated with increased carotid intima media tickness (a measure of preclinical atherosclerosis[40]),[41] increased blood pressure,[22,42-43] and increased arterial stiffness (quantified by Pulse Wave Velocity).[44] However, controversies still exist, and the underlying cause of this increased risk for CVD in adults born preterm remains unknown. It might well lay in different early growth trajectories in infants born preterm.[12,45-47] Like in term infants, alterations in growth during this highly dynamic developmental time window might have programming effects on later health outcomes.

2. Determinants of cardiovascular disease and type 2 diabetes

In order to determine risk for cardiovascular disease and type 2 diabetes in early adulthood, many measurements and biomarkers were investigated in the studies described in this thesis.

Blood pressure

Increased blood pressure is an important determinant of CVD.[48] Next to increased diastolic and systolic blood pressure, an increased pulse pressure (the difference between systolic blood pressure and diastolic blood pressure), and blood pressure variability in time have also been associated with CVD.[49-51]

Intima Media Thickness and Pulse Wave Velocity

Atherosclerosis is an important contributor to CVD. The presence of atherosclerotic changes in the carotid arteries can be determined by investigating the intima media thickness (IMT, Appendix A) in the vessel wall of the carotid arteries by non-invasive ultrasound measurements.[40] A greater thickness is associated with the development of atherosclerotic plaques and is positively correlated with cardiovascular events.[52-53] Because development of atherosclerosis already starts in childhood, determining carotid IMT in early adulthood might give more insight in the risk of cardiovascular events in later life.

Arterial stiffness is another important determinant of CVD, which can be quantified by assessing Pulse Wave Velocity (PWV, Appendix A).[54-55]

Inflammatory markers

The role of inflammation in preclinical as well as advanced stages of atherosclerosis has been widely acknowledged.[56] There are many biomarkers of inflammation known to date. Some inflammatory biomarkers, related to preclinical stages of atherosclerosis, are: high sensitivity C-reactive protein (CRP), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), soluble vascular adhesion molecule 1 (sVCAM-1), and soluble intracellular adhesion molecule 1 (sICAM-1). CRP is an acute phase protein, which is an important predictor of future atherosclerotic events, such as myocardial infarction, stroke, and peripheral vascular disease.[57] MCP-1 and IL-8 are pro-inflammatory chemoattractant proteins (chemokines) which cause recruitment of leukocytes to the arterial endothelium,[58-59] while VCAM-1 and ICAM-1 are pro-inflammatory adhesion molecules which cause adhesion of leukocytes to the arterial endothelium.[60]

Lipid metabolism

Raised serum levels of total cholesterol (TC), low-density lipoprotein (LDLc), and apolipoprotein B (ApoB) together with reduced levels of high-density lipoprotein (HDLc) and apolipoprotein A-I (ApoA1) increase the risk for CVD.[61-63] Acylation stimulating protein (ASP) is a hormone which stimulates uptake of glucose and free fatty acids (FFA) in adipocytes and inhibits triglyceride (TG)

lipolysis in adipocytes.[64-65] Increased FFA, TG, and ASP levels have also been associated with CVD risk.[66-68]

Body composition

Obesity is an important determinant of type 2 diabetes and CVD. The prevalence of obesity is rising in both children and adults.[69-71] Body composition can be measured by Dual-Energy X-ray Absorptiometry (DXA), which is explained in Appendix A. DXA gives insight in the total amount of fat mass (FM) and lean body mass (LBM). It is well known that a higher fat percentage in adults results in an increased risk for CVD and type 2 diabetes.[72]

Insulin sensitivity

Glucose homeostasis can be measured by a Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test (Appendix A). In normal condition, variations of insulin sensitivity are compensated proportionally by insulin secretion; reduced insulin sensitivity leads to increased insulin secretion by the beta cells.[73] If insulin secretion does not change appropriately, impaired glucose tolerance will develop, which might eventually lead to type 2 diabetes.[74]

Metabolic syndrome

The metabolic syndrome (MetS) is a combination of closely related cardiovascular risk factors (visceral obesity, dyslipidemia, hyperglycemia, and hypertension). Several definitions of the MetS exist. One of these comprises the revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III)[75], which defines MetS as having 3 or more of the following risk factors: abdominal obesity, high TG, low HDLc, high systolic or diastolic blood pressure, and high fasting glucose.

3. Gonadal function

Early life factors may affect many organ functions (Figure 1). Therefore, it is also of interest to determine the effect of birth size and growth trajectories on gonadal function.

Male gonadal function

The testis fulfils two essential functions: the synthesis and secretion of sex hormones and the production and maturation of male gametes. Functionally and anatomically, the testis can be divided into an interstitial compartment and a tubular compartment (Figure 6). The interstitial compartment, consisting of Leydig cells which produce testosterone, is situated between the seminiferous tubules. Leydig cell function is influenced by Luteinizing Hormone (LH) levels. The tubular compartment consists of the seminiferous tubules containing two types of cells: Sertoli cells and germ cells in different stages of differentiation. Each Sertoli cell can harbor a limited number of germ cells.

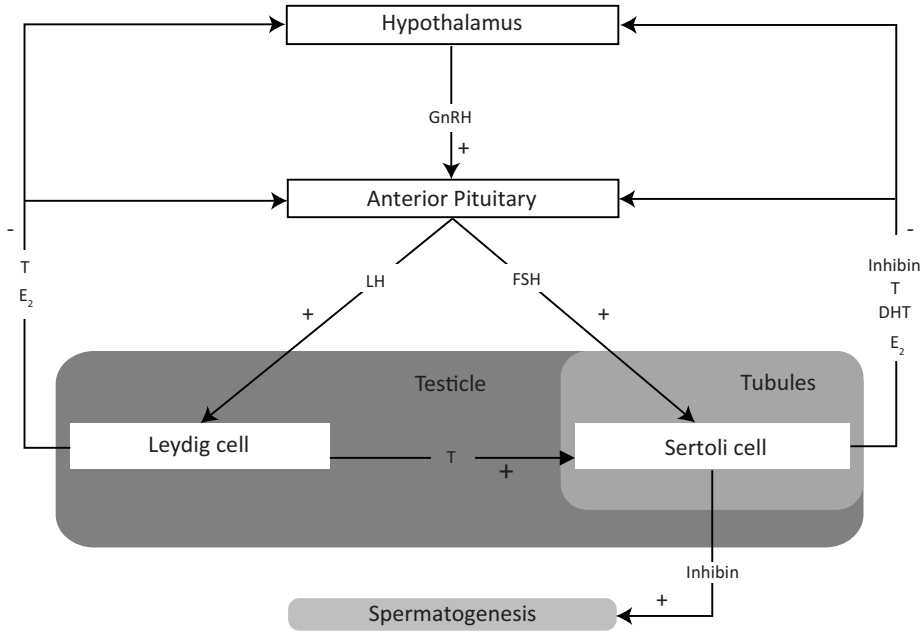


Figure 6. Hypothalamic-pituitary-gonadal axis in males.
GnRH= Gonadotropin-Releasing Hormone, LH= Luteinizing Hormone, FSH= Follicle Stimulating Hormone, T= Testosterone, E2= Estradiol, DHT= Dihydrotestosterone

The number of Sertoli cells is quantitatively determinative for the sperm production. In adulthood, Sertoli cells produce Inhibin B. Therefore, serum level of Inhibin B is an important marker of Sertoli cell function in adulthood.[76] Anti-Müllerian hormone (AMH) is also produced by the Sertoli cells, but is stimulated by intratesticular testosterone rather than FSH.[77]

The "testicular dysgenesis syndrome" is based on the hypothesis that developmental disorders in fetal life may lead to abnormal spermatogenesis, cryptorchidism, penile malformations (e.g. hypospadias) and testicular cancer.[78] In several studies increased risk of hypospadias, cryptorchidism, and testicular cancer were found to be associated with small birth size for gestational age.[79] Furthermore, a longitudinal study in Norway showed a reduced reproduction rate in men born preterm; the reproduction improved with increasing gestational age.[80] It is unknown whether this effect was related to marital status, lower socioeconomic status, or reduced gonadal function. Thus, it remains a question whether small birth size for gestational age and preterm birth associate with less gonadal function in men.

Female gonadal function

The ovary fulfils two essential functions: the synthesis and secretion of sex hormones and the development and release of the mature oocyte. Human follicle development starts in the twelfth week of intrauterine life and in the fifth month the maximum size of the ovarian follicle pool is reached. During fetal life and childhood, follicles develop through primordial and primary stage, to pre-antral and small antral follicles.[81-82] The loss of primordial follicles, which begins already prior to birth, continues throughout childhood so that at the time of menarche approximately 500 000 follicles remain.

From the onset of puberty, the small antral follicles will develop to antral follicles. At the antral stage, most follicles undergo atresia whereas a few develop into a Graafian follicle and will reach the preovulatory stage.[83] Granulosa cells of pre-antral and small antral follicles produce the dimeric glycoprotein anti-Müllerian hormone (AMH), which is involved in the regulation of early folliculogenesis.[83] Serum AMH level is a good marker of the ovarian pool size, as this hormone reflects the number of pre-antral and small antral follicles (Figure 7).[84-85]

Controversies exist regarding the association between preterm and small for gestational age (SGA) birth and abnormal ovarian function in adulthood. Some studies showed smaller ovaries and uterus in infants and adolescent women born SGA.[86-87] Others found no differences in ultrasonic measurements of the uterus and ovaries in girls born SGA;[88] neither did intrauterine growth retardation affect the ovarian volume of fetuses nor the volume percentage of follicles in the ovaries.[82]

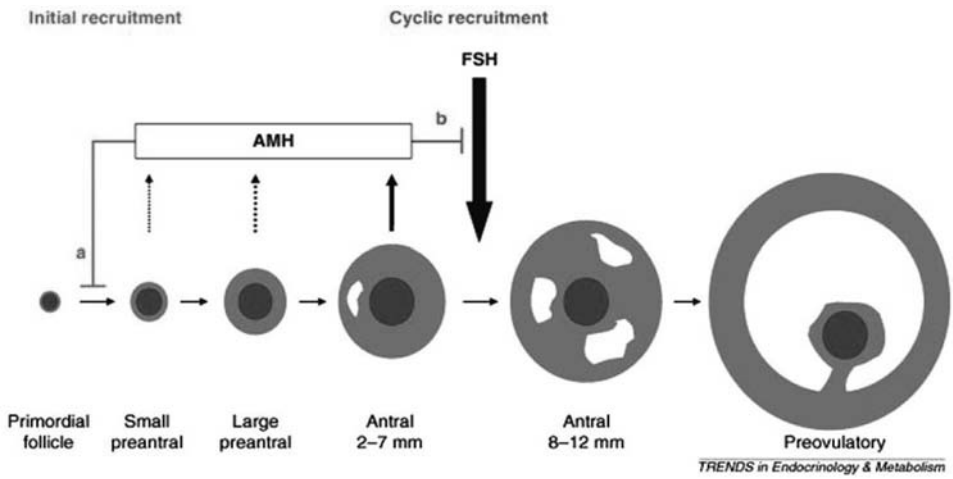


Figure 7. The role of AMH in normal ovarian follicle development. Broekmans FJ et al. Anti-Müllerian hormone and ovarian dysfunction. *Trends Endocrinol Metab.* Nov 2008;19(9):340-347.[89]

4. Hypotheses

We hypothesized that accelerated postnatal gain in weight relative to length, rather than preterm birth and small size at birth, leads to risk factors for preclinical atherosclerosis, type 2 diabetes and MetS, which is in line with the growth acceleration hypothesis. We, furthermore, hypothesized that preterm and small size at birth do not affect gonadal function in early adulthood.

5. The PROGRAM and PREMS study cohorts

To investigate these hypotheses the PROgramming factors for Growth And Metabolism (PROGRAM) and Prematurity and Small for Gestational Age (PREMS) study cohorts were initiated. The inclusion and exclusion criteria are described in Appendix B. The PROGRAM study consists of 323 healthy young adults born term and the PREMS study of 169 healthy young adults born preterm (gestational age <36 weeks). In these participants, many determinants for CVD, type 2 diabetes and gonadal function were determined, including blood pressure, intima media thickness, pulse wave velocity, inflammatory markers, lipid metabolism, body composition, and insulin sensitivity.

6. Study design

To investigate the influence of different growth patterns during childhood on determinants of adult disease and gonadal function, we oversampled subjects with extreme variants of normal growth, such as subjects born small for gestational age (SGA) (with and without catch-up growth) and subjects with unknown growth retardation during childhood (idiopathic short stature (ISS)). This design created greater contrast in the study population, which contributed to more statistical power. The definitions of SGA and ISS are explained in Appendix C.

For subgroup analyses, the total study population was divided into four clinically relevant subgroups based on birth length and adult height. The criteria for being included into one of the subgroups are described in Appendix D. Two subgroups consisted of small for gestational age born adults, one without catch-up growth (SGA-S) and one with catch-up growth (SGA-CU). The last two subgroups consisted of young adults born appropriate for gestational age: One with short adult stature without known reason (ISS) and one with normal adult height (controls).

7. Aims of the study

This thesis describes results of eight studies performed in young adults, aged 18-24 years, who participated in the PROGRAM or PREMS study. These studies were started to investigate if size at birth, preterm birth, and different growth patterns during childhood influence determinants of adult disease and gonadal function in early adulthood. We also investigated mechanisms involved in the development of atherosclerosis.

Birth size, preterm birth and CVD-risk in young adults

We investigated if small size at birth and preterm birth were associated with blood pressure, pulse pressure, blood pressure variability, carotid intima media thickness, pulse wave velocity, inflammatory markers, and lipid levels in young adults. We also investigated whether other factors influenced these determinants in early adulthood. In addition, subgroup analyses were performed to evaluate whether determinants differed between the clinically relevant subgroups.

Growth in early life and risk for CVD and type 2 diabetes

First year growth data were collected to investigate if gain in weight relative to length during a specific time period in the first year had an influence on determinants of cardiovascular disease and type 2 diabetes in young adults born preterm. Furthermore, we investigated whether gain in weight relative to length was associated with MetS, and combinations of specific type 2 diabetes determinants determined by Principal Component Analysis (Appendix E), in young adults born term.

Mechanisms involved in the development of atherosclerosis

We explored pathways resulting from increased fat mass, through several determinants of atherosclerosis, including lipid levels and blood pressure, using Structural Equation Modeling (Appendix E). We aimed particularly at fat mass, because fat mass accumulation during childhood is an important risk factor for CVD in adulthood.

Birth size, preterm birth and gonadal function in young adults

We aimed to investigate the influence of birth size and preterm birth on several markers of gonadal function in males and females. We were also interested in the effect of other (environmental) factors, such as socioeconomic status, maternal smoking during gestation and fat mass in early adulthood.

8. Outline of the thesis

Chapter 1 gives an introduction in the topics described in this thesis.

Chapter 2 describes the effect of preterm birth on several determinants of CVD in early adulthood, including effects of birth size and growth patterns.

Chapter 3 reports the association of gain in weight relative to length during different periods, and weight trajectories in early life after preterm birth with determinants of CVD and type 2 diabetes in early adulthood.

Chapter 4 describes mechanisms underlying metabolic syndrome in later life, including birth weight, gain in weight for length during early life and adult IGF-I SDS.

Chapter 5 reports associations between low birth weight, preterm birth, adult body size and a broad range of biomarkers related to early stage atherosclerosis in early adulthood. Principal Component Analysis (PCA) was applied to identify combinations of biomarkers associated with early stage atherosclerosis.

Chapter 6 presents associations of growth trajectories in early childhood with combinations of type 2 diabetes determinants, also using PCA.

Chapter 7 reports a novel approach to study pathways involved in the development of atherosclerosis in early adulthood. A model is presented of complex direct and indirect effects of fat mass leading to atherosclerosis, using Structural Equation Modeling.

Chapter 8 and Chapter 9 present the studies on the influence of preterm birth and small birth size for gestational age on gonadal function of respectively male and female participants of the PROGRAM and PREMS study. In addition, subgroup analyses were performed to investigate differences in gonadal function with regard to different growth patterns.

Chapter 10 discusses the results of the studies in relation to the current literature and presents conclusions and clinical implications of the study results.

Chapter 11 summarizes the findings of the study in English and Dutch.

Appendix A

Dual-Energy X-ray Absorptiometry

Dual-Energy X-ray Absorptiometry (DXA) is a method used to measure bone mineral density and body composition. The participant being assessed lies still for approximately 15 minutes while a scanner slides over the participant. DXA uses X-ray to assess these measures, but the radiation dose is approximately 1/10 of a chest X-ray. In the present thesis, DXA measurements were used to determine total body fat and lean body mass.[90]

Carotid Intima media thickness

Intima media thickness (IMT) is the thickness of the two inner layers of an arterial wall. The thickness of the intima media of the carotid artery is related to atherosclerosis in later life.[40,91] Carotid IMT was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using a 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Tech. Laboratories, Bethel Washington, USA). On the R wave of the electrocardiogram, three longitudinal images of the near and far wall of the common carotid artery were frozen and stored on videotape. These frozen images were digitalized and displayed on the screen of a computer using a frame grabber (VP 1400-KIT-512-E-AT, Imaging Technology). The common carotid IMT was determined as the mean of the mean near and far wall measurements of both the left and right side common carotid artery.[91]

Frequently Sampled Intravenous Glucose Tolerance test

Several values of glucose homeostasis can be measured measured by Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT): Insulin sensitivity index, which is the ability of insulin to increase net glucose disposal; acute insulin response to glucose (AIR), which is the integrated insulin release during the first 10 minutes after the glucose infusion; and the disposition index (DI), the product of insulin sensitivity and acute insulin response indicating the degree of glucose homeostasis. These indicators of glucose regulation were determined by the Bergman's minimal model (MINMOD 6.01 copyright RN Bergman) calculating paired glucose and insulin data obtained by frequent measurements during an FSIGT[92-94] with Tolbutamide.[95]

Appendix B

Inclusion criteria of the PROGRAM and PREMS study

- Chronological age at inclusion: 18.00-23.99 years
- Neonatal period without signs of severe asphyxia (defined as an Apgar score below three after five minutes), no serious diseases such as long-term artificial ventilation and oxygen supply, broncho-pulmonary dysplasia or other chronic lung disease
- Well documented growth data
- Caucasian
- Born singleton
- Signed informed consent
- PROGRAM study: gestational age of 36 weeks or more
- PREMS study: gestational age of less than 36 weeks

Exclusion criteria of the PROGRAM and PREMS study

- Chromosomal disorders, known syndromes and serious dysmorphic symptoms suggestive for a yet unknown syndrome, except Silver-Russell Syndrome
- Any disease, endocrine or metabolic disorder that could interfere with growth during childhood (such as diabetes, growth hormone deficiency, malignancies, severe chronic disease)
- Treatment that could have interfered with growth (such as radiotherapy or growth hormone treatment)
- Serious suspicion of psychosocial dwarfism (emotional deprivation) during childhood

Appendix C

Definition of Small for Gestational Age (SGA)

In 2001, the International SGA Advisory Board Panel formulated a consensus statement on the definition of SGA, by defining SGA as a birth length and/or birth weight below -2 standard deviation score (SDS), adjusted for gestational age and gender.[96] Data from an appropriate reference population are necessary to take differences in ethnicity into account.

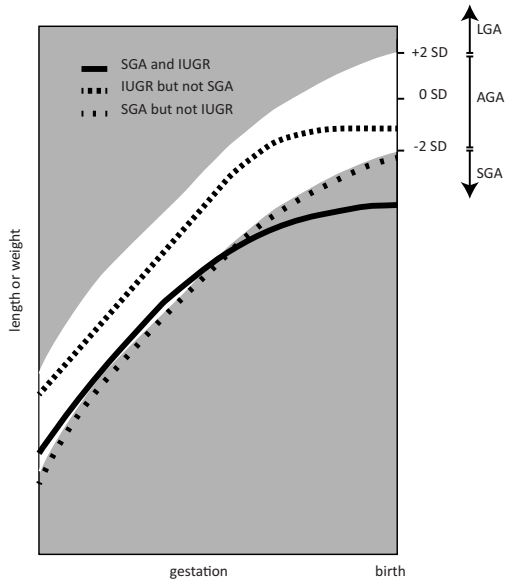


Figure 8. Fetal growth chart showing different growth patterns in IUGR and SGA newborns.

SGA is a term used for size at birth and does not refer to intrauterine growth. Intrauterine growth is used to describe growth velocity in fetal life, which is determined by at least two ultrasound measurements. A child born SGA might have been small from the beginning of fetal life, or could have experienced Intrauterine Growth Retardation (IUGR) later in gestation, resulting in a small size at birth. However, children with IUGR late in gestation can be born with a normal birth size. Figure 8 shows these different fetal growth patterns.

Prevalence and etiology of SGA

When SGA is defined as birth length and/or birth weight below -2 SDS, 2.3% of all live-born children are born SGA. For the Netherlands this means that in 2005, of all 187 910 live-born children, 4322 were born SGA (Central Bureau of Statistics, Voorburg, The Netherlands).

Several factors influence intrauterine growth and may therefore cause SGA birth, including fetal, maternal, placental and demographic factors. Although many factors are known, in 40% of the cases, no cause can be found. Identification of the cause of SGA is important as underlying

mechanisms may influence the prognosis and treatment. Table 1 lists factors associated with IUGR .

Approximately 85% of the SGA children show catch-up growth in the first two years of life to a height above -1.88 SDS.[97-99] Catch-up is most pronounced in the first six months of life, but this might be prolonged in prematurely born SGA children. Although subjects born SGA with catch-up growth (SGA-CU) attain a normal adult stature, they remain significantly shorter than subjects born appropriate for gestational age (AGA).

Idiopathic short stature (ISS)

ISS is defined as a condition in which height is <-2 SDS without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities. ISS subjects have a normal size at birth and do not have growth hormone deficiency. The group of ISS subjects is heterogeneous, with an unknown cause of short stature. Familial short stature is a common feature in this group.

Table 1. Factors associated with intrauterine growth retardation

Fetal factors	
Multiple births	
Congenital malformations	
Chromosomal anomalies	Turner syndrome Down syndrome
Inborn errors of metabolism	
Intrauterine infections	TORCHES (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex, Syphilis)
Maternal factors	
Medical conditions	Hypertension Pre-eclampsia Severe chronic disease of infections Systemic lupus erythematosus Antiphospholipid syndrome Anemia Malignancies Abnormalities of the uterus
Social conditions	Maternal nutrition Low prepregnancy BMI Low maternal weight gain Delivery at <16 or >35 yrs Low socioeconomic status Use of drugs
Placental factors	
Reduced blood flow	
Reduced area for exchange of nutrients and oxygen	Infarcts Hematomas Partial abruption
Environmental factors	
High altitude	
Toxic substances	

Adapted from Bryan and Hindmarch [100]

Appendix D

Additional criteria for inclusion into one of the four subgroups

Normal birth length and adult height were set at an SDS >-1 (± 0.1 SDS)

- Born small for gestational age (birth length <-2 SDS) with a short adult height (<-2 SDS) (SGA-short, SGA-S)
- Born small for gestational age (birth length <-2 SDS) with catch-up growth resulting in a normal adult height (>-1 SDS) (SGA catch-up (SGA-CU))
- Born appropriate for gestational age (birth length >-1 SDS) with growth retardation resulting in a short adult stature (<-2 SDS) (Idiopathic short stature (ISS))
- Born appropriate for gestational age (birth length >-1 SDS) with a normal adult height (>-1 SDS) (controls)

Appendix E

Principal Component Analysis

Principal Component Analysis (PCA) is a multivariate correlation technique that is used to reduce a large number of intercorrelated variables to a smaller set of independent principal components.[101-102] Subsequently, component scores can be calculated for each principal component identified, to use in multiple regression analysis and subgroup comparisons.

Structural Equation Modeling

Structural Equation Modeling (SEM) is a powerful statistical tool for non-hypothesis-driven path analyses, using maximum likelihood estimations.[103-104] SEM has been used in psychological, social, educational, and management fields and is applicable in clinical research, specifically to visualize pathways and calculate the magnitudes of direct and indirect effects on human diseases.[103]

REFERENCES

1. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. *BMJ (Clinical research ed.* 1990;301:259-262.
2. Barker DJ. The fetal and infant origins of adult disease. *BMJ (Clinical research ed.* 1990;301:1111.
3. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia.* 1993;36:62-67.
4. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993;341:938-941.
5. Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH. Growth in utero and serum cholesterol concentrations in adult life. *BMJ (Clinical research ed.* 1993;307:1524-1527.
6. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia.* 1994;37:150-154.
7. Martyn CN, Gale CR, Jespersen S, Sherriff SB. Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. *Lancet.* 1998;352:173-178.
8. de Bruin JP, Dorland M, Bruinse HW, Spliet W, Nikkels PG, Te Velde ER. Fetal growth retardation as a cause of impaired ovarian development. *Early human development.* 1998;51:39-46.
9. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. *Hypertension.* 2000;36:790-794.
10. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ (Clinical research ed.* 2000;320:967-971.
11. Law CM, Shiell AW, Newsome CA, Syddall HE, Shinebourne EA, Fayers PM, Martyn CN, de Swiet M. Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. *Circulation.* 2002;105:1088-1092.
12. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet.* 2003;361:1089-1097.
13. Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health? *Circulation.* 2004;109:1108-1113.
14. Ong KK, Dunger DB. Birth weight, infant growth and insulin resistance. *Eur J Endocrinol.* 2004;151 Suppl 3:U131-139.
15. Gale CR, Martyn CN. Birth weight and later risk of depression in a national birth cohort. *Br J Psychiatry.* 2004;184:28-33.
16. Sayer AA, Cooper C. Fetal programming of body composition and musculoskeletal development. *Early human development.* 2005;81:735-744.
17. Ong KK, Loos RJ. Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. *Acta Paediatr.* 2006;95:904-908.
18. Levy-Marchal C, Czernichow P. Small for gestational age and the metabolic syndrome: which mechanism is suggested by epidemiological and clinical studies? *Horm Res.* 2006;65 Suppl 3:123-130.
19. Mericq V. Low birth weight and endocrine dysfunction in postnatal life. *Pediatr Endocrinol Rev.* 2006;4:3-14.
20. Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A. Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation.* 2007;115:213-220.
21. Tideman E, Marsal K, Ley D. Cognitive function in young adults following intrauterine growth restriction with abnormal fetal aortic blood flow. *Ultrasound Obstet Gynecol.* 2007;29:614-618.

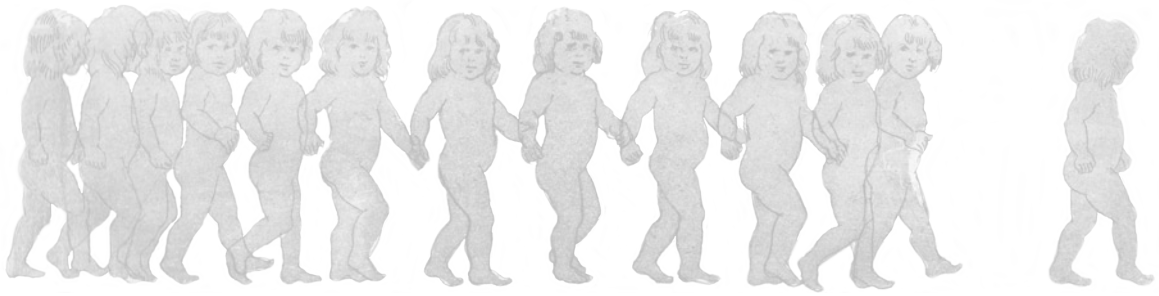
22. Evensen KA, Steinshamn S, Tjonna AE, Stolen T, Hoydal MA, Wisloff U, Brubakk AM, Vik T. Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early human development.* 2009;85:239-245.
23. Wells JC. Historical cohort studies and the early origins of disease hypothesis: making sense of the evidence. *Proc Nutr Soc.* 2009;68:179-188.
24. Vanbillemont G, Lapauw B, Bogaert V, De Naeyer H, De Bacquer D, Ruige J, Kaufman JM, Taes YE. Birth weight in relation to sex steroid status and body composition in young healthy male siblings. *J Clin Endocrinol Metab.* 2010;95:1587-1594.
25. Singhal A. Does weight gain in infancy influence the later risk of obesity? *J Pediatr Gastroenterol Nutr.* 2010;51 Suppl 3:S119-120.
26. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Circulation.* 2001;104:2855-2864.
27. *Global Atlas on cardiovascular disease prevention and control*: World Health Organization; 2011.
28. Stern MP. Diabetes and cardiovascular disease. The "common soil" hypothesis. *Diabetes.* 1995;44:369-374.
29. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. *J Hypertens.* 2008;26:1049-1057.
30. Barker DJ. Fetal origins of coronary heart disease. *BMJ (Clinical research ed.* 1995;311:171-174.
31. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet.* 1999;353:1789-1792.
32. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet.* 2004;363:1642-1645.
33. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab.* 2008;93:445-451.
34. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC. Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. *J Clin Endocrinol Metab.* 2008;93:4307-4314.
35. Leunissen RW, Gao Y, Cianflone K, Stijnen T, Hokken-Koelega AC. Growth patterns during childhood and the relationship with acylation-stimulating protein. *Clin Endocrinol (Oxf).* 2010;72:775-780.
36. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301:2234-2242.
37. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* 2008;371:75-84.
38. Thomas EL, Parkinson JR, Hyde MJ, Yap IK, Holmes E, Dore CJ, Bell JD, Modi N. Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. *Pediatr Res.* 2011;70:507-512.
39. Crump C, Sundquist K, Sundquist J, Winkleby MA. Gestational age at birth and mortality in young adulthood. *JAMA.* 2011;306:1233-1240.
40. Bots ML, Grobbee DE. Intima media thickness as a surrogate marker for generalised atherosclerosis. *Cardiovasc Drugs Ther.* 2002;16:341-351.
41. Skilton MR, Viikari JS, Juonala M, Laitinen T, Lehtimaki T, Taittonen L, Kahonen M, Celermajer DS, Raitakari OT. Fetal growth and preterm birth influence cardiovascular risk factors and arterial health in young adults: the Cardiovascular Risk in Young Finns Study. *Arterioscler Thromb Vasc Biol.* 2011;31:2975-2981.
42. Keijzer-Veen MG, Dulger A, Dekker FW, Nauta J, van der Heijden BJ. Very preterm birth is a risk factor for increased systolic blood pressure at a young adult age. *Pediatr Nephrol.* 2010;25:509-516.
43. Irving RJ, Belton NR, Elton RA, Walker BR. Adult cardiovascular risk factors in premature babies. *Lancet.* 2000;355:2135-2136.

44. Oren A, Vos LE, Bos WJ, Safar ME, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML. Gestational age and birth weight in relation to aortic stiffness in healthy young adults: two separate mechanisms? *Am J Hypertens.* 2003;16:76-79.
45. Euser AM, de Wit CC, Finken MJ, Rijken M, Wit JM. Growth of preterm born children. *Horm Res.* 2008;70:319-328.
46. Kytnarova J, Zlatohlavkova B, Kubena A, Markova D, Dokoupilova M, Plavka R, Zeman J. Post-natal growth of 157 children born as extremely premature neonates. *J Paediatr Child Health.* 2011;47:111-116.
47. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics.* 2003;111:986-990.
48. Whelton PK. Epidemiology of hypertension. *Lancet.* 1994;344:101-106.
49. Raitakari OT, Juonala M, Taittonen L, Jula A, Laitinen T, Kahonen M, Viikari JS. Pulse pressure in youth and carotid intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *Stroke.* 2009;40:1519-1521.
50. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet.* 2010;375:938-948.
51. Kikuya M, Ohkubo T, Metoki H, Asayama K, Hara A, Obara T, Inoue R, Hoshi H, Hashimoto J, Totsune K, Satoh H, Imai Y. Day-by-day variability of blood pressure and heart rate at home as a novel predictor of prognosis: the Ohasama study. *Hypertension.* 2008;52:1045-1050.
52. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med.* 1999;340:14-22.
53. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. *Stroke.* 1995;26:386-391.
54. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation.* 2006;113:657-663.
55. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation.* 2006;113:664-670.
56. Hansson GK. Atherosclerosis--an immune disease: The Anitschkov Lecture 2007. *Atherosclerosis.* 2009;202:2-10.
57. de Ferranti SD, Rifai N. C-reactive protein: a nontraditional serum marker of cardiovascular risk. *Cardiovasc Pathol.* 2007;16:14-21.
58. Braunersreuther V, Mach F, Steffens S. The specific role of chemokines in atherosclerosis. *Thromb Haemost.* 2007;97:714-721.
59. Libby P. Inflammation in atherosclerosis. *Nature.* 2002;420:868-874.
60. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007;27:2292-2301.
61. Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang KY, Levine DM. Serum cholesterol in young men and subsequent cardiovascular disease. *N Engl J Med.* 1993;328:313-318.
62. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet.* 2001;358:2026-2033.
63. Ballantyne CM, Hoogeveen RC. Role of lipid and lipoprotein profiles in risk assessment and therapy. *Am Heart J.* 2003;146:227-233.
64. Germinario R, Sniderman AD, Manuel S, Lefebvre SP, Baldo A, Cianflone K. Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. *Metabolism.* 1993;42:574-580.

65. Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Walsh M, Rodriguez MA. Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. *Lipids.* 1991;26:495-499.
66. Cianflone K, Xia Z, Chen LY. Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta.* 2003;1609:127-143.
67. Cianflone K, Zakarian R, Couillard C, Delplanque B, Despres JP, Sniderman A. Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance. *J Lipid Res.* 2004;45:124-131.
68. Rader DJ. Effect of insulin resistance, dyslipidemia, and intra-abdominal adiposity on the development of cardiovascular disease and diabetes mellitus. *Am J Med.* 2007;120:S12-18.
69. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *Jama.* 2004;291:2847-2850.
70. Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. *J Clin Endocrinol Metab.* 2008;93:S9-30.
71. Ogden CL, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003-2006. *Jama.* 2008;299:2401-2405.
72. Allende-Vigo MZ. Pathophysiologic mechanisms linking adipose tissue and cardiometabolic risk. *Endocr Pract.* 2010;16:692-698.
73. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia.* 2003;46:3-19.
74. Cnop M, Vidal J, Hull RL, Utzschneider KM, Carr DB, Schraw T, Scherer PE, Boyko EJ, Fujimoto WY, Kahn SE. Progressive loss of beta-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. *Diabetes Care.* 2007;30:677-682.
75. Grundy SM, Cleeman JI, Merz CN, Brewer HB, Jr., Clark LT, Hunninghake DB, Pasternak RC, Smith SC, Jr., Stone NJ, National Heart L, Blood I, American College of Cardiology F, American Heart A. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation.* 2004;110:227-239.
76. Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, Bremner WJ. Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. *J Clin Endocrinol Metab.* 1996;81:3341-3345.
77. Rey R. Endocrine, paracrine and cellular regulation of postnatal anti-mullerian hormone secretion by sertoli cells. *Trends Endocrinol Metab.* 1998;9:271-276.
78. Joensen UN, Jorgensen N, Rajpert-De Meyts E, Skakkebaek NE. Testicular dysgenesis syndrome and Leydig cell function. *Basic & clinical pharmacology & toxicology.* 2008;102:155-161.
79. Main KM, Jensen RB, Asklund C, Hoi-Hansen CE, Skakkebaek NE. Low birth weight and male reproductive function. *Hormone research.* 2006;65 Suppl 3:116-122.
80. Swamy GK, Ostbye T, Skjaerven R. Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. *JAMA.* 2008;299:1429-1436.
81. Macklon NS, Fauser BC. Aspects of ovarian follicle development throughout life. *Hormone research.* 1999;52:161-170.
82. de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW, te Velde ER. Morphometry of human ovaries in normal and growth-restricted fetuses. *Early Hum Dev.* 2001;60:179-192.
83. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev.* 2000;21:200-214.
84. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril.* 2002;77:357-362.
85. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod.* 2003;18:323-327.
86. Ibanez L, Potau N, Enriquez G, Marcos MV, de Zegher F. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. *Hum Reprod.* 2003;18:1565-1569.
87. Ibanez L, Potau N, Enriquez G, de Zegher F. Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatr Res.* 2000;47:575-577.

88. Hernandez MI, Martinez A, Capurro T, Pena V, Trejo L, Avila A, Salazar T, Asenjo S, Iniguez G, Mericq V. Comparison of clinical, ultrasonographic, and biochemical differences at the beginning of puberty in healthy girls born either small for gestational age or appropriate for gestational age: preliminary results. *J Clin Endocrinol Metab.* 2006;91:3377-3381.
89. Broekmans FJ, Visser JA, Laven JS, Broer SL, Themmen AP, Fauser BC. Anti-Mullerian hormone and ovarian dysfunction. *Trends Endocrinol Metab.* 2008;19:340-347.
90. Genton L, Hans D, Kyle UG, Pichard C. Dual-energy X-ray absorptiometry and body composition: differences between devices and comparison with reference methods. *Nutrition.* 2002;18:66-70.
91. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation.* 1997;96:1432-1437.
92. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest.* 1981;68:1456-1467.
93. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed.* 1986;23:113-122.
94. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther.* 2003;5:1003-1015.
95. Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes.* 1989;38:1512-1527.
96. Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics.* 2003;111:1253-1261.
97. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? *Pediatr Res.* 1995;38:267-271.
98. Albertsson-Wikland K, Karlberg J. Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl.* 1994;399:64-70; discussion 71.
99. Karlberg J, Albertsson-Wikland K. Growth in full-term small-for-gestational-age infants: from birth to final height. *Pediatr Res.* 1995;38:733-739.
100. Bryan SM, Hindmarsh PC. Normal and abnormal fetal growth. *Hormone research.* 2006;65 Suppl 3:19-27.
101. DiStefano C, Zhu M, Mîndila D. Understanding and Using Factor Scores: Considerations for the Applied Researcher. *Practical Assessment, Research & Evaluation.* 2009;14.
102. Meigs JB. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am J Epidemiol.* 2000;152:908-911; discussion 912.
103. Rabe-Hesketh S, Skrondal A. Classical latent variable models for medical research. *Stat Methods Med Res.* 2008;17:5-32.
104. Jöreskog K, Sörbom D. LISREL8: structural equation modeling with the SIMPLIS command language. Chicago: Scientific Software International, Inc; 1993.

Chapter 2



Does Preterm Birth Influence Cardiovascular Risk in Early Adulthood?

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Abstract

Introduction: Both preterm birth and small birth size for gestational age (SGA) have been associated with an increased risk for developing cardiovascular disease (CVD), but controversies still exist. Our aim was to investigate the effect of preterm birth on risk factors for CVD, independent of birth size.

Patients and Methods: Observational study using data of 406 healthy participants aged 18 to 24 years, from the PROGRAM/PREMS study. Associations between gestational age and systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure, blood pressure variability, heart rate, Pulse Wave Velocity (PWV), and carotid Intima Media Thickness (cIMT) were studied. To study the differential effects of preterm and SGA birth, these parameters were also analyzed in subgroups born either preterm or term: young adults born SGA with short or normal adult stature, and young adults born appropriate for gestational age with normal adult stature.

Results: Subjects born preterm (gestational age <36 weeks) had higher unadjusted SBP, pulse pressure, systolic and diastolic blood pressure variability, and heart rate, but a lower DBP than subjects born term. Gestational age was inversely associated with SBP, pulse pressure, blood pressure variability, and heart rate, and positively associated with DBP, also after adjustment for confounders. There was no effect of gestational age on PWV and cIMT, a marker of atherosclerosis. Of all the CVD risk factors measured, higher pulse pressure affected cIMT the most.

Conclusions: Young adults born preterm might have a higher risk for CVD than those born term.

Introduction

Small size at birth has been associated with an increased risk for developing cardiovascular disease (CVD) in later life.[1] Both preterm birth and poor fetal growth can lead to small birth size. Thus, in unraveling the mechanism of this association, independent effects of gestational age as well as small birth size for gestational age (SGA) are important to determine.

Increased blood pressure and arterial stiffness (quantified by Pulse Wave Velocity (PWV)[2-3]) are major determinants of CVD, and both preterm birth and SGA birth have been related to these CVD risk factors.[1, 4-9]

A recent study showed increased carotid Intima Media Thickness (cIMT), which is a measure of atherosclerosis,[10] in subjects born preterm, however, this was restricted to those with fetal growth restriction.[11] Furthermore, low birth weight has been associated with increased cIMT in young adulthood.[12] Although these results were not adjusted for gestational age, it was shown that exclusion of young adults born preterm strengthened the association, indicating that the effect of small birth size on cIMT was due to SGA rather than preterm birth. In contrast, others showed that birth weight SDS did not associate with cIMT in young adulthood.[13]

We investigated differences between young adults born either preterm or term, using the following variables: systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure,[14] blood pressure variability,[15-16] heart rate,[17] PWV, and cIMT. We also investigated the influence of gestational age on these outcomes after adjustment for several confounders, including birth weight SDS and birth length SDS. Additionally, we studied the differential effects of preterm and SGA birth on CVD risk, by subdividing the total population in clinically relevant groups: born small for gestational age (either preterm or term) with short (SGA-S) or normal adult stature (SGA-CU), and born appropriate for gestational age (either preterm or term) with normal adult stature (AGA).

Methods

The PROGRAM (n=323) and PREMS (n=169) study cohorts consist of 492 healthy participants, aged 18-24 years. The PROGRAM- and PREMS study cohorts had similar inclusion and exclusion criteria, study center (Erasmus University Medical Centre in Rotterdam), and measurements, the only difference was that the PREMS study consists of participants born preterm (gestational age <36 weeks). Participants were recruited from several hospitals in the Netherlands, where they had been registered because of their small birth size (birth length<-2SDS),[18] short stature (adult height<-2SDS),[19] or being born preterm. By using advertisement, healthy subjects born AGA were asked to participate. The participation rate of the PROGRAM/PREMS study cohort was 79.5%.[20] The study population has been previously described in detail.[20-21] Birth data were taken from medical records of hospitals, community health services and general practitioners.

Information regarding socioeconomic status (SES), smoking and alcohol use was obtained using questionnaires. Education level of the participant was used as socioeconomic indicator to determine SES.[22] The Medical Ethics Committee of Erasmus Medical Centre approved the study. Written informed consent was obtained from all participants.

Of the 492 participants who entered the study, 86 had incomplete data because the devices to measure blood pressure, cIMT and PWV were not available at all times, resulting in a total number of 406 eligible subjects for analyses.

Additionally, based on SD-scores of birth length and adult height, the subjects were assigned to one of three subgroups. In order to increase the statistical power for subgroup comparison, the cut-off values for small birth size and short adult height were set at -2 SDS, and the cut-off values for normal birth size and normal adult height were set at -1 SDS. This resulted in a total of 246 participants who were included in one of the three subgroups:

- SGA (birth length<-2 SDS) with a short adult height (<-2 SDS)(SGA-S):n=44,
- SGA (birth length<-2 SDS) with catch-up growth resulting in normal adult height (>-1 SDS) (SGA-CU):n=75,
- AGA (birth length>-1 SDS) with normal adult height (>-1 SDS)(AGA):n=127.

All participants fasted for 12 hours and abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer, weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice; the mean was used for analysis.

Blood pressure and heart rate were measured after 10 minutes at rest, in the supine position, using the nondominant arm with an automatic device (Accutorr Plus, Datascope Corp., Montvale NJ, USA[23]) every five minutes for one hour and the mean values of these 13 measurements were taken to reflect resting blood pressure and resting hear rate (HR). Measuring blood pressure using an automatic device has many advantages, however some factors influence the measurement accuracy such as the underlying algorithms used and size and material of the cuff.[24] The device used in the present study has been validated by the Association for the Advancement of Medical Instruments (AAMI) and the British Hypertension Society (BHS), concluding that the device gives accurate measurements in greatest agreement with the mercury standard.[25] The 13 blood pressure measurements were also used to calculate the coefficient of variation.[15-16] Pulse pressure was calculated as the difference between mean systolic and diastolic blood pressure.[14]

Carotid-femoral PWV was measured in supine position using SphygmoCor (AtCor Medical, Sydney Australia).[26] A pressure tonometer was used to simultaneously record carotid pulse wave and ECG. The femoral pulse wave and ECG were also recorded. Distance travelled by the pulse wave was determined by measuring the distances from sternal notch to the femoral location and from sternal notch to the carotid location of pulse wave recording.[27]

Carotid IMT was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using one 7.5 MHz linear array transducer (ATL Ultramark IV,

Advanced Tech. Laboratories, Bethel Washington, USA).[28] On the R-wave of the electrocardiogram, three longitudinal images of the near and far wall of the common carotid artery were frozen and stored on videotape. These frozen images were digitalized and displayed on the screen of a computer using a frame grabber (VP 1400-KIT-512-E-AT, Imaging Technology). The common cIMT was determined as the mean of the mean near and far wall measurements of both the left and right side common carotid artery.[28]

Statistical analysis

SD-scores for birth length and birth weight were calculated to correct for gestational age and sex.[18] SD-scores for adult height, and adult weight were calculated to correct for sex, and age.[19] Variables were log-transformed (natural logarithm) if not normally distributed. ANOVA was used to determine if there were differences between participants born either preterm or term. Using the 13 blood pressure measurements, the coefficient of variation (CV) was calculated to determine the within-subject variation in SBP and DBP with time (blood pressure variability).[15, 29]

Multiple linear regression (MR)-analysis was performed to determine the association of gestational age with SBP, DBP, pulse pressure, blood pressure variability, HR, PWV, and cIMT independent of birth size. In all MR-models, adjustments were made for birth length SDS, birth weight SDS, adult height SDS, age, sex, SES, smoking, alcohol use, and the interaction term birth length SDS∗adult height SDS because the study group had been selected on birth length and adult height (Model A). To study the association with SBP, DBP, pulse pressure, and blood pressure variability, we additionally adjusted for weight SDS (model B), and heart rate (model C). To study PWV, we additionally adjusted for mean arterial pressure (MAP) (model B), weight SDS, the interaction term sex∗weight SDS and age∗weight SDS (model C), and heart rate (model D). To study cIMT, we additionally adjusted for artery diameter (model B), and weight SDS (model C). We tested which parameter (SBP, DBP, pulse pressure, blood pressure variability, HR, PWV) was the most important determinant of cIMT, by adding the parameters alternately to the final cIMT-model. All regression coefficients are presented as a percentage for better interpretation of the results. A positive value indicates that the dependent variable is increased by that % for every unit increase of the independent variable.

ANCOVA was used to determine differences in blood pressure among the subgroups corrected for age and sex (model 1), and additionally adjusted for alcohol use, smoking, SES, and weight SDS (model 2). In blood pressure analyses, heart rate was added to model 2. In HR analyses, systolic blood pressure was added to model 1. In PWV analyses, MAP and HR were added to model 1, and height SDS was added to model 2 (model 3). In cIMT analyses, artery diameter was added to model 1. AGA subjects born term served as reference group and SGA-S preterm, SGA-S term, SGA-CU preterm, SGA-CU term, and AGA preterm were added as dummy variables. Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for analyses. Results were regarded statistically significant if p was <0.05.

Results

The clinical characteristics of the total study population are shown in Table 1. Young adults born preterm had higher unadjusted SBP (p=0.007), pulse pressure (p<0.001), systolic and diastolic blood pressure variability (p=0.002, and p<0.001 respectively), and heart rate (p<0.001) than subjects born term. Unadjusted DBP was lower in subjects born preterm (p<0.001).

Gestational age was inversely associated with SBP (p=0.026) and pulse pressure (p=0.001) after correction for age, sex, SES, smoking, alcohol use, adult height SDS, and birth size. These associations remained significant after additional correction for adult weight SDS (Table 2). In contrast with the association between gestational age and pulse pressure, which remained significant after additional correction for heart rate, the association of gestational age with SBP disappeared after correction for heart rate. Heart rate on itself was positively associated with SBP (p<0.001) (Table 2).

Gestational age was positively associated with DBP after correction for age, sex, SES, smoking, alcohol use, adult height SDS and birth size (p=0.001) (Table 2). This association remained significant after additional adjustment for weight SDS and heart rate (p<0.001), which were both positively associated with DBP (p<0.001).

Lower gestational age was associated with a higher coefficient of variation of both systolic (β(%)=-1.67, p=0.003, adj. R^2=0.058) and diastolic blood pressure (β(%)=-2.85, p<0.001, adj. R^2=0.149), after adjustment for age, sex, birth length SDS, birth weight SDS, adult height SDS, SES, smoking, alcohol use, heart rate, and weight SDS (data not shown).

In MR-analyses, gestational age was inversely associated with heart rate after adjustment for age, sex, birth size, adult height SDS, SES, smoking, and alcohol use (β(%)=-0.86, p<0.001, adj. R^2=0.176). This association remained significant after additional adjustment for weight SDS and SBP (β(%)=-0.76, p<0.001, adj. R^2=0.213) (data not shown).

After adjustments, gestational age was not significantly associated with PWV (Table 3). Adult height SDS showed a significant positive association with PWV (p=0.029) after adjustment for weight SDS. Smoking, higher mean arterial pressure, and higher heart rate, were also related to a higher PWV.

Lower gestational age showed a trend towards lower cIMT after adjustment for age, sex, SES, smoking, alcohol use, adult height SDS, and birth size (p=0.074) (Table 3). However, this disappeared after adjustment for artery diameter, which was positively associated with cIMT.

Because gestational age had an effect on several markers that have been previously associated with atherosclerosis, we tested which marker was the most important determinant of cIMT, by adding the markers alternately to model C (data not shown). The effects of SBP (β(%)=0.16, p=0.002, adj.R^2=0.198), DBP (β(%)=0.02, p=0.818, adj.R^2=0.172), pulse pressure (β(%)=0.48, p<0.001, adj.R^2=0.228), SBP variability (β(%)=0.015, p=0.475, adj.R^2=0.174), DBP variability (β(%)=0.28, p=0.072, adj.R^2=0.181), HR (β(%)=-0.06, p=0.352, adj.R^2=0.176), and PWV (β=-0.62, p=0.259, adj.R^2=0.172) on cIMT were determined. The model with the highest adjusted R^2, thus explaining the largest proportion of variation in cIMT, was the model including pulse pressure.

Table 1. Unadjusted clinical characteristics of the total study population and subgroups

	Total study population		Subgroups					
			SGA-S		SGA-CU		AGA	
	Preterm (n=163)	Term (n= 243)	Preterm (n=9)	Term (n=34)	Preterm (n=31)	Term (n=44)	Preterm (n=63)	Term (n=64)
Male/female #	83/80§	92/151	5/4	10/24	15/16	17/27	37/26	26/38
Age (yrs)	20.8(1.7)	20.9(1.7)	21.6(1.8)	20.6(1.7)	20.4(1.9)§	21.4(1.4)	21.0(1.6)	20.7(1.8)
Gestational age (wks)	32.0(2.2)†	39.2(1.7)	32.3(1.5)†	39.3(1.6)	32.3(2.1)†	38.3(1.6)	32.3(2.4)†	39.4(1.6)
Birth length SDS	-1.22(1.9)	-1.46(1.5)	-3.58(1.0)	-2.99(0.9)	-3.16(0.8)	-2.85(0.8)	0.38(0.9)	0.14(0.7)
Birth weight SDS	-0.42(1.8)†	-1.12(1.4)	-2.49(0.9)	-2.02(0.9)	-2.11(1.1)	-2.36(0.7)	0.79(1.1)§	0.08(1.2)
Adult height SDS	-0.42(1.0)†	-1.03(1.4)	-2.31(0.3)	-2.61(0.6)	-0.10(0.6)	-0.11(0.8)	0.13(0.6)	0.38(0.9)
Adult weight SDS	-0.28(1.2)‡	-0.63(1.4)	-1.08(1.2)	-1.50(1.6)	-0.32(1.2)	0.21(1.1)	0.27(0.8)	0.10(0.9)
SBP(mmHg)*	112.3(8.0)§	110.0(9.0)	109.2(6.6)	107.8(10.2)	113.4(7.2)	112.4(10.2)	113.1(8.2)‡	110.1(7.2)
DBP(mmHg)	63.3(5.3)†	66.1(5.9)	58.2(3.3)§	66.2(8.0)	64.5(5.2)	66.6(6.1)	63.6(5.4)§	66.1(5.0)
PP(mmHg)*	48.9(6.2)†	43.8(5.8)	51.0(6.1)†	41.7(5.2)	48.9(6.0)‡	45.8(6.7)	49.5(6.1)†	43.9(5.5)
CV SBP(%)*	5.17(1.8)§	4.77(2.7)	5.90(1.9)	5.49(4.4)	5.07(1.7)	4.42(1.8)	5.00(1.5)	4.62(2.1)
CV DBP(%)*	9.77(3.2)†	7.98(3.7)	9.00(2.1)	8.36(3.1)	9.64(2.8)§	7.87(3.1)	9.21(3.0)§	7.59(4.3)
HR (beats/ minute)	70(9.1)†	65(9.0)	67(9.1)	71(9.6)	72(11.0)§	65(9.1)	69(8.6)§	64(8.4)
PWV (m/sec)*	7.60(1.0)	7.59(0.9)	8.00(0.8)	7.16(1.0)	7.65(1.1)	7.62(1.1)	7.67(0.9)	7.76(1.2)
cIMT (mm)*	0.52(0.1)	0.52(0.05)	0.52(0.1)	0.50(0.05)	0.52(0.1)	0.53(0.1)	0.53(0.1)	0.52(0.05)
Supplement								
GA Median (IQR)	32(29-34)	40(38-40)	32(32-34)	40(38-41)	33(31-34)	38(37-40)	34(32-36)	40(40-41)
BMI	22.2(3.4)	22.4(3.5)	23.5(3.6)	23.1(5.0)	21.5(3.7)	23.2(3.6)	22.7(2.7)	21.8(2.8)
Alcohol users (%)#	84.5‡	75.7	77.8	76.5	80.7	80.0	88.9	78.1
Smokers (%)#	27.0	25.5	22.2	20.6	19.4	35.0	27.0	20.3
SES(%) 1	13.0	9.4	14.3	13.3	14.3	15.6	7.5	3.2
2	30.5‡	20.8	28.6	33.3	39.3	21.9	26.4§	6.5
3	56.5	69.8	57.1	53.3	46.4	62.5	66.0	90.3
MAP(mmHg)*	83.4(7.3)	83.3(7.7)	81.7(5.2)	81.9(8.2)	84.0(7.7)	86.3(9.4)	84.4(7.4)	83.0(5.8)
AD (mm)	6.66(0.4)	6.66(0.5)	6.49(0.4)	6.38(0.4)	6.65(0.4)	6.76(0.4)	6.77(0.4)	6.79(0.5)
cIMT/AD	0.08(0.01	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)

Values are given as means (SD). GA: gestational age, SDS= standard deviation score, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure, CV= Coefficient of Variation, HR= heart rate, PWV= Pulse Wave Velocity, cIMT= carotid Intima Media Thickness.

*log transformed for ANOVA, # Chi-square test used to determine differences between subjects born preterm and term.

†: p<.001 compared to term (same subgroup), ‡: p<.05 compared to term (same subgroup), §: p<.01 compared to term (same subgroup)

Table 2. Multiple regression for Systolic blood pressure, Diastolic blood pressure and Pulse pressure in early adulthood

	Systolic blood pressure						Diastolic blood pressure						Pulse pressure					
	Model A		Model B		Model C		Model A		Model B		Model C		Model A		Model B		Model C	
	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P
Gestational age	-0.246	**0.026**	-0.230	**0.026**	-0.127	0.223	0.451	**0.001**	0.462	**0.001**	0.631	**<0.001**	-1.207	**<0.001**	-1.184	**<0.001**	-1.167	**<0.001**
Birth length SDS	-0.257	0.556	-0.496	0.227	-0.413	0.302	-0.703	0.193	-0.866	0.104	-0.733	0.153	0.293	0.664	-0.058	0.928	-0.045	0.944
Birth weight SDS	0.060	0.883	0.155	0.690	0.106	0.781	0.213	0.677	0.361	0.475	0.280	0.563	-0.466	0.465	-0.152	0.802	-0.159	0.792
Adult height SDS	0.121	0.774	-1.021	**0.018**	-0.956	**0.023**	-0.599	0.249	-1.379	**0.014**	-1.272	**0.018**	1.254	0.056	-0.431	0.520	-0.421	0.531
SES 1	5.180	**<0.001**	4.557	**0.001**	3.408	**0.013**	3.678	**0.043**	3.256	0.068	1.415	0.414	7.354	**0.001**	6.425	**0.003**	6.238	**0.005**
SES 2	1.629	0.112	1.159	0.228	0.815	0.385	2.019	0.113	1.695	0.176	1.133	0.348	1.299	0.412	0.634	0.681	0.559	0.709
Smoking	-1.057	0.286	-0.531	0.569	-0.096	0.917	-1.949	0.111	-1.592	0.187	-0.890	0.448	0.447	0.772	1.228	0.403	1.298	0.380
Alcohol use	2.118	**0.045**	1.596	0.107	1.449	0.133	2.319	0.077	1.960	0.128	1.720	0.164	1.850	0.256	1.089	0.479	1.065	0.489
Adult weight SDS			2.108	**<0.001**	2.110	**<0.001**			1.442	**0.001**	1.447	**<0.001**			3.099	**<0.001**	3.099	**<0.001**
HR					0.176	**<0.001**					0.288	**<0.001**					0.028	0.688
Overall P-value	<0.001		<0.001		<0.001		0.004		<0.001		<0.001		<0.001		<0.001		<0.001	
R^2 adjusted	0.172		0.272		0.307		0.046		0.079		0.149		0.386		0.452		0.451	

Regression coefficients are shown as a percentage, a positive value indicates that the dependent variable is increased with that % for every unit increase of the independent variable.
Bolded p-values are p-values below 0.05.
Adjusted for age, sex and the interaction term birth length SDS * adult height SDS.
SES 3 (highest socioeconomic class) is used as reference for SES analyses.
SDS= standard deviation score, SES= socioeconomic status, HR= heart rate

Unadjusted differences between the subgroups are shown in Table 1. Comparisons of preterm and term SGA-subgroups, after adjustment for age, sex, alcohol use, smoking, SES, heart rate and weight SDS, showed that SGA-S subjects born preterm had a significantly lower diastolic blood pressure (p=0.002), and a higher pulse pressure (p=0.016) than those born term. Also, SGA-CU subjects born preterm had a lower diastolic blood pressure (p=0.046), and a higher pulse pressure (p=0.028) and systolic and diastolic blood pressure variability (p=0.035 and p=0.004 respectively) than those born term. There were no significant differences in systolic blood pressure between preterm and term SGA-subgroups.

After adjustment for age, sex, alcohol use, smoking, SES, systolic blood pressure, and weight SDS, SGA-CU subjects born preterm had a higher heart rate than those born term (p=0.009). There was, however, no significant difference in heart rate between SGA-S subjects born preterm or term. After adjustment for confounders, PWV and cIMT did also not differ significantly between subjects born preterm or term, in any of the subgroups.

Table 4 shows comparisons of systolic blood pressure, diastolic blood pressure, pulse pressure, blood pressure variability, heart rate, PWV, and cIMT of the subgroups after adjustment for possible confounders, with AGA subjects born term as reference group. In the final model, all preterm subgroups had a significantly lower diastolic blood pressure, but higher pulse pressure and diastolic blood pressure variability than the reference group. After correction, there were no differences in systolic blood pressure variability and cIMT. SGA-CU and AGA subjects born preterm had a higher heart rate than the reference group (AGA, born term). SGA-S and SGA-CU subjects born term had a lower PWV than the reference group, but this significant difference disappeared after correction for adult height SDS.

Table 3. Multiple regression for PWV and cIMT in early adulthood

	PWV								cIMT					
	Model A		Model B		Model C		Model D		Model A		Model B		Model C	
	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P
Gestational age	0.147	0.460	0.141	0.442	0.145	0.405	0.261	0.145	0.263	0.074	0.095	0.481	0.091	0.506
Birth length SDS	-0.062	0.935	-0.061	0.932	0.400	0.553	0.493	0.461	0.113	0.847	-0.084	0.875	-0.063	0.906
Birth weight SDS	-0.101	0.890	0.193	0.776	-0.104	0.872	-0.173	0.787	-0.330	0.550	-0.234	0.643	-0.254	0.615
Adult height SDS	0.005	0.995	-0.041	0.952	1.556	**0.032**	1.574	**0.029**	0.502	0.372	-0.337	0.519	-0.235	0.676
SES1	2.064	0.259	0.740	0.755	2.540	0.268	1.365	0.555	0.531	0.775	0.432	0.799	0.461	0.786
SES2	-1.814	0.317	-3.084	0.066	-2.453	0.126	-2.725	0.087	1.488	0.271	1.538	0.213	1.575	0.204
Smoking	2.343	0.185	4.006	**0.017**	3.770	**0.017**	4.136	**0.009**	0.645	0.623	1.214	0.313	1.179	0.328
Alcohol use	-0.116	0.950	-0.552	0.749	0.600	0.716	0.443	0.787	0.757	0.586	1.001	0.431	1.053	0.410
Artery diameter											10.66	**<0.001**	10.85	**<0.001**
Adult weight SDS					5.496	0.376	6.247	0.312					-0.202	0.636
MAP			0.638	**<0.001**	0.740	**<0.001**	0.692	**<0.001**						
HR							0.192	**0.015**						
Overall P-value	<0.001		<0.001		<0.001		<0.001		0.099		<0.001		<0.001	
R^2 adjusted	0.108		0.236		0.317		0.329		0.019		0.183		0.181	

Regression coefficients are shown as a percentage, a positive value indicates that the dependent variable is increased with that % for every unit increase of the independent variable.
Adjusted for age, sex and the interaction term birth length SDS∗adult height SDS, the model with PWV as dependent variable is additionally adjusted for the interaction terms age∗adult weight SDS and gender∗adult weight SDS
SES 3 (highest socioeconomic class) is used as reference for SES analyses.
PWV= pulse wave velocidy, cIMT= carotid intima media thickness, SES= socioeconomic status, SBP= systolic blood pressure, HR= heart rate, MAP= mean arterial pressure

Table 4. Subgroup analyses of blood pressure, pulse pressure, blood pressure variability, heart rate, PWV, and cIMT compared to AGA term controls

		SGA-S Preterm		SGA-S Term		SGA-CU preterm		SGA-CU term		AGA preterm		
		β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	R^2 adjusted
Systolic BP	Model 1	-2.67	0.355	-0.45	0.802	2.47	0.149	1.20	0.464	1.02	0.486	0.129
	Model 2[1,2]	-3.00	0.256	0.09	0.959	-0.12	0.937	-0.34	0.819	-1.33	0.300	0.314
Diastolic BP	Model 1	-12.9	**<0.001**	0.27	0.901	-1.45	0.469	0.63	0.745	-4.57	0.005	0.107
	Model 2[1,2]	-14.3	**<0.001**	-0.75	0.721	-5.57	**0.003**	-1.02	0.554	-7.66	**<0.001**	0.318
Pulse Pressure	Model 1	12.9	**0.008**	-1.95	0.483	8.34	0.002	2.87	0.371	9.36	**<0.001**	0.358
	Model 2[1,2]	14.4	**0.003**	0.71	0.817	8.08	**0.005**	0.87	0.729	8.17	**<0.001**	0.396
CV SBP	Model 1	34.2	**0.038**	24.4	**0.013**	8.73	0.303	-9.94	0.182	8.91	0.204	0.057
	Model 2[1,2]	32.5	0.055	20.4	0.060	6.60	0.470	-11.1	0.145	7.55	0.300	0.051
CV DBP	Model 1	36.4	**0.042**	23.0	**0.028**	30.4	**0.003**	2.95	0.730	29.8	<0.001	0.109
	Model 2[1,2]	38.5	**0.037**	22.5	0.053	29.1	**0.007**	0.89	0.917	28.2	0.001	0.127
Heart rate	Model 1[3]	9.13	0.083	10.1	**0.002**	13.5	<0.001	0.67	0.811	8.72	**0.001**	0.234
	Model 2[1,3]	4.52	0.377	5.11	0.138	9.58	**0.002**	-0.79	0.774	7.70	**0.002**	0.283
PWV	Model 1[4]	0.07	0.990	-6.96	**0.019**	-4.73	0.101	-5.59	**0.033**	-4.47	0.060	0.275
	Model 2[1,4]	-2.24	0.681	-11.2	**<0.001**	-5.10	0.070	-4.90	0.055	-3.65	0.114	0.342
	Model 3[1,4,5]	2.48	0.691	-6.41	0.145	-4.48	0.114	-4.18	0.106	-3.22	0.163	0.348
cIMT	Model 1[6]	2.25	0.374	1.35	0.525	1.75	0.409	2.28	0.261	1.82	0.298	0.132
	Model 2[1,6]	2.57	0.503	0.03	0.989	1.43	0.522	2.31	0.265	2.02	0.260	0.131

Regression coefficients are shown as a percentage, a positive % indicates that the dependent variable is increased with that %, compared to AGA term controls.

BP= blood pressure, CV= coefficient of variation, Systolic blood pressure, DBP= diastolic blood pressure, PWV= pulse wave velocity, cIMT= carotid Intima Media Thickness.

All models are adjusted for age and sex and additionally adjusted for: [1] Alcohol use, smoking, SES and adult weight SDS, [2] Heart rate, [3] Systolic blood pressure, [4] Mean arterial pressure, [5] Adult Height SDS, [6] Artery diameter

Discussion

Higher blood pressure in adults born preterm than in healthy controls has been reported.[30] Also in the present study lower gestational age was associated with higher systolic blood pressure, but this disappeared after adjustment for heart rate. These findings suggest that the reported elevated systolic blood pressure in subjects born preterm is associated with an increased heart rate, indicating that both might share an underlying determinant. The mechanisms underlying these associations remain unknown,[31] but might be explained by preterm birth being associated with an increased cardiac output, which might eventually lead to hypertension.[32] In contrast, we showed a lower diastolic blood pressure in young adults born preterm, which remained significant after adjustment for several confounders. Lower diastolic blood pressure has been associated with less risk for CVD,[33] although this was controversial in other studies.[34]

To our knowledge, we are the first to report an increased pulse pressure in young adults born preterm. This new finding is in line with a study showing an inverse association between gestational age and pulse pressure in children.[35] Elevated pulse pressure has been associated with increased risk for atherosclerosis, already in early adulthood.[14,33] This was confirmed by our study showing that of all determinants of CVD examined, the effect of pulse pressure on cIMT was most pronounced, in contrast to the non-significant effect of DBP on cIMT. In addition, variability of systolic and diastolic blood pressure was higher in participants born preterm. Higher variability of blood pressure in time has also been associated with CVD.[15-16]

Although one would expect a lower heart rate in combination with a higher pulse pressure, young adults born preterm had a higher heart rate than those born term. This finding is supported by previous studies.[36-38] Johansson et al. hypothesized that an increased heart rate could be ascribed to altered sympathoadrenal function in subjects born small, either preterm or SGA.[38] In the present study, higher heart rate was only found in subjects born preterm, regardless of birth weight. This implies that there is an effect of preterm birth on heart rate, rather than an effect of SGA birth. Determination of resting heart rate is of importance since it is associated with CVD.[17] Unfortunately, the present study does not include tests to determine neural regulatory mechanisms. For future research it would be interesting to carry out spectral analyses in young adults born preterm, in order to determine whether the increased heart rate and blood pressure variability are due to sympathovagal imbalance.[39-40]

We did not find an association of preterm birth with PWV. Adult height SDS was, however, positively associated with PWV. This association also explains the difference in PWV between SGA-S subjects born term and AGA subjects born term, as that difference disappeared after correction for height SDS. Only limited studies investigated the association between adult height SDS and PWV. One study showed a positive association between height and PWV in healthy children.[41]

There was also no effect of preterm birth on cIMT. Previous studies reported controversial results regarding the association of cIMT with gestational age, preterm birth, and birth size.[12-13]

These studies, however, did not adjust for artery diameter, which is likely to be a confounder in the relationship of gestational age and birth size with cIMT. Also, it might well be that an effect of gestational age on cIMT will arise at an older age.

The great contrasts in birth size and adult stature in our study population enabled performing comparisons of clinically relevant subgroups. These comparisons showed that the effect of preterm birth on CVD risk can not be ascribed to SGA birth and/or catch up growth. We found significant differences in DBP, pulse pressure, and DBP variability, between the preterm subgroups and term AGA controls, irrespectively of SGA birth. The preterm groups had a significantly higher resting heart rate, except for the preterm SGA-S subgroup. There were no differences in CVD risk parameters between the SGA-groups born term and the healthy controls.

We acknowledge that the Datascope Accutorr Plus to determine blood pressure during one hour uses an algorithm to compute systolic and diastolic blood pressure. Although it has shown to be in greatest agreement with the mercury standard, this should be taken into account. Future studies are warranted to reproduce our results using directly measured systolic and diastolic blood pressure. We also acknowledge that our study population consists of subjects without serious postnatal complications and did not include extreme prematurely born subjects. Whether our results can be generalized to subjects with complications, such as broncho-pulmonary dysplasia, requires further research. Furthermore, it would be of additional value to include family history, as a risk factor of atherosclerosis, in our analyses. Unfortunately, we did not have sufficient information to assess family history in our cohort of young adults. However, none of the subjects who fully completed the questionnaires mentioned a family history of cardiovascular disease.

Our data show that young adults born preterm might have a higher risk to develop CVD due to a higher systolic blood pressure, resting heart rate, and a higher pulse pressure and blood pressure variability in time. Although we show that young adults born preterm have a lower diastolic blood pressure than adults born term, the lower diastolic blood pressure contributes to an increased pulse pressure in these subjects. Because the prevalence of preterm birth and survival is rapidly increasing, our results are of clinical relevance for an increasing number of subjects and are thus of major importance for public health.

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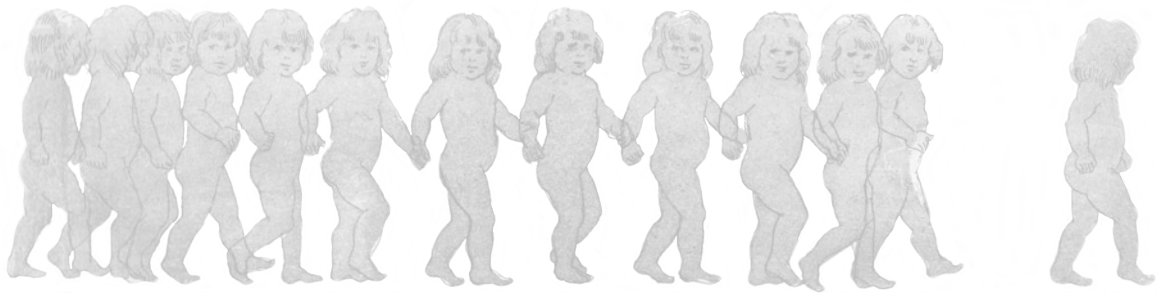
References

1. Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ.* Feb 13 1999;318(7181):427-431.
2. Willum-Hansen T, Staessen JA, Torp-Pedersen C, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation.* Feb 7 2006;113(5):664-670.
3. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation.* Feb 7 2006;113(5):657-663.
4. Evensen KA, Steinshamn S, Tjonna AE, et al. Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early Hum Dev.* Apr 2009;85(4):239-245.
5. Keijzer-Veen MG, Dulger A, Dekker FW, Nauta J, van der Heijden BJ. Very preterm birth is a risk factor for increased systolic blood pressure at a young adult age. *Pediatr Nephrol.* Mar 2010;25(3):509-516.
6. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. *Hypertension.* Nov 2000;36(5):790-794.
7. Law CM, Shiell AW. Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. *J Hypertens.* Aug 1996;14(8):935-941.
8. Irving RJ, Belton NR, Elton RA, Walker BR. Adult cardiovascular risk factors in premature babies. *Lancet.* Jun 17 2000;355(9221):2135-2136.
9. Oren A, Vos LE, Bos WJ, et al. Gestational age and birth weight in relation to aortic stiffness in healthy young adults: two separate mechanisms? *Am J Hypertens.* Jan 2003;16(1):76-79.
10. Bots ML, Grobbee DE. Intima media thickness as a surrogate marker for generalised atherosclerosis. *Cardiovasc Drugs Ther.* Jul 2002;16(4):341-351.
11. Skilton MR, Viikari JS, Juonala M, et al. Fetal growth and preterm birth influence cardiovascular risk factors and arterial health in young adults: the cardiovascular risk in young Finns study. *Arterioscler Thromb Vasc Biol.* Dec 2011;31(12):2975-2981.
12. Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML. Birth weight and carotid intima-media thickness: new perspectives from the atherosclerosis risk in young adults (ARYA) study. *Ann Epidemiol.* Jan 2004;14(1):8-16.
13. Finken MJ, Inderson A, Van Montfoort N, et al. Lipid profile and carotid intima-media thickness in a prospective cohort of very preterm subjects at age 19 years: effects of early growth and current body composition. *Pediatr Res.* Apr 2006;59(4 Pt 1):604-609.
14. Raitakari OT, Juonala M, Taittonen L, et al. Pulse pressure in youth and carotid intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *Stroke.* Apr 2009;40(4):1519-1521.
15. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet.* Mar 13 2010;375(9718):938-948.
16. Kikuya M, Ohkubo T, Metoki H, et al. Day-by-day variability of blood pressure and heart rate at home as a novel predictor of prognosis: the Ohasama study. *Hypertension.* Dec 2008;52(6):1045-1050.
17. Fox K, Borer JS, Camm AJ, et al. Resting heart rate in cardiovascular disease. *J Am Coll Cardiol.* Aug 28 2007;50(9):823-830.
18. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* Jun 1969;74(6):901-910.
19. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* Mar 2000;47(3):316-323.
20. Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T, Hokken-Koelega AC. Influence of preterm birth and birth size on gonadal function in young men. *J Clin Endocrinol Metab.* Nov 2009;94(11):4243-4250.
21. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* Jun 3 2009;301(21):2234-2242.

22. *Dutch Standard Classification of Education, 2006*: Centraal Bureau van de Statistiek, Statistics Netherlands.
23. Anwar YA, Tendler BE, McCabe EJ, Mansoor GA, White WB. Evaluation of the Datascope Accutorr Plus according to the recommendations of the Association for the Advancement of Medical Instrumentation. *Blood Press Monit.* Apr 1997;2(2):105-110.
24. Tholl U, Forstner K, Anlauf M. Measuring blood pressure: pitfalls and recommendations. *Nephrol Dial Transplant.* Apr 2004;19(4):766-770.
25. O'Brien E, Waeber B, Parati G, Staessen J, Myers MG. Blood pressure measuring devices: recommendations of the European Society of Hypertension. *BMJ.* Mar 3 2001;322(7285):531-536.
26. Sigrist MK, Chiarelli G, Levin A, Romann A, Weber C. Pulse Wave Velocity Measurements Are Reproducible in Multiple Trained Observers: A Short Report. *Nephron Clin Pract.* May 21 2010;116(1):c60-c64.
27. Weber T, Ammer M, Rammer M, et al. Noninvasive determination of carotid-femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. *J Hypertens.* Aug 2009;27(8):1624-1630.
28. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation.* Sep 2 1997;96(5):1432-1437.
29. Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. *Lancet.* Mar 13 2010;375(9718):906-915.
30. Norman M. Preterm birth--an emerging risk factor for adult hypertension? *Semin Perinatol.* Jun 2010;34(3):183-187.
31. Alexander BT, Intapad S. Preterm birth: a novel risk factor for higher blood pressure in later life. *Hypertension.* Feb 2012;59(2):189-190.
32. Messerli FH, Frohlich ED, Suarez DH, et al. Borderline hypertension: relationship between age, hemodynamics and circulating catecholamines. *Circulation.* Oct 1981;64(4):760-764.
33. Strandberg TE, Salomaa VV, Vanhanen HT, Pitkala K, Miettinen TA. Isolated diastolic hypertension, pulse pressure, and mean arterial pressure as predictors of mortality during a follow-up of up to 32 years. *J Hypertens.* Mar 2002;20(3):399-404.
34. Benetos A, Thomas F, Bean K, Gautier S, Smulyan H, Guize L. Prognostic value of systolic and diastolic blood pressure in treated hypertensive men. *Arch Intern Med.* Mar 11 2002;162(5):577-581.
35. Relton CL, Pearce MS, O'Sullivan JJ. The relationship between gestational age, systolic blood pressure and pulse pressure in children. *J Hum Hypertens.* May 2008;22(5):352-357.
36. Bonamy AK, Martin H, Jorneskog G, Norman M. Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *J Intern Med.* Dec 2007;262(6):635-642.
37. Bonamy AK, Andolf E, Martin H, Norman M. Preterm birth and carotid diameter and stiffness in childhood. *Acta Paediatr.* Apr 2008;97(4):434-437.
38. Johansson S, Norman M, Legnevall L, Dalmaz Y, Lagercrantz H, Vanpee M. Increased catecholamines and heart rate in children with low birth weight: perinatal contributions to sympathoadrenal overactivity. *J Intern Med.* May 2007;261(5):480-487.
39. Malliani A, Pagani M, Lombardi F. Physiology and clinical implications of variability of cardiovascular parameters with focus on heart rate and blood pressure. *Am J Cardiol.* Apr 7 1994;73(10):3C-9C.
40. Pal GK, Adithan C, Amudharaj D, et al. Assessment of sympathovagal imbalance by spectral analysis of heart rate variability in prehypertensive and hypertensive patients in Indian population. *Clin Exp Hypertens.* 2011;33(7):478-483
41. Kis E, Cseprekal O, Horvath Z, et al. Pulse wave velocity in end-stage renal disease: influence of age and body dimensions. *Pediatr Res.* Jan 2008;63(1):95-98

Chapter 3



Health Profile of Young Adults Born Preterm: Negative Effects of Rapid Weight Gain in Early Life

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Submitted

Abstract

Context: Early postnatal weight gain is associated with determinants of cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2) in adults born term. However, this association remains to be elucidated in adults born preterm.

Objectives: To investigate the association of weight gain during different periods, and weight trajectories in early life after preterm birth with determinants of CVD and DM2 in early adulthood.

Design, Setting, and Participants: Observational study using longitudinal data collected in the PREMS study in 162 healthy participants born preterm (gestational age <36 weeks), aged 18 to 24 years at a medical center in the Netherlands between March 2006 and September 2007.

Main Outcome Measures: Associations between early and first-year growth, tempo of weight gain, and determinants of CVD and DM2 in young adults born preterm.

Results: Weight gain, adjusted for length, in the period from birth up to term age and in the first three months after term age, was positively associated with body fat percentage and waist circumference at 21 years. Weight gain in the first three months after term age was also positively associated with total cholesterol and LDLc levels at 21 years. Subjects with the highest gain in weight from birth to term age (highest quartile) had significantly higher body fat percentage, waist circumference, acute insulin response, and disposition index in early adulthood than the subgroups with moderate and low gain in weight. Rapid catch-up in weight during the first three months after term age resulted in a higher fat percentage, waist circumference and serum triglycerides level than slower catch-up in weight.

Conclusion: Accelerated neonatal weight gain compared to gain in length after preterm birth (immediately after birth and during the first three months after term age) is associated with risk factors for cardiovascular disease in early adulthood, and should therefore be avoided.

Introduction

Nowadays, 5-13% of all newborns in developed countries is being born preterm.[1] Because of advances in neonatal intensive care, survival of preterm infants has improved, and most of these children reach adulthood. Young adults born preterm are at increased risk for developing cardiovascular diseases (CVD),[2-3] and have increased cardiovascular mortality.[4] The underlying cause of this increased cardiovascular risk is unknown, but might well lay in different early growth trajectories in infants born preterm.[5-7] Alterations in growth during this highly dynamic developmental time window might have programming effects on later health outcomes.

In young adults born term, accelerated neonatal weight gain has been associated with increased risk for CVD and type 2 diabetes (DM2).[8-10] Few studies have investigated this relationship in subjects born preterm, and most of these studies did not have prospective detailed first year growth data of the preterm participants, as well as control data of subjects born term.[11-13]

In the present study, we investigated associations between weight gain during different time windows from preterm birth up to one year after term age and determinants of CVD and DM2 in early adulthood. Also, the effect of rapid weight gain during the first three months after term age was studied and compared to the effect in subjects born term.[8]

Studying this relationship in subjects born preterm also enabled to investigate whether a negative effect of accelerated postnatal weight gain is related to the first period after birth (regardless of gestational age) or to a maturational stage (term age).

Methods

Subjects

The study population consisted of 169 healthy young adults (PREMS study[14]) who were registered because of being born preterm (gestational age <36 weeks). Data of the participants were compared with data of 217 young adults born term (PROGRAM study).[8] Both cohorts had similar in- and exclusion criteria, research centre, and measurements. The clinical characteristics and first year growth data of the PROGRAM study have been reported.[8] All included subjects were aged 18-24 years, Caucasian, born singleton and had an uncomplicated neonatal period without severe asphyxia (defined as an Apgar score below three after five minutes), sepsis, or long-term complications of respiratory ventilation and/or oxygen supply. Birth data were obtained from hospital records, primary health care records, and general practitioner records. Data on educational level were obtained using questionnaires to determine socioeconomic status (SES).[15] Weight and length at term age (age at which gestational age would have reached 40 weeks), 3, 6, 9, and 12 months after term age had been prospectively measured at primary health care centers

or hospitals. These data were collected during the study period March 2006-September 2007 from the records of the health care centers and hospitals.

The medical ethics committee of Erasmus Medical Center approved the study. Written informed consent was obtained from all participants. Of 169 study participants of the PREMS study, data on first-year growth were available for 162 young adults, 43 were small at birth (birth length < -2SDS) of whom 9 had short stature (adult height <-2SDS), and 119 had normal size at birth (birth length >-2SDS) of whom 5 had short stature.

Measurements

Participants were invited to visit Erasmus University Medical Center and were reimbursed for travel expenses. Prior to the visit, participants fasted for at least 12 hours and abstained from smoking and drinking alcohol for at least 16 hours. All anthropometric measurements were performed twice; the mean value was used for analyses.

Lean body mass and fat mass were measured on one Dual-energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, England).[16] Insulin sensitivity index (capacity of insulin to promote glucose disposal), acute insulin response to glucose (estimate of insulin secretory capacity), and the disposition index (the product of insulin sensitivity and acute insulin response indicating the degree of glucose homeostasis) were determined using the Bergman minimal model (MINMOD Millenium version 6.01, MINMOD Inc, Los Angeles, California), which calculated the paired glucose and insulin data obtained by frequent measurements during an intravenous glucose tolerance test[17-19] with Tolbutamide.[20] Blood pressure was measured after 10 minutes at rest, in the sitting position, using the non-dominant arm with an automatic device (Accutorr Plus, Datascope Corp, Montvale, New Jersey) every five minutes for one hour and the mean value was taken to reflect resting blood pressure.

Laboratory Methods

After centrifugation, all samples were kept frozen until assayed (-80°C). The assays have been previously described in detail.[21] Briefly, plasma glucose levels were determined on a VITROS analyzer 750 (Ortho-Clinical Diagnostics, Johnson & Johnson Company, Beerse, Belgium). Plasma insulin levels were measured using an immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). Total cholesterol level was measured using the CHOD-PAP and the GPO-PAP reagent kit (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol level was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula: LDL cholesterol level in mmol/L = total cholesterol level – HDL cholesterol level – 0.45 x level of triglycerides. Apolipoprotein A-I and apolipoprotein B were determined by rate of nephelometry on the Image Immunochemistry System (Beckman Coulter, Mijdrecht, the Netherlands) according to the manufacturer's instructions.

Statistical analysis

SD-scores for birth length, birth weight, and first-year growth were calculated to correct for gestational age and sex.[22] SD-scores for adult height and weight were calculated to correct for sex and age.[23] All SD-scores were calculated using growth analyser software (http://www.growthanalyser.org). Multiple linear regression analyses were performed to investigate the association between weight gain from birth up to term age, per each three months during one year after term age, and several determinants in young adults born preterm. The five periods were analyzed separately from each other. Adjustments were made for gestational age, sex, age, and socioeconomic status (SES). To investigate the association between weight and the outcome variables independently of length, adjustments were made for length growth during the same period. When residuals deviated from homogeneity, outcome variables were log transformed. This applied to waist circumference, insulin sensitivity, acute insulin response, disposition index, and serum levels of total cholesterol, ApoB, ratio of ApoB to ApoA1, and triglycerides.

To study the period from birth up to term age, we subdivided the study population of 162 participants in quartiles based on their gain in weight SDS in that period. This resulted in three subgroups: the lowest quartile, the second and third quartile, and the highest quartile of gain in weight SDS. Adjusted subgroup comparisons were performed by calculating the estimated marginal means (EMM), with adjustments for sex, age, gestational age, SES, birth weight SDS, and gain in length SDS from birth to term age. We could not study catch-up from birth to term age (defined as >0.67 SDS weight gain) because most participants decreased in weight in this period. Only selecting subjects with catch-up from birth to term age would result in a very small group size.

Catch-up in weight in the first year after term age was defined as an SD-score of more than 0.67 of weight gain because this represents the width of each percentile band on standard growth curves (second to ninth percentile, ninth to 25th percentile, etc.).[8,24] Of the group with catch-up in weight, two subgroups were formed based on rapid (SD≥0.5) or slow (SD<0.5) weight gain during the first three months after term age. Additionally, young adults born preterm with rapid catch-up and slow catch-up in weight were compared to those born term. Differences in clinical characteristics were determined by an independent t-test. Differences in determinants of CVD and DM2 were determined by regression analyses, with corrections for first-year length growth, gestational age, sex, age, and SES.

SPSS statistical package version 17.0 (SPSS Inc, Chicago, Illinois) was used. All statistical tests were performed two-sided and results were regarded statistically significant if P-value was <0.05.

Results

Clinical characteristics and determinants of CVD and DM2 of the total study group are shown in Table 1 and 2. The mean (SD) age was 20.8(1.68) years. Mean weight SDS declined from birth up to term age and increased from term age up to three months after term age.

Table 1. Clinical characteristics of the total preterm group and subjects with fast versus slow catch-up growth in weight in the first year after term age

		Preterm				Term			Preterm vs. Term	
		Total group (n=162)	**Rapid (n=55)**	**Slow (n=36)**	**p-value***	**Rapid (n=65)**	**Slow (n=22)**	**p-value***	**Rapid p-value**	**Slow p-value**
Male (%)		50	41.8	50.0	0.443	35.4	50.0	0.225	0.470	1.000
Gestational age (weeks)		32.0(2.22)	32.7(2.07)	32.1(2.07)	0.146	38.5(1.51)	39.0(1.97)	0.164	<0.001	<0.001
Gestational age (IQR)		30.3-34.0	31.6-34.0	30.3-34.0		37.5-40.0	37.0-40.7			
Age (yr)		20.8(1.68)	20.5(1.81)	20.8(1.76)	0.595	20.6(1.66)	21.0(1.90)	0.358	0.754	0.559
SES (%)	1	12.6	14.9	3.4		10.5	10.5			
	2	31.1	29.8	37.9	0.269	40.4	21.1	0.290	0.449	0.342
	3	56.3	55.3	58.6		49.1	68.4			
Birth length SDS		-1.27(1.88)	-1.37(1.96)	-1.95(1.72)	0.178	-2.22(1.28)	-2.13(1.62)	0.777	**0.005**	0.697
Birth weight SDS		-0.51(1.80)	-0.88(1.75)	-1.16(1.56)	0.439	-2.23(0.74)	-2.05(1.04)	0.376	<0.001	0.021
Term age length SDS		-1.82(1.51)	-1.74(1.51)	-2.56(1.34)	**0.013**	–	–	–	–	–
Term age weight SDS		-1.41(1.17)	-1.58(1.18)	-1.99(0.98)	0.088	–	–	–	–	–
3mo length SDS		-1.30(1.22)	-0.98(1.18)	-1.95(1.11)	**<0.001**	-1.13(1.06)	-1.66(1.23)	0.058	0.465	0.355
3mo weight SDS		-1.01(1.38)	-0.34(1.38)	-1.99(1.07)	**<0.001**	-0.76(0.93)	-2.12(1.05)	**<0.001**	0.051	0.662
Delta length SDS birth-term age		-0.52(1.10)	-0.36(1.04)	-0.61(0.99)	0.293	–	–	–	–	–
Delta weight SDS birth-term age		-0.91(1.10)	-0.70(1.02)	-0.83(0.90)	0.539	–	–	–	–	–
Delta length SDS term age - 3mo		0.53(0.65)	0.79(0.64)	0.51(0.54)	**0.039**	1.07(1.02)	0.47(1.01)	**0.020**	0.091	0.855
Delta weight SDS term age - 3mo		0.40(0.86)	1.24(0.61)	0.00(0.43)	**<0.001**	1.47(0.62)	-0.07(0.47)	**<0.001**	**0.045**	0.574
1yr length SDS		-0.55(1.02)	0.02(0.91)	-0.72(0.81)	**<0.001**	-0.55(0.97)	-1.08(1.09)	**0.033**	**0.001**	0.154
1yr weight SDS		-0.66(1.17)	0.06(1.12)	-0.69(0.91)	**0.001**	-0.33(0.79)	-0.74(1.02)	0.054	**0.026**	0.854
Adult height SDS		-0.42(0.95)	-0.18(0.97)	-0.30(0.91)	0.553	-0.74(1.02)	-0.79(1.14)	0.833	**0.003**	0.080
Adult weight SDS		-0.28(1.22)	0.19(1.09)	-0.57(1.02)	**0.001**	-0.09(1.16)	-0.59(1.05)	0.075	0.171	0.939
Adult weight minus height SDS		0.14(1.21)	0.38(1.23)	-0.27(0.96	**0.009**	0.65(1.25)	0.19(0.91)	0.125	0.242	0.075

Data are given as mean(sd), except of gestational age in inter quartile range (IQR). SES= socioeconomic status. All subjects had a first year catch-up growth in weight of more than 0.67 SDS. They were divided in two subgroups based on fast (>0.5SDS) or slow (<0.5SDS) catch-up in growth in weight in the first three months. * rapid vs. slow

Table 2. Determinants of CVD and DM2 of the preterm group and subjects with fast versus slow catch-up growth in weight in the first year after term age

	Preterm				Term			Preterm vs. Term	
	Total group (n=162)	**Rapid (n=55)**	**Slow (n=36)**	**p-value**	**Rapid (n=65)**	**Slow (n=22)**	**p-value**	**Rapid p-value**	**Slow p-value**
Body fat %	24.2	28.3(9.8)	21.2(10.4)	**<0.001**	27.4(9.2)	19.4(11.6)	**0.006**	0.608	0.540
Ratio trunk fat to total fat	0.49	0.49(0.05)	0.49(0.05)	0.174	0.49(0.05)	0.48(0.07)	**0.040**	0.969	0.574
Waist circumference*(cm)	77.2	79.8(11.0)	74.4(6.5)	**0.009**	80.6(10.6)	75.0(8.3)	**0.028**	0.662	0.813
Insulin sensitivity*(µU/mL)	7.71	6.96(5.08)	7.77(6.06)	0.991	5.38(3.75)	8.13(4.76)	**0.048**	0.153	0.756
Acute insulin response*(mU/L)	512	571(230)	488(312)	0.425	920(853)	864(324)	0.069	**0.042**	0.327
Disposition index*	3081	3121(1756)	3085(2043)	0.434	3371(2102)	3566(1245)	0.713	0.750	0.237
Total cholesterol*(mg/dl)	4.27	4.31(0.79)	4.06(0.75)	0.053	4.64(1.10)	4.39(0.90)	0.543	0.108	0.181
LDLc(mg/dl)	2.54	2.26(0.73)	2.46(0.91)	0.292	2.76(1.05)	2.51(0.63)	0.383	0.253	0.815
HDLc(mg/dl)	1.38	1.39(0.33)	1.31(0.27)	0.222	1.38(0.34)	1.45(0.47)	0.280	0.838	0.146
ApoB*(mg/dl)	0.81	0.83(0.22)	0.77(0.23)	0.236	0.85(0.27)	0.75(0.18)	0.117	0.826	0.345
ApoA1(mg/dl)	1.46	1.47(0.25)	1.43(0.24)	0.219	1.29(0.21)	1.27(0.26)	0.730	<0.001	0.025
Ratio of ApoB to ApoA1*	0.57	0.58(0.18)	0.55(0.17)	0.815	0.68(0.26)	0.60(0.16)	0.288	**0.023**	0.446
Triglycerides*(mg/dl)	0.98	1.04(0.57)	0.85(0.35)	**0.049**	1.11(0.51)	0.95(0.33)	0.662	0.387	0.226
Systolic blood pressure(mmHg)	112.4	112.6(8.3)	110.7(8.12)	0.672	110.7(9.5)	109.6(10.0)	0.672	0.291	0.664

Data are given as mean(sd). All subjects had a first year catch-up gain in weight of more than 0.67 SDS. They were divided in two subgroups based on rapid (>0.5SDS) or slow (<0.5SDS) catch-up in in weight in the first three months. Comparisons are adjusted for gestational age, sex, age, SES, delta length SDS in the first year. * log transformed

Relationship between period of postnatal weight gain SDS and health profile at 21 years

Associations between infant weight gain after preterm birth and determinants of CVD and DM2 in early adulthood are shown in Table 3. Adjustments were made for gestational age, sex, age, SES, and length gain SDS in the same period, to investigate the association of weight gain independently of length gain. Weight gain from birth to term age was positively associated with adult body fat percentage (R^2=0.474) and waist circumference (R^2=0.138). Similarly, weight gain in the first three months after term age had a positive association with body fat percentage (R^2=0.489), waist circumference (R^2=0.165), and in addition serum levels of total cholesterol and LDLc at 21 years. The other periods did not show significant associations, except for weight gain during 9 to 12 months after term age which was inversely associated with serum levels of total cholesterol, LDLc, and ApoB.

Influence of postnatal weight trajectories

Because of evident differences in weight trajectory before and after term age, these periods were studied separately. To study the period from birth to term age, the study population was subdivided in quartiles with regard to their gain in weight SDS from birth to term age. We could not study catch-up from birth to term age as most participants decreased in weight during this period (80.2%). Only selecting subjects with catch-up from birth to term age would result in a very small group size.

Birth to term age

Adjusted for gestational age, sex, age, SES, birth weight SDS, and length gain SDS from birth to term age, the subgroup with the highest gain in weight SDS (highest quartile), had a significantly higher body fat percentage, waist circumference, acute insulin response, and disposition index in early adulthood than the other subgroups (Table 4). In addition, they had a significantly higher ratio of trunk fat to total body fat than the subgroup with weight gain in the middle quartiles. The subjects with the lowest gain in weight SDS had a lower body fat percentage and lower HDLc at the age of 21 years than those with weight gain in the middle quartiles. At the age of 21 years, there were no significant differences in SES defined by educational level.

First year after term age

To assess if tempo of weight gain after term age was associated with determinants of CVD and DM2 in young adults born preterm, those with catch-up in weight were divided in two subgroups with rapid or slow catch-up in weight. Of all young adults with a clinically relevant catch-up in weight of at least 0.67 SDS in the first year after term age, some had a weight gain of more than 0.5 SDS in the first three months (rapid catch-up, n=55), while others had a weight gain of less than 0.5 SDS in the first three months (slow catch-up, n=36). The clinical characteristics of these two subgroups are shown in Table 1. Gestational age, sex, age, birth length SDS, birth weight SDS and

Table 3. Regression coefficients for gain in weight SDS from birth to 1 year after term age and determinants of CVD and type 2 diabetes in young adults born preterm

	Gain in weight SDS in the total preterm group (n=162)#											
	Birth - term age		Term age - 3 mo		Birth – 3mo		3 – 6 mo		6 - 9 mo		9 - 12 mo	
Outcome	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Body fat %	1.734	**0.028**$	2.385	**0.012**$	2.261	**<0.001**$	-1.130	0.393	-1.787	0.353	-0.252	0.903
Ratio trunk fat to total fat	0.005	0.260	0.004	0.497$	0.005	0.205	-0.001	0.875	-0.004	0.715	0.005	0.683
Waist circumference*(cm)	0.023	**0.028$**	0.036	**0.004**$	0.032	**<0.001**$	-0.029	0.088	-0.042	0.088	0.028	0.296
Insulin sensitivity*(µU/mL)	-0.061	0.339	0.048	0.533	-0.013	0.814	-0.150	0.157$	-0.158	0.313	0.202	0.263
Acute insulin response*(mU/L)	0.102	0.117	-0.049	0.573	0.059	0.326	-0.073	0.535	0.077	0.657	0.117	0.560
Disposition index*	0.055	0.432	-0.001	0.990	0.046	0.435	-0.223	0.055	-0.081	0.639	0.319	0.107
Total cholesterol*(mg/dl)	0.008	0.640	0.048	**0.032**	0.023	0.123	-0.042	0.189	-0.087	0.053	-0.115	**0.013**
LDLc(mg/dl)	0.040	0.563	0.176	**0.042**	0.056	0.132$	-0.114	0.358	-0.201	0.258	-0.417	**0.023**
HDLc(mg/dl)	0.023	0.466	0.068	0.069	0.050	**0.050**	-0.018	0.728	-0.110	0.153	-0.011	0.889
ApoB*(mg/dl)	0.001	0.977	0.047	0.111$	0.015	0.421	-0.053	0.210	-0.044	0.470	-0.139	**0.025**
ApoA1(mg/dl)	0.018	0.409	0.015	0.567	0.021	0.241	-0.008	0.833	-0.052	0.342	-0.045	0.443
Ratio of ApoB to ApoA1*	-0.012	0.641	0.035	0.287$	0.001	0.969	-0.045	0.328	-0.009	0.889	-0.109	0.106
Triglycerides*(mg/dl)	0.001	0.984	0.048	0.388$	0.013	0.731	-0.007	0.927	-0.187	0.096	-0.185	0.118
Systolic blood pressure(mm Hg)	0.383	0.596	0.425	0.630	0.263	0.657	-0.093	0.941	-0.883	0.623	1.268	0.509

β= unstandardized regression coefficient. # Adjusted for gestational age, sex, age, SES, gain in length SDS in the same period. * log transformed. $ p<0.05 if not corrected for gain in length SDS in the same period

SES were not different between the rapid and slow catch-up group. First-year growth patterns and SD-scores for adult height, weight, and weight SDS minus height SDS are shown in Figure 1. Both catch-up subgroups attained a similar adult height SDS, but the rapid catch-up group attained a significantly higher adult weight SDS, and weight SDS compared to height SDS than the slow catch-up group. The rapid catch-up group also had a significantly higher percentage of body fat, waist circumference, and serum triglyceride levels, and had a trend for higher total cholesterol levels after adjustment for sex, age, gestational age, SES, and length gain in the first year after term age (Table 2).

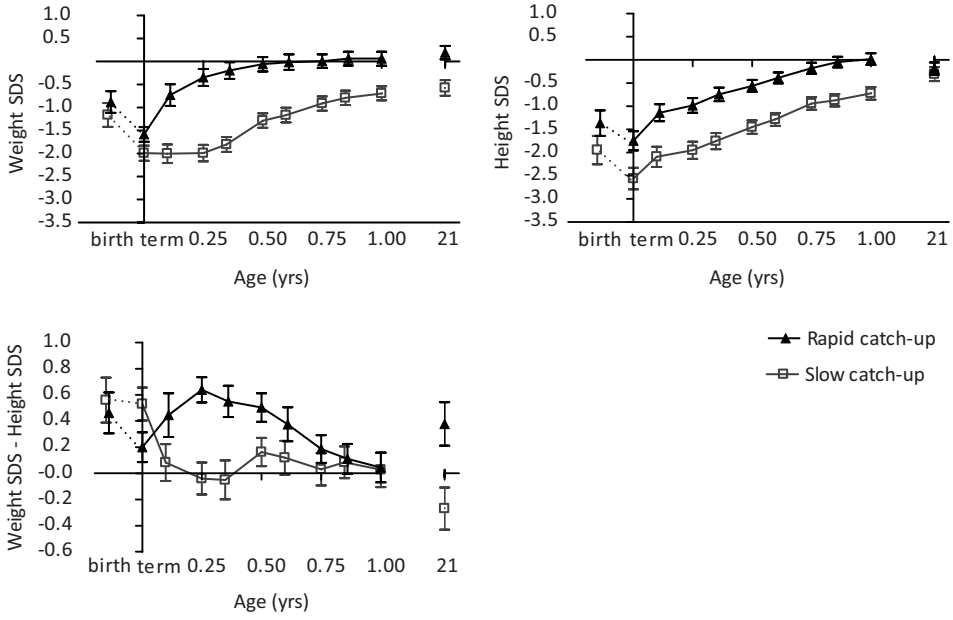

Figure 1. Weight SDS, height SDS and weight SDS relative to height SDS in the first year after term age and in early adulthood, in subjects born preterm with slow and rapid catch-up growth

To investigate whether the effect of rapid growth in subjects born preterm was similar to that in subjects born term, we compared our data with those of young adults born term (Table 1 and 2).[8] In both groups, subjects with rapid catch-up in weight after term age showed higher body fat percentage and higher waist circumference in early adulthood. However, in adults born preterm, rapid catch-up was not associated with higher ratio of trunk fat to total fat, and lower insulin sensitivity, which was the case in adults born term. Furthermore, compared to adults born preterm with rapid catch-up, those born term had lower ApoA1 levels and higher acute insulin response and ApoB/ApoA1 ratio.

Table 4. Differences in subgroups of young adults born preterm, based on weight gain SDS from birth to term age

	Gain in Weigth SDS from birth to term age					
	Lowest quartile (n=40)	2nd and 3rd quartile (n=82)	Highest quartile (n=40)	p-value Low vs. High	p-value high vs. middle	p-value low vs. middle
Determinants of CVD and DM2*						
Body fat %	21.67(17.4-25.9)	24.02(22.1-26.0)	31.30(27.6-35.0)	**0.006**	**0.001**	**0.006**
Ratio trunk fat to total fat	0.488(0.47-0.51)	0.474(0.46-0.48)	0.519(0.50-0.54)	0.093	**<0.001**	0.248
Waist circumference(cm)	71.3(67.0-75.7)	75.7(73.7-77.8)	85.7(82.0-89.4)	**<0.001**	**<0.001**	0.072
Insulin sensitivity(µU/mL)	7.87(5.07-10.68)	7.63(6.32-8.94)	6.84(4.55-9.20)	0.648	0.576	0.876
Acute insulin response(mU/L)	373(182-563)	494(405-582)	686(528-844)	**0.038**	**0.039**	0.259
Disposition index	2478(1509-3446)	2755(2304-3206)	4017(3213-4820)	**0.045**	**0.008**	0.609
Total cholesterol(mg/dl)	4.11(3.68-4.54)	4.32(4.12-4.53)	4.49(4.12-4.85)	0.272	0.444	0.379
LDLc(mg/dl)	2.67(2.29-3.05)	2.56(2.38-2.74)	2.65(2.32-2.97)	0.940	0.637	0.600
HDLc(mg/dl)	1.20(1.03-1.37)	1.39(1.31-1.47)	1.46(1.32-1.60)	0.052	0.386	**0.046**
ApoB(mg/dl)	0.82(0.70-0.93)	0.82(0.77-0.88)	0.85(0.75-0.95)	0.685	0.609	0.902
ApoA1(mg/dl)	1.38(1.26-1.50)	1.46(1.41-1.52)	1.51(1.40-1.61)	0.185	0.454	0.224
Ratio of ApoB to ApoA1	0.60(0.512-0.69)	0.57(0.53-0.61)	0.58(0.51-0.66)	0.762	0.752	0.479
Triglycerides(mg/dl)	0.90(0.62-1.18)	0.93(0.79-1.06)	1.17(0.92-1.41)	0.234	0.088	0.865
Systolic blood pressure(mmHg)	109.5(105.6-113.5)	112.9(111.0-114.8)	113.8(110.5-117.2)	0.173	0.646	0.129
Anthropometrics and SES						
Height SDS	0.30(-0.57- -0.02)	-0.33(-0.55- -0.12)	-0.73(-1.02- -0.44)	0.116	0.086	>0.999
Weight SDS	-0.29(-0.52- -0.07)	-0.46(-0.76 - -0.16)	0.11(-0.28-0.51)	0.329	**0.041**	>0.999
Weight SDS – Height SDS	0.00(-0.30-0.30)	-0.13(-0.38-0.12)	0.85(0.42-1.28)	**0.003**	**<0.001**	>0.999
SES (%) 1	10.0	11.3	17.6			
2	16.7	38.0	29.4	0.243	0.546	0.084
3	73.3	50.7	52.9			

*Estimated marginal means(95% CI)

Determinants of CVD and DM2 are corrected for gestational age, sex, age, SES, birth weight SDS, and gain in length SDS from birth to term age.

Discussion

Our study shows that accelerated gain in weight relative to length during the period from birth to term age, as well as during the first three months after term age, has adverse effects on body composition in early adulthood. Thus, the time window for effects of accelerated weight gain on body composition is wider in subjects born preterm than term. Most children born preterm have a decrease in weight SDS from birth to term age. We also demonstrate that rapid weight gain after term age in subjects born preterm is an important determinant of body composition in later life, which is in line with results in subjects born term.

The period after preterm birth up to term age was studied separately, because of the characteristic growth trajectory in that period. We showed that subjects with the highest gain in weight SDS in that period had a higher body fat percentage, waist circumference, acute insulin response (AIR) and disposition index (DI) at the age of 21 years, than those with less weight gain, while insulin sensitivity was similar. These new findings are consistent with previous findings of Singhal and co-workers, who reported higher fasting 32-33 proinsulin levels in adolescents born preterm with weight gain in the first two weeks of life, compared to those who lost weight.[11] Proinsulin levels and acute insulin response are positively associated in normoglycemic subjects.[25] Insulin is a crucial growth factor during the intrauterine as well as neonatal period,[26-27] whereas growth hormone does not have an effect on neonatal growth.[28] It might well be that subjects born preterm with the highest neonatal gain in weight relative to length have a well functioning pancreatic beta cell function, which allows easy catch-up in weight after birth, persisting into young adulthood with higher AIR and DI at the age of 21 years. It might well be that nutrition and (epi)genetic variations contribute to this mechanism.

Our data suggest that an imbalance in neonatal gain in weight compared to length after preterm birth should be avoided to reduce the risk for a less favorable body composition in later life. This may be achieved by modifying nutritional intervention according to weight for length trajectories, pointing out that both weight and length should be routinely measured during infancy. Our data are in line with previous studies,[11, 29-30] demonstrating that rapid weight gain mediated by nutrition-enriched diets have subsequent adverse effects on cardiovascular risk factors in later life. Furthermore, our data suggest that it might be beneficial to inform parents on the long term effects of accelerated gain in weight for length, as the period after discharge (usually around term age) of preterm infants seems to be a critical window for programming of later body composition.

Postnatal growth impairment in infants born preterm has been associated with adverse neurodevelopment outcome.[31] This has led to concerns with regard to cognitive function after premature birth.[32-33] We did not find evidence that catch-up in weight after preterm birth is associated with educational level at 21 years. However, our study was not designed to investigate that association. Further research is warranted to determine the optimal postnatal weight trajectory after preterm birth to guarantee optimal neurodevelopment but avoid adverse effects on later body composition.

Although our data in young adults born preterm showed a similar association of weight gain in the first three months after term age with body composition as in young adults born term, there were differences in the associations with lipoprotein levels (total cholesterol and LDLc in adults born preterm, versus HDLc, total cholesterol/HDLc ratio, and triglycerides in adults born term).[8] When investigating differences between subjects born preterm and term, early nutritional intake should be taken into account. The prevalence and duration of breastfeeding is likely altered in infants born preterm because coordination of sucking, swallowing, and respiration is not yet established.[34] Early nutrition has an effect on later lipoprotein levels,[35-36] and it might be that alterations in nutritional intake of preterm infants modify or mask the effect of early weight gain on certain lipoprotein levels. Our study did not have nutritional data to investigate differences in nutritional intake between subjects born preterm and term, and its relationship with growth in infancy and cardiovascular determinants later in life, but our results warrant further investigations.

We acknowledge that our study population consists of subjects without serious postnatal complications and did not include extreme prematurely born subjects. Whether our results can be generalized to subjects with complications, such as broncho-pulmonary dysplasia, requires further research.

In conclusion, accelerated weight gain compared to gain in length after preterm birth is associated with risk factors for CVD and DM2 in early adulthood. Furthermore, the critical window it is not only fixed to the period immediately after birth, but also to the period during the first three months after term age, as accelerated weight gain during both periods affects body composition at 21 years. Our findings point to the need for new data to investigate the optimal target of postnatal weight gain after preterm birth, with regard to neurodevelopment as well as health profile in adulthood. This could lead to public-health interventions based on recommendations for infant feeding after preterm birth, thereby decreasing the risk for development of cardiovascular disease and obesity in later life.

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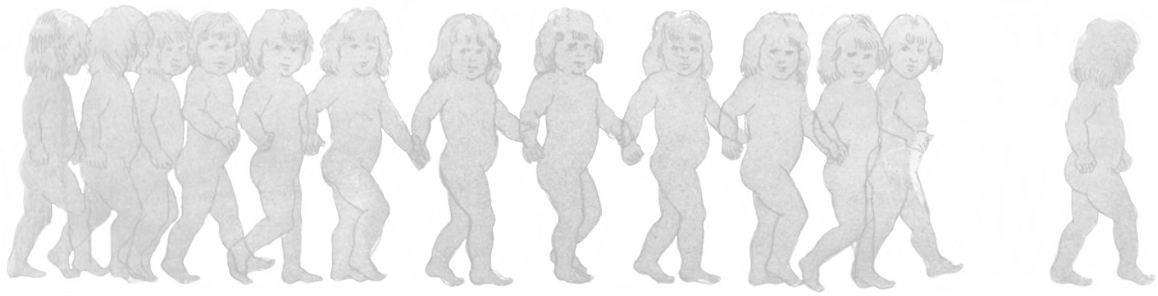
References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* Jan 5 2008;371(9606):75-84.
2. Evensen KA, Steinshamn S, Tjonna AE, et al. Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early Hum Dev.* Apr 2009;85(4):239-245.
3. Thomas EL, Parkinson JR, Hyde MJ, et al. Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. *Pediatr Res.* Nov 2011;70(5):507-512.
4. Crump C, Sundquist K, Sundquist J, Winkleby MA. Gestational age at birth and mortality in young adulthood. *JAMA.* Sep 21 2011;306(11):1233-1240.
5. Euser AM, de Wit CC, Finken MJ, Rijken M, Wit JM. Growth of preterm born children. *Horm Res.* 2008;70(6):319-328.
6. Kytnarova J, Zlatohlavkova B, Kubena A, et al. Post-natal growth of 157 children born as extremely premature neonates. *J Paediatr Child Health.* Mar 2011;47(3):111-116.
7. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics.* May 2003;111(5 Pt 1):986-990.
8. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* Jun 3 2009;301(21):2234-2242.
9. Ekelund U, Ong KK, Linne Y, et al. Association of weight gain in infancy and early childhood with metabolic risk in young adults. *J Clin Endocrinol Metab.* Jan 2007;92(1):98-103.
10. Slining MM, Kuzawa CW, Mayer-Davis EJ, Adair LS. Evaluating the indirect effect of infant weight velocity on insulin resistance in young adulthood: a birth cohort study from the Philippines. *Am J Epidemiol.* Mar 15 2011;173(6):640-648.
11. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet.* Mar 29 2003;361(9363):1089-1097.
12. Fewtrell MS, Doherty C, Cole TJ, Stafford M, Hales CN, Lucas A. Effects of size at birth, gestational age and early growth in preterm infants on glucose and insulin concentrations at 9-12 years. *Diabetologia.* Jun 2000;43(6):714-717.
13. Rotteveel J, van Weissenbruch MM, Twisk JW, Delemarre-Van de Waal HA. Infant and childhood growth patterns, insulin sensitivity, and blood pressure in prematurely born young adults. *Pediatrics.* Aug 2008;122(2):313-321.
14. Willemsen RH, Leunissen RW, Stijnen T, Hokken-Koelega AC. Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab.* May 2009;94(5):1695-1700.
15. *Dutch Standard Classification of Education, 2006*: Centraal Bureau van de Statistiek, Statistics Netherlands.
16. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P. The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res.* Dec 2004;12(12):1925-1929.
17. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed.* Oct 1986;23(2):113-122.
18. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest.* Dec 1981;68(6):1456-1467.
19. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther.* 2003;5(6):1003-1015.
20. Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes.* Dec 1989;38(12):1512-1527.

21. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC. Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. *J Clin Endocrinol Metab.* Nov 2008;93(11):4307-4314.
22. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* Jun 1969;74(6):901-910.
23. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* Mar 2000;47(3):316-323.
24. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ.* Apr 8 2000;320(7240):967-971.
25. Mykkanen L, Haffner SM, Hales CN, Ronnemaa T, Laakso M. The relation of proinsulin, insulin, and proinsulin-to-insulin ratio to insulin sensitivity and acute insulin response in normoglycemic subjects. *Diabetes.* Dec 1997;46(12):1990-1995.
26. Fowden AL. The role of insulin in fetal growth. *Early Hum Dev.* Jun-Jul 1992;29(1-3):177-181.
27. Menon RK, Sperling MA. Insulin as a growth factor. *Endocrinol Metab Clin North Am.* Sep 1996;25(3):633-647.
28. Huysman MW, Hop WC, Cromme-Dijkhuis AH, Sauer PJ, Hokken-Koelega AC. A randomized, placebo-controlled GH trial in very preterm infants who were at risk for bronchopulmonary dysplasia and were treated with dexamethasone. *Pediatr Res.* Oct 2005;58(4):705-712.
29. Fewtrell MS, Morley R, Abbott RA, et al. Catch-up growth in small-for-gestational-age term infants: a randomized trial. *Am J Clin Nutr.* Oct 2001;74(4):516-523.
30. Singhal A, Cole TJ, Fewtrell M, et al. Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation.* Jan 16 2007;115(2):213-220.
31. Fanaro S. Which is the ideal target for preterm growth? *Minerva Pediatr.* Jun 2010;62(3 Suppl 1):77-82.
32. Yeung MY. Postnatal growth, neurodevelopment and altered adiposity after preterm birth--from a clinical nutrition perspective. *Acta Paediatr.* Aug 2006;95(8):909-917.
33. Morsing E, Asard M, Ley D, Stjernqvist K, Marsal K. Cognitive function after intrauterine growth restriction and very preterm birth. *Pediatrics.* Apr 2011;127(4):e874-882.
34. Mizuno K, Ueda A. The maturation and coordination of sucking, swallowing, and respiration in preterm infants. *J Pediatr.* Jan 2003;142(1):36-40.
35. Ravelli AC, van der Meulen JH, Osmond C, Barker DJ, Bleker OP. Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. *Arch Dis Child.* Mar 2000;82(3):248-252.
36. Owen CG, Whincup PH, Odoki K, Gilg JA, Cook DG. Infant feeding and blood cholesterol: a study in adolescents and a systematic review. *Pediatrics.* Sep 2002;110(3):597-608.

Chapter 4



Early Origins of the Metabolic Syndrome: Role of Small Size at Birth, Early Postnatal Weight Gain and Adult IGF-I

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Abstract

Background: The relationship between low birth weight and increased risk for Metabolic Syndrome (MetS) in later life has been frequently described, but mechanisms underlying this association remain unknown.

Methods: In 280 young adults of the PROGRAM study, aged 18-24 yr, we investigated associations of birth weight, gain in weight for length during early life, and adult Insulin like Growth Factor-I (IGF-I) SDS, with number of MetS components (ordinal regression analyses), prevalence of MetS components and MetS (logistic regression analyses), and other metabolic parameters (linear regression analyses). Revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III) were used to determine components of MetS. The other metabolic parameters were C-reactive protein (CRP), insulin sensitivity, trunk fat mass, total cholesterol, and LDL cholesterol.

Results: More gain in weight for length SDS in the first three months of life was significantly associated with an increased number of MetS components (Odds ratio: 1.34), prevalence of low HDLc (Odds ratio: 1.49), prevalence of MetS (Odds ratio: 2.51), increased CRP levels and lower insulin sensitivity (p=0.007) at the age of 21 years. Low birth weight SDS was associated with lower insulin sensitivity (p=0.036), but low birth weight SDS and adult IGF-I SDS were not significantly associated with any of the MetS components, or MetS prevalence at 21 years.

Conclusion: Our study demonstrates that higher gain in weight for length in the first three months of life is associated with a higher prevalence of MetS at 21 years, whereas low birth weight and low adult IGF-I are not.

Introduction

The relationship between low birth weight and increased risk for metabolic syndrome (MetS) in later life has been frequently described.[1-3] Adults born small for gestational age (SGA) have lower insulin sensitivity, higher abdominal fat mass, and lipoprotein levels are more often disturbed, all contributing to a higher prevalence of MetS.[4] The majority of children born SGA shows catch-up growth within two years after birth, resulting in a normal stature in childhood and adulthood.[5] Because accelerated gain in weight for length in early life has been associated with adverse health profile in adulthood,[6-9] it might well be that the association between small size at birth and MetS in later life can be ascribed to accelerated early weight gain.

Another factor contributing to the relationship between SGA birth and later risk for MetS might be insulin like growth factor-I (IGF-I). Decreased serum levels of IGF-I have been reported in adults born SGA.[10] Furthermore, low IGF-I levels have been associated with each of the components of MetS (waist circumference, triglycerides (TG), HDL cholesterol (HDLc), blood pressure, and fasting glucose[11]).[12]

Our aim was to unravel mechanisms involved in the association between small size at birth and components of MetS in early adulthood. We hypothesized that, in contrast to lower weight at birth, accelerated early weight gain for length and/or lower IGF-I levels are associated with an increased risk for MetS. We therefore investigated associations of birth weight, first year gain in weight for length, and serum IGF-I levels with MetS components according to revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III)[11]. MetS criteria were defined for use in clinical practice, however, additional metabolic parameters are also relevant to study with regard to cardiovascular disease risk, such as insulin sensitivity determined by Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test, and trunk fat mass determined by DXA scan.

Study design

Subjects

The PROGRAM study cohort consists of 323 healthy participants with an age between 18 and 24 years. Participants were recruited from hospitals in the Netherlands, where they had been registered because of being small at birth (SGA with a birth length <-2SD (n=102))[13] or showing short stature (with an adult height <-2SD after being born SGA (n=42 of 102) or appropriate for gestational age (n=40)).[14] In addition, healthy subjects (neither small at birth nor having short stature) from schools with different educational levels were randomly asked to participate. This design was purposely chosen to increase the contrast within the study population regarding birth size and adult stature. All participants fulfilled the same inclusion criteria: 1) age 18-24 years, 2) born singleton, 3) born at term (≥36 weeks of gestational age), 4) Caucasian, 5) uncomplicated

neonatal period without signs of severe asphyxia (defined as an Apgar score below three after five minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia, 6) maximum duration of respiratory ventilation and/or oxygen supply in the neonatal period of two weeks. Subjects were excluded if they had been suffering from any serious complication or condition (including necrotizing enterocolitis, intra-ventricular hemorrhage with a degree of three or more, spastic hemiplegia or quadriplegia), from any disease or had received any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome.

Birth data were taken from records of hospitals, community health services and general practitioners. Information regarding socioeconomic status (SES) was obtained using questionnaires. Education level of the participant was used as socioeconomic indicator to determine SES.[15] In the Netherlands, periodical measurements of weight and length are performed for each child. Thus, weight and length at 3, 6, 9, and 12 months after birth had been measured prospectively at primary health care centers or hospitals. These data were collected from the records of the health care centers and hospitals during the study period March 2006-September 2007.

The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam The Netherlands, approved the study. Written informed consent was obtained from all participants. Of 323 subjects data on each component of the metabolic syndrome were available for 280 subjects (of whom n=87 were born SGA, and n=70 had short stature). Of these 280 subjects, data on first-three month's growth were available for 184 subjects.

Measurements

All participants visited the Erasmus Medical Centre in Rotterdam and were reimbursed for travel expenses. Prior to the visit, participants fasted for at least 12 hours and abstained from smoking and drinking alcohol for at least 16 hours. All anthropometric measurements were performed twice; the mean value was used for analyses.

Height was measured to the nearest 0.1cm by a Harpenden stadiometer and weight to the nearest 0.1kg by a scale (Servo Balance KA-20-150S). Lean body mass and fat mass were measured on one Dual-Energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, England).[16] Insulin sensitivity index (capacity of insulin to promote glucose disposal) was determined using the Bergman minimal model (MINMOD Millenium version 6.01, MINMOD Inc, Los Angeles, California), which calculated the paired glucose and insulin data obtained by frequent measurements during an intravenous glucose tolerance test[17-19] with Tolbutamide.[20] Blood pressure was measured after 10 minutes at rest, in the sitting position, using the non-dominant arm with an automatic device (Accutorr Plus, Datascope Corp, Montvale, New Jersey)

three times with five minutes in between, and the mean value was taken to reflect resting blood pressure.

Assays

All fasting blood samples were drawn between 08.00-13.00 h, centrifuged after clotting, and were kept frozen until assayed (-80°C). Briefly, plasma glucose levels were determined on a VITROS analyzer 750 (Ortho-Clinical Diagnostics, Johnson & Johnson Company, Beerse, Belgium). Plasma insulin levels were measured using an immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). TG was measured using an automated enzymatic method with the GPO-PAP reagent kit (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein cholesterol (HDLc) level was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics). Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula: LDLc = total cholesterol – HDLc – 0.45 x triglycerides. For hsCRP an in-house high-sensitivity ELISA with polyclonal rat CRP antibodies for catching and tagging (DAKO, Denmark) was used. Serum IGF-I and IGFBP-3 levels were measured in one laboratory using an automated chemiluminescence immunometric assay (Immulite-1000systems, Siemens Healthcare Diagnostics, Tarrytown, NY). Serum levels were expressed as SDS to adjust for age and sex using reference data from a healthy Dutch population.[21] The assays have been previously described in detail.[22]

Statistical analysis

SD-scores for birth length, birth weight, and first year weight and length were calculated to correct for gestational age and sex.[13] SD-scores for adult height and weight were calculated to correct for sex and age.[14] Revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III) were used to determine components of MetS.[11,23] MetS was defined as having three or more of the following risk factors:

- Abdominal obesity (waist circumference): Men >102 cm, women >88 cm
- Triglycerides: ≥1.7 mmol/L
- HDLc: Men ≤1.03, women ≤1.3 (mmol/L)
- Blood pressure: ≥130/ ≥85 mm Hg
- Fasting glucose: ≥5.6 mmol/L

Ordinal regression analyses were performed to determine associations of birth weight SDS, weight gain during the four three-month periods in the first year of life, and IGF-I SDS, with the number of MetS components per individual. All regression analyses were adjusted for age, gender, gestational age, and SES. We additionally adjusted for smoking and alcohol use (after removing SES), which did not change our results (data not shown). Weight gain analyses were additionally adjusted for gain in length SDS in the same period, and IGF-I analyses were additionally adjusted for IGF-BP3. We additionally tested whether adult height SDS was a significant confounder in the

associations studied, because IGF-I is related to adult height and subjects with short stature were oversampled in the study population.

Next, logistic regression analyses were performed to investigate associations between birth weight SDS, gain in weight for length in the first three months of life, and IGF-I SDS with prevalence of each of the components of MetS and prevalence of MetS (three or more of the components).

Finally, to identify associations of birth weight SDS, gain in weight for length in the first three months of life, and IGF-I SDS with other metabolic parameters, we performed linear regression analyses with the dependent variables C-reactive protein, insulin sensitivity, trunk fat mass, total cholesterol, and LDLc.

Statistical package SPSS version 17.0 (SPSS, Inc., Chicago, IL) was used for analyses. Results were regarded statistically significant if p was <0.05.

Results

Clinical characteristics, components of the MetS and additional metabolic parameters are shown in Table 1. (MetS, according to the revised NCEP criteria, was present in 5.4% of all participants.

Table 2 shows results of ordinal regression analyses. Gain in weight SDS in the first three months of life was associated with an increased number of MetS components at the age of 21 years (odds ratio (OR): 1.34, 95% confidence interval (CI): 1.01-1.78), adjusted for age, gender, gestational age, SES and gain in length in the same period. Thus, per one SDS increase in weight gain, the chance of having a higher number of MetS components increases with 34%. There were no significant associations of birth weight SDS, weight gain during the other three-month periods in the first year of life, or serum IGF-I SDS at 21 years with number of MetS components. Subjects with short stature were oversampled in the study population. We, therefore, additionally adjusted for adult height SDS. After adjustment for adult height SDS, results were similar and adult height SDS itself was no significant determinant of the number of MetS components. Gain in weight relative to length in the first three months of life was significantly associated with a higher prevalence of low HDLc (OR: 1.49, 95%CI: 1.06-2.08), and prevalence of MetS (OR: 2.51, 95%CI: 1.20-5.25). When we additionally adjusted for adult height SDS in the analyses including birth weight and early weight gain, results were similar. Furthermore, adult height SDS itself was no significant determinant.

Table 1. Clinical characteristics and components of the metabolic syndrome in the study group

Clinical characteristics	N	Mean(SD)	MetS components	N	Mean(SD)
Age	280	20.9(1.6)	Waist (cm)	280	77.2(9.98)
Gender (M/F)	280	112/168	% high*	280	8.6
Gestational age	280	39.2(1.7)	TG (mmol/L)	280	1.03(0.51)
Birth length SDS	280	-1.48(1.5)	% high*	280	10.4
Birth weight SDS	280	-1.18(1.4)	HDLc (mmol/L)	280	1.37(0.37)
3 months length SDS	183	-1.13(1.25)	% low*	280	34.6
6 months length SDS	182	-1.03(1.20)	Systolic BP (mmHG)	280	119.6(11.5)
9 months length SDS	181	-1.00(1.27)	% high*	280	17.1
12 months length SDS	179	-1.02(1.21)	Diastolic BP (mmHG)	280	74.1(7.62)
3 months weight SDS	184	-0.96(1.17)	% high*	280	8.2
6 months weight SDS	183	-0.91(1.09)	Glucose (mmol/L)	280	4.91(0.47)
9 months weight SDS	181	-0.88(1.13)	% high*	280	7.9
12 months weight SDS	175	-0.79(1.08)			
Adult height SDS	280	-1.05(1.37)	**Metabolic syndrome (%)***	280	5.4
Adult weight SDS	280	-0.60(1.42)			
IGF-I SDS	280	-0.32(0.83)	**Additional metabolic parameters**		
IGF-BP3 SDS	280	-1.03(0.68)	C-reactive protein (mg/L)	273	3.79 (6.6)
			Insulin sensitivity (µU/ml)	130	6.82 (4.4)
			Trunk fat (kg)	273	7.65 (4.7)
			Total cholesterol (mmol/L)	279	4.54 (0.9)
			LDLc (mmol/L)	279	2.70 (0.8)

SDS: Standard deviation score, IGF: Insulin like growth factor, waist: waist circumference, TG: triglycerides, HDLc: HDL-cholesterol, BP: blood pressure, LDLc: LDL-cholesterol. *Based on revised criteria of the National Cholesterol Educational Program.

Table 2. Associations of birth size, first year gain in weight for length SDS and adult IGF-I SDS, with the number of MetS components per individual

	Number of components MetS	
	Odds ratio	**95% CI**
Birth weight SDS[1]	1.09	0.91-1.30
Gain in weight SDS		
0-3 months[2]	**1.34**	**1.01-1.78**
3-6 months[2]	1.10	0.64-1.89
6-9 months[2]	0.80	0.37-1.73
9-12 months[2]	0.47	0.19-1.13
IGF-I SDS[3]	1.06	0.78-1.45

CI: confidence interval. [1]Adjusted for age, gender, gestational age, SES. [2]Adjusted for age, gender, gestational age, SES, and gain in length SDS in the same period. [3]Adjusted for age, gender, gestational age, SES, and IGF-BP3 SDS.

Table 3. Associations of birth size, early weight gain, IGF-I and IGF-BP3, with MetS components and prevalence of MetS

		High Waist		High TG		Low HDLc		High BP		High Glucose		MetS	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Model 1	Birth weight SDS	0.760	0.439-1.316	0.933	0.590-1.477	1.091	0.810-1.470	0.995	0.702-1.410	1.269	0.753-2.139	0.492	0.224-1.080
	Birth length SDS	1.124	0.714-1.767	0.972	0.643-1.469	0.945	0.719-1.242	1.097	0.790-1.524	0.743	0.445-1.242	0.795	0.600-1.948
Model 2	Delta weight 0-3mo	1.618	0.921-2.841	1.336	0.794-2.248	**1.485**	**1.060-2.080**	1.022	0.715-1.459	0.911	0.517-1.607	**2.509**	**1.200-5.247**
	Delta length 0-3mo	0.998	0.533-1.867	1.022	0.569-1.835	0.843	0.572-1.241	0.952	0.615-1.473	1.366	0.675-2.764	0.987	0.432-2.255
Model 3	Adult IGF-I SDS	0.656	0.373-1.151	0.664	0.395-1.116	1.069	0.752-1.520	1.476	0.930-2.341	1.820	0.861-3.849	0.857	0.409-1.795

OR: Odds ratio, CI: confidence interval, waist: waist circumference, TG: triglycerides, HDLc: HDL-cholesterol, BP: blood pressure, delta weight 0-3 mo: Gain in weight SDS from birth until three months of age, delta length 0-3 mo: Gain in length SDS from birth until three months of age
Model 1: Birth weight SDS and birth length SDS are entered simultaneously
Model 2: Delta weight 0-3 mo and delta length 0-3 mo are entered simultaneously
All analyses are additionally adjusted for gender, age, SES, and gestational age
IGF-I SDS analyses are additionally adjusted for IGF-BP3

Table 4. Associations of birth size, early weight gain, IGF-I and IGF-BP3, with MetS related parameters

		CRP			Si			Trunk fat mass			Chol			LDLc		
		β (%)	p	Adj. R^2	β (%)	p	Adj. R^2	β (%)	p	Adj. R^2	β (%)	p	Adj. R^2	β (%)	p	Adj. R^2
Model 1	Birth weight SDS	-5.32	0.159	0.184	16.8	**0.036**	0.055	-5.32	0.181	0.114	-0.69	0.607	0.152	-1.61	0.430	0.057
	Birth length SDS	2.32	0.625		-4.73	0.502		2.32	0.540		-1.25	0.310		-1.38	0.463	
Model 2	Delta weight 0-3mo	30.9	**0.009**	0.216	-21.0	**0.007**	0.072	8.11	0.064	0.153	-0.28	0.844	0.150	1.54	0.494	0.049
	Delta length 0-3mo	-0.16	0.989		19.2	0.085		-0.08	0.986		2.96	0.085		1.20	0.655	
Model 3	Adult IGF-I SDS	-16.6	0.133	0.188	-8.71	0.262	0.047	-2.97	0.538	0.107	1.80	0.269	0.153	3.96	0.115	0.051

β: regression coefficient in %, p: p-value, CRP: c-reactive protein, Si: insulin sensitivity, Chol: total cholesterol, delta weight 0-3 mo: Gain in weight SDS from birth until three months of age, delta length 0-3 mo: Gain in length SDS from birth until three months of age
Model 1: Birth weight SDS and birth length SDS are entered simultaneously
Model 2: Delta weight 0-3 mo and delta length 0-3 mo are entered simultaneously
All analyses are additionally adjusted for gender, age, SES, and gestational age
IGF-I SDS analyses are additionally adjusted for IGF-BP3

There was no significant association of IGF-I SDS with any of the MetS components, also after adjustment for IGF-BP3 SDS. However, after additional adjustment for adult height SDS, we found a borderline significant positive association (OR: 2.013, 95%CI: 0.950-4.264, p-value: 0.068). In that model, adult height SDS itself was inversely associated with prevalence of increased fasting glucose levels (OR: 0.643, 95%CI: 0.427-0.967, p-value: 0.034).

In Table 4, results from linear regression analyses are shown with additional metabolic parameters as dependent variables, namely C-reactive protein (CRP), insulin sensitivity (Si), trunk fat mass, total cholesterol, and LDLc. Lower birth weight SDS (16.8% lower Si, per SDS decrease, p=0.036) as well as accelerated gain in weight SDS in the first three months of life (21% lower Si, per SDS increase in weight gain, p=0.007), showed significant associations with lower insulin sensitivity at 21 years. There was no association of birth weight SDS with CRP, trunk fat mass, total cholesterol, or LDLc.

Accelerated gain in weight SDS in the first three months of life was associated with higher levels of CRP (30.9% higher CRP, per SDS increase in weight gain, p=0.009) and was borderline significantly associated with trunk fat mass (8.11% higher trunk fat mass, per SDS increase in weight gain, p=0.064), adjusted for gender, age, SES, and gestational age. IGF-I was not associated with any of the additional metabolic parameters. Additional adjustment for adult height SDS did not change the results.

Discussion

In this study we investigated mechanisms involved in the reported association of small size at birth with metabolic syndrome (MetS) in later life. We studied associations of low birth weight, early life gain in weight for length, and adult serum levels of IGF-I, with components of MetS, prevalence of MetS (having three or more of the components), and several other metabolic parameters. Our results imply that gain in weight relative to length SDS in the first three months of life is the most important determinant of MetS, as that was associated with a higher number of MetS components at the age of 21 years, in contrast with birth weight SDS, weight gain during the other three-month periods in the first year of life, and adult IGF-I levels. Furthermore, gain in weight relative to length SDS in the first three months of life was associated with increased prevalence of MetS, higher prevalence of low HDLc, increased levels of CRP, and lower insulin sensitivity in early adulthood.

To our knowledge, this is the first study investigating the relationship of small size at birth, accelerated weight gain in early life, and adult IGF-I SDS with MetS and several other metabolic parameters in early adulthood. The association of small size at birth with MetS has been frequently described, but often postnatal weight gain and other factors associated with SGA birth, such as serum level of IGF-I, have not been taken into account. Beardsall et al. previously reported that postnatal weight gain from birth, rather than birth weight, was associated with risk factors for

metabolic diseases in childhood.[24] Our study shows that more gain in weight than length SDS in the first three months of life is related to prevalence of the MetS and low birth weight SDS is not. Furthermore, low birth weight SDS was not related with any of the components of MetS and other metabolic parameters, except for an association with lower insulin sensitivity. This association is, however, possibly due to catch-up growth after being born small, as increased gain in weight for length from birth to three months of age, which often follows small size at birth, was also more strongly associated with decreased insulin sensitivity. We previously showed that subjects born small for gestational age with catch-up growth have lower insulin sensitivity which was not found in those without catch-up growth.[25]

Increased gain in weight for length from birth to three months of age was also associated with one of the components of MetS, namely with prevalence of decreased HDLc levels. Furthermore, it was associated with increased levels of CRP, which has been previously associated with both MetS and low HDLc.[26] Our findings are in line with previous studies[6,27] and suggest that an imbalance in neonatal gain in weight compared to length after birth should be avoided to reduce the risk for MetS in later life. Animal studies showed that early life catch-up in weight is associated with skeletal muscle insulin resistance and adipose tissue insulin hyperresponsiveness accompanied by suppressed thermogenesis.[28-29] The authors hypothesized that this phenomenon exists for the purpose of sparing glucose in order to catch-up in fat, which might be the link between early life accelerated weight gain and risk for later MetS.[28]

The term metabolic syndrome refers to clustering of risk factors, being a pathophysiological condition underlying CVD and type 2 diabetes.[30] Our study population consists of healthy young adults, and it was not possible to study hard endpoints such as CVD and type 2 diabetes. Therefore, we used MetS as an outcome variable. One limitation of this approach is the arbitrary character of the MetS definition and the subsequent possibility to miss critical information, which has been debated.[30-31] We, therefore, decided to also study the components of MetS separately, and in addition to investigate CRP, insulin sensitivity, trunk fat mass, total cholesterol, and LDLc, which are also determinants of CVD.[32-35]

Previous studies reported an association between IGF-I and components of the MetS.[12,36-37] We, however, did not find significant associations of adult IGF-I SDS with any of the components or prevalence of MetS. We also did not find a significant association between IGF-I and insulin sensitivity. It might, however, still be that lower IGF-I levels affect the risk for developing MetS, but that we could not prove this because the variation in IGF-I levels was not large enough in our study population of health young adults. Although we oversampled subjects with short stature, who are likely to have lower levels of IGF-I, we did not include subjects with known growth hormone deficiency.

Adults with short stature have a higher a priori chance of having a smaller waist circumference than normal statured subjects. The NCEP criteria of the MetS do not take this into account, as waist circumference is defined as one of the MetS components. Therefore, in the present study we also investigated associations of birth size, early weight gain and adult IGF-I with MetS

after adjustment for adult height SDS. Shorter stature was associated with a higher prevalence of increased fasting glucose levels, when adjusted for IGF-I and IGF-BP3. An explanation of this finding might be the adverse effect of low/subnormal growth hormone levels on glucose homeostasis,[38-39] assuming that the subjects with short stature in our study population have lower levels of growth hormone than those with normal stature.

In conclusion, our study indicates that the reported association of small size at birth with MetS in adulthood is mainly due to an increased gain in weight for length in the first three months after birth. Although our results show the importance of balanced weight gain during the early postnatal period, we recognize that there might be other critical windows later in childhood that remained unstudied in the present study. Our findings point to the need to investigate the optimal target of postnatal weight gain after birth in all infants, regardless whether they are born SGA or AGA.

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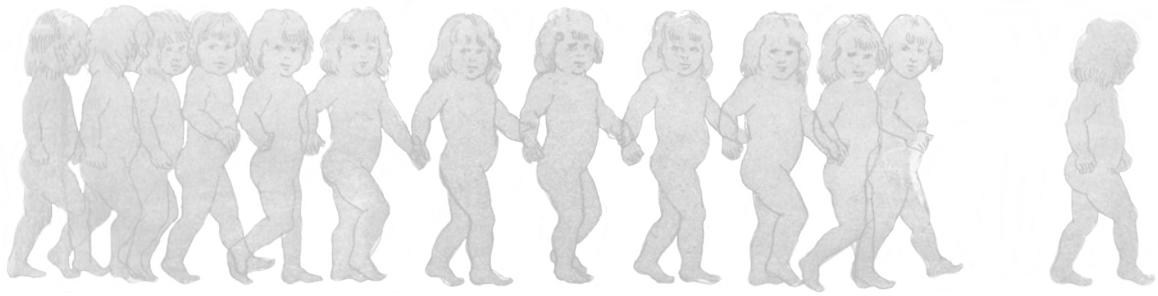
References

1. Levy-Marchal C, Czernichow P 2006 Small for gestational age and the metabolic syndrome: which mechanism is suggested by epidemiological and clinical studies? *Horm Res 65 Suppl* 3:123-130
2. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-67
3. Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP 1994 Birthweight and adult health outcomes in a biethnic population in the USA. *Diabetologia* 37:624-631
4. Meas T, Deghmoun S, Alberti C, Carreira E, Armoogum P, Chevenne D, Levy-Marchal C 2010 Independent effects of weight gain and fetal programming on metabolic complications in adults born small for gestational age. *Diabetologia* 53:907-913
5. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? *Pediatr Res* 38:267-271
6. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A 2009 Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. JAMA 301:2234-2242
7. Singhal A, Lucas A 2004 Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet* 363:1642-1645
8. Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A 2007 Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation* 115:213-220
9. Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A 2004 Is slower early growth beneficial for long-term cardiovascular health? *Circulation* 109:1108-1113
10. Verkauskiene R, Jaquet D, Deghmoun S, Chevenne D, Czernichow P, Levy-Marchal C 2005 Smallness for gestational age is associated with persistent change in insulin-like growth factor I (IGF-I) and the ratio of IGF-I/IGF-binding protein-3 in adulthood. *J Clin Endocrinol Metab* 90:5672-5676
11. Grundy SM, Cleeman JI, Merz CN, Brewer HB, Jr., Clark LT, Hunninghake DB, Pasternak RC, Smith SC, Jr., Stone NJ, National Heart L, Blood I, American College of Cardiology F, American Heart A 2004 Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* 110:227-239
12. Saydah S, Ballard-Barbash R, Potischman N 2009 Association of metabolic syndrome with insulin-like growth factors among adults in the US. *Cancer Causes Control* 20:1309-1316
13. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-910
14. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-323
15. 2006 Dutch standard classification of education. Heerlen, The Netherlands: Centraal Bureau van de Statistiek, Statistics Netherlands
16. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P 2004 The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res* 12:1925-1929
17. Pacini G, Bergman RN 1986 MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 23:113-122
18. Bergman RN, Phillips LS, Cobelli C 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* 68:1456-1467

19. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN 2003 MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther* 5:1003-1015
20. Bergman RN 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 38:1512-1527
21. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 50:166-176
22. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC 2008 Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. *J Clin Endocrinol Metab* 93:4307-4314
23. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F, American Heart A, National Heart L, Blood I 2005 Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 112:2735-2752
24. Beardsall K, Ong KK, Murphy N, Ahmed ML, Zhao JH, Peeters MW, Dunger DB 2009 Heritability of childhood weight gain from birth and risk markers for adult metabolic disease in prepubertal twins. *J Clin Endocrinol Metab* 94:3708-3713
25. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC 2008 Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab* 93:445-451
26. Rein P, Saely CH, Beer S, Vonbank A, Drexel H 2010 Roles of the metabolic syndrome, HDL cholesterol, and coronary atherosclerosis in subclinical inflammation. *Diabetes Care* 33:1853-1855
27. Singhal A 2006 Early nutrition and long-term cardiovascular health. *Nutr Rev* 64:S44-49; discussion S72-91
28. Cettour-Rose P, Samec S, Russell AP, Summermatter S, Mainieri D, Carrillo-Theander C, Montani JP, Seydoux J, Rohner-Jeanrenaud F, Dulloo AG 2005 Redistribution of glucose from skeletal muscle to adipose tissue during catch-up fat: a link between catch-up growth and later metabolic syndrome. *Diabetes* 54:751-756
29. Crescenzo R, Samec S, Antic V, Rohner-Jeanrenaud F, Seydoux J, Montani JP, Dulloo AG 2003 A role for suppressed thermogenesis favoring catch-up fat in the pathophysiology of catch-up growth. *Diabetes* 52:1090-1097
30. Kahn R, Buse J, Ferrannini E, Stern M 2005 The metabolic syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia* 48:1684-1699
31. Reaven GM 2011 The metabolic syndrome: time to get off the merry-go-round? *J Intern Med* 269:127-136
32. Jeppesen J, Hansen TW, Olsen MH, Rasmussen S, Ibsen H, Torp-Pedersen C, Hildebrandt PR, Madsbad S 2008 C-reactive protein, insulin resistance and risk of cardiovascular disease: a population-based study. *Eur J Cardiovasc Prev Rehabil* 15:594-598
33. Despres JP, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C 1990 Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis* 10:497-511
34. Libby P 2002 Inflammation in atherosclerosis. *Nature* 420:868-874
35. Ballantyne CM, Hoogeveen RC 2003 Role of lipid and lipoprotein profiles in risk assessment and therapy. *Am Heart J* 146:227-233
36. Lam CS, Chen MH, Lacey SM, Yang Q, Sullivan LM, Xanthakis V, Safa R, Smith HM, Peng X, Sawyer DB, Vasan RS 2010 Circulating insulin-like growth factor-1 and its binding protein-3: metabolic and genetic correlates in the community. *Arterioscler Thromb Vasc Biol* 30:1479-1484
37. Parekh N, Roberts CB, Vadiveloo M, Puvananayagam T, Albu JB, Lu-Yao GL 2010 Lifestyle, anthropometric, and obesity-related physiologic determinants of insulin-like growth factor-1 in the Third National Health and Nutrition Examination Survey (1988-1994). *Ann Epidemiol* 20:182-193

38. Oliveira CR, Salvatori R, Barreto-Filho JA, Rocha IE, Mari A, Pereira RM, Campos VC, Menezes M, Gomes E, Meneguz-Moreno RA, Araujo VP, Leite NT, Nascimento-Junior AC, Farias MI, Viscente TA, Araujo RD, Melo EV, Aguiar-Oliveira MH 2011 Insulin Sensitivity and beta-Cell Function in Adults with Lifetime, Untreated Isolated Growth Hormone Deficiency. *J Clin Endocrinol Metab*
39. Yuen KC, Dunger DB 2007 Therapeutic aspects of growth hormone and insulin-like growth factor-I treatment on visceral fat and insulin sensitivity in adults. *Diabetes Obes Metab* 9:11-22

Chapter 5



Biomarkers of Early Stage Atherosclerosis in Young Adults: Effects of Small Size at Birth, Prematurity, and Adult Body Size

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Abstract

Background: Small birth size, either due to prematurity or fetal growth restriction, has been associated with increased risk for atherosclerosis in later life. There are many biomarkers known to date which relate to preclinical stages of atherosclerosis, such as acute-phase proteins, pro-inflammatory cytokines, and lipoproteins.

Objective: To investigate associations between small birth size, prematurity, adult body size and a broad range of biomarkers related to early stage atherosclerosis

Methods: In 474 healthy participants of the PREMS/PROGRAM study, 18-24 years of age, we determined serum levels of high-sensitivity C-reactive protein (hsCRP), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), soluble vascular adhesion molecule 1 (sVCAM-1), soluble intracellular adhesion molecule (sICAM-1), and several lipoproteins. Principal component analysis was applied to identify combinations of biomarkers and their component-scores. Regression analysis was used to study the effect of birth size, gestational age, and adult body size on the principal components. Also, the effect of components on insulin sensitivity, systolic blood pressure and carotid intima media thickness was investigated.

Results: Three principal components of biomarkers were identified. Birth size and gestational age, were not associated with any of the components. Adult fat mass was positively associated with component Adverse Lipids and hsCRP, and component Inflammatory Markers, but not associated with component HDLc and ApoA1. Short stature was associated with higher scores for component Adverse Lipids and hsCRP.

Conclusion: Higher fat mass and short stature in early adulthood are independently associated with combined biomarkers related to early stage atherosclerosis, also after adjustment for confounders, whereas small birth size and prematurity are not.

Introduction

Low birth weight has been associated with risk factors for cardiovascular diseases (CVD) in later life.[1-2] Furthermore, several studies demonstrated that subsequent catch-up in weight for height is an important independent determinant of CVD-risk.[3-6] So far, many determinants of CVD have been studied in relation to small for gestational age (SGA) birth and accelerated weight gain during childhood, but the relationship with a broad range of biomarkers related to atherosclerosis, including inflammatory markers, remains to be elucidated.

The role of inflammation in preclinical as well as advanced stages of atherosclerosis has been widely acknowledged.[7] There are many markers of inflammation known to date. Because of the young age of our study population, we focused on biomarkers which relate to preclinical stages of atherosclerosis, such as acute-phase proteins, pro-inflammatory cytokines, and lipoproteins, namely: high sensitivity C-reactive protein (hsCRP), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), soluble vascular adhesion molecule 1 (sVCAM-1), soluble intracellular adhesion molecule 1 (sICAM-1), high-density lipoprotein (HDLc), low-density lipoprotein (LDLc), apolipoprotein B (ApoB), apolipoprotein A1 (ApoA1), and triglycerides (TG).[8-12]

CRP is an important predictor of future atherosclerotic events, also without prior CVD.[9] MCP-1 and IL-8 are pro-inflammatory chemo-attractant proteins (chemokines) which cause recruitment of leukocytes to the arterial endothelium,[10-11] while VCAM-1 and ICAM-1 are pro-inflammatory adhesion molecules which cause adhesion of leukocytes to the arterial endothelium.[12]

We hypothesized that adult body size, specifically adult weight SDS adjusted for height SDS, rather than small birth size (either due to SGA- or preterm birth), is associated with serum biomarkers of early stage atherosclerosis in young adulthood. We first identified combinations of biomarkers, using principal component analysis as it might well be that combinations of certain parameters tell more about the risk of developing CVD by reflecting a risk profile, than separate serum levels of biomarkers.[13] Subsequently, we tested our hypotheses by investigating the effect of birth size, gestational age, and adult size on the principal components, using multiple regression analysis with the component scores as dependent variable. Also, the effect of the components on insulin sensitivity, systolic blood pressure, and carotid intima media thickness (cIMT) was investigated.

In addition, we evaluated differences in component scores between three clinically relevant subgroups of young adults, born small for gestational age with either short adult height (SGA-S), or normal adult height (SGA-CU), or born appropriate for gestational age (AGA) with normal adult height (controls).

Methods

Subjects

The study population consists of 492 healthy participants, 18 to 24 years of age (PROGRAM/PREMS study cohort). All participants had similar in- and exclusion criteria, visiting centre and measurements, and were recruited from the same source-population, but participants from the PROGRAM study were born term whereas those from the PREMS study were born preterm (gestational age <36 weeks). Participants who were registered in one of several hospitals because of their small size at birth (SGA with a birth length <-2 SDS or being born preterm), or short adult stature (adult height <-2 SDS), were randomly selected for this study. In addition, by using advertisement at several schools with different educational levels, healthy AGA subjects born term were asked to participate. The participation rate of the PROGRAM/PREMS study cohort was 79.5%. All participants fulfilled the same inclusion criteria: 1) age 18-24 yr; 2) born singleton, 3) Caucasian; 4) uncomplicated neonatal period. The study population has been described previously in detail.[6,14] Additionally to the total group analyses, the subjects were assigned to one of three subgroups based on SD-scores of birth length and adult height. In order to increase the statistical power for subgroup comparison by increasing the contrast in birth size and adult height between the subgroups, the cut-off-values for small birth size and short adult height were set at <-2 SDS, and the cut-off-values for normal birth size and normal adult height were set at >-1 SDS. This resulted in a total of 286 participants who were included in one of the three subgroups:

- Subjects born SGA (birth length <-2 SDS) with a short adult height (<-2 SDS) (SGA-S): n=50 (of whom n=9 had a gestational age <36 weeks),
- Subjects born SGA (birth length <-2 SDS) with catch-up growth resulting in a normal adult height (>-1 SDS) (SGA-CU): n=92 (of whom 34 had a gestational age <36 weeks),
- Subjects born AGA (birth length >-1 SDS) with a normal adult height (>-1 SDS) (controls): n=144 (of whom 64 had a gestational age <36 weeks).

Measurements

Participants were invited to visit Erasmus University Medical Centre. Prior to the visit, participants fasted for at least 12 hours and abstained from smoking and alcohol for at least 16 hours. Height was measured to the nearest 0.1 cm (Harpenden stadiometer), weight to the nearest 0.1 kg (Servo Balance KA-20-150S). All anthropometric measurements were performed twice; the mean value was used for analysis. Lean body mass and fat mass were measured on one Dual-Energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Chalfon St Giles, England). A frequent sampled intravenous glucose tolerance (FSIGT) test with Tolbutamide was performed,[15] and insulin sensitivity was determined by the Bergman's minimal model (MINMOD 6.01, copyright R.N. Bergman). Brachial blood pressure was measured after 10 minutes of rest, in the supine position, using the nondominant arm with an automatic device (Accutorr Plus, Datascope Corp., Montvale NJ, USA)[16] every five minutes for one hour and the mean value of

these 13 measurements was taken to reflect resting blood pressure. A standard cuff size was used unless a large cuff was necessary. Carotid intima media thickness (cIMT) was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using one 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Tech. Laboratories, Bethel Washington, USA).[17]

Assays

After centrifugation, all blood samples were kept frozen until assayed (-80 C). All inflammatory markers were analyzed in the same laboratory. Serum levels of MCP-1, IL-8, sVCAM-1 and sICAM-1 were measured using Cytometric Bead Array kits (eBioscience, USA) according to the manufacturer's protocol. For hsCRP an in-house high-sensitivity ELISA with polyclonal rat CRP antibodies for catching and tagging (DAKO, Denmark) was used. Fasting levels of total cholesterol (TC), triglycerides (TG), and HDLc were measured. LDLc was calculated using the Friedewald formula: LDLc = TC-HDLc-0.45xTG. TC and TG were measured using an automated enzymatic method with the CHOD-PAP reagent kit and with the GPO-PAP reagent kit, respectively (Roche Diagnostics, Mannheim, Germany). HDLc was measured using a homogenous enzymatic colorimetric assay (Roche Diagnostics).

Statistical analysis

Standard deviation scores for birth length and birth weight were calculated using the Growth Analyser program (http://www.growthanalyser.org), in order to correct for gestational age.[18] SD-scores for adult height, and adult weight were calculated to correct for age and gender.[19] Because of statistical collinearity of birth length SDS and birth weight SDS (r=0.78), that pair of variables was combined as one variable: of both birth length SDS and birth weight SDS, the one with the lowest SDS was used in analyses (called 'birth size SDS').

To identify combinations of biomarkers we performed principal component analysis (PCA) on metric data. The principal components with eigenvalues (the variance in all variables which is accounted for by that component) greater than 1.0 were retained.[20] Varimax rotation was used for sake of interpretation. Varimax rotation is orthogonal, thus the components were uncorrelated. Component scores were calculated for each component (three scores for each participant) to use in multiple regression analyses and subgroup comparisons. Component loadings >0.40 and <-0.40 were considered to characterize components. Components were given names based on the variables characterizing them.

Multiple regression (MR) analysis was performed in the total study population (n=474) to determine associations of birth size SDS, gestational age, adult weight SDS, and adult height SDS with the principal components to be identified. Additionally, all analyses were performed after exclusion of the participants of the PREMS cohort, to investigate if oversampling of subjects born preterm might affect the results. Because results were similar, all analyses reported in this paper include both cohorts. All MR models and adjusted subgroup comparisons were corrected for the

duration of storage of the samples until determination of MCP-1, IL-8, sVCAM-1 and sICAM-1. Additionally, all MR models were adjusted for age, gender, gestational age, oral contraceptive use (OC-use), and smoking (Model 1). The MR model with the component including HDLc and ApoA1 as dependent variable was additionally adjusted for alcohol use. Next, adult weight SDS was added to the model (Model 2). Finally, adult weight SDS was replaced by fat mass (kg) and lean body mass (kg) (Model 3). MR analysis was also performed to investigate the associations between the principal components and insulin sensitivity (295 participants), systolic blood pressure (381 participants), and carotid itima media thickness (cIMT) (392 participants).

ANOVA was used to determine unadjusted differences between subgroups, with regard to the group characteristics. Bonferroni correction was used for pair-wise group comparisons. Mann-Whitney U was used to test unadjusted differences between subgroups with regard to the inflammatory markers, lipid levels, insulin sensitivity, systolic blood pressure, and cIMT because of a non-normal distribution. Adjusted subgroup comparisons were performed by calculating the estimated marginal means (EMM) of the component scores, with adjustments for the covariates with a p-value below 0.10 resulting from the MR analyses.

Statistical package SPSS version 17.0 (SPSS, Inc., Chicago, IL) was used for analyses. Results were regarded statistically significant if p was <0.05 (two-tailed).

Results

The clinical characteristics and levels of biomarkers of the total study population are shown in Table 1. The mean age (standard deviation) was 20.8 (1.7) years. The correlation matrix of the parameters entered in principal component analysis (PCA) is shown in the supplement (S1).

Principal Component Analysis (PCA)

PCA was performed and resulted in three principal components (Table 2, S2). The first was characterized by ApoB, LDLc, TG, and hsCRP (in the following text referred to as component Adverse Lipids and hsCRP). The second was characterized by HDLc and ApoA1 (referred to as component HDLc and ApoA1). The third was characterized by MCP-1, sICAM-1, IL-8, and sVCAM-1 (referred to as component Inflammatory Markers).

Table 1. Clinical characteristics and inflammatory parameters of the total group and comparison between subgroups

	Total group (n=474)	SGA-S (n=50)	SGA-CU (n=92)	Controls (n=144)
Age (yr)[1,3]	20.8(1.7)	20.9(1.8)	20.8(1.7)	20.8(1,7)
Male/Female	203/271	18/32	37/55	68/76
Birth length SDS[1,3]	-1.27(1.7)	-3.04(0.88)*	-2.97(0.81)*	0.21(0.8)
Birth weight SDS[1,3]	-0.83(1.66)	-2.08(0.92)*	-2.29(0.86)*	0.29(1.24)
Gestational age (wks)[1,3]	36.8(3.79)	38.2(3.15)‡§	36.0(3.36)	36.3(4.11)
Adult height SDS[1,3]	-0.65(1.35)	-2.56(0.54)*†	-0.13(0.68)§	0.19(0.8)
Adult weight SDS[1,3]	-0.34(1.42)	-1.39(1.49)*†	-0.00(1.19)	0.18(0.96)
Smoking (%)	28.0	26.0	29.2	24.3
OC-use (% females)	74.2	75.0	72.7	71.1
Alcohol use (%)	78.1	78.0	76.4	81.9
Fat mass (kg)[1,3]	16.1(9.1)	16.1(8.5)	17.8(10.3)	17.0(9.5)
LBM (kg)[1,3]	46.3(9.9)	39.0(7.7)*‡	47.9(9.8)	50.8(9.6)
LDLc (pg/ml)[2]	2.58(2.11-3.08)	3.12(2.45-3.66)*†	2.52(2.13-3.06)	2.52(2.13-2.84)
ApoB (g/l)[2]	0.80(0.67-0.93)	0.91(0.72-1.06)*||	0.80(0.68-0.94)	0.77(0.66-0.90)
TG (mmol/l)[2]	0.86(0.66-1.22)	0.85(0.68-1.17)	0.91(0.71-1.32)	0.85(0.66-1.20)
HDLc (mmol/l)[2]	1.34(1.13-1.57)	1.29(1.17-1.46)	1.33(1.11-1.56)	1.39(1.17-1.61)
ApoA1 (g/l)[2]	1.32(1.20-1.47)	1.27(1.18-1.48)	1.29(1.20-1.50)	1.32(1.20-1.46)
hsCRP (mg/l)[2]	1.43(0.42-4.09)	1.16(0.35-2.73)	1.85(0.66-5.24)	1.30(0.41-3.83)
MCP-1 (pg/ml)[2]	331.5(282.2-398.2)	322.0(260.5-400.5)	328.8(279.8-402.0)	336.9(289.7-417.0)
IL-8 (ng/ml)[2]	125.3(54.9-180.1)	93.2(32.5-178.4)	133.7(60.7 -188.4)	133.7(68.2-173.4)
sICAM-1 (ng/ml)[2]	383.5(300.6-535.5)	459.7(288.2-759.3)	389.0(326.4- 509.7)	391.8(316.7-576.1)
sVCAM-1 (ng/ml)[2]	1090.9(867.0-1320)	974.5(713.3-1244)§||	1105.0(932.8-1256)	1143.0(913.4-1385)
Si *10^{-4}/min (μU/ml)[2]	6.25(3.85-10.02)	5.93(3.93-10.35)	5.05(3.18-9.09)#	7.03(4.69-10.64)
Systolic blood pressure (mmHg)[2]	110.3(105.2-116.2)	107.5(103.8-113.1)‡#	112.5(108.0-117.8)	111.6(105.7-117.1)
cIMT (mm)[2]	0.52(0.48-0.55)	0.51(0.47-0.54)	0.53(0.49-0.56)	0.52(0.48-0.55)
Component scores				
Adverse Lipids / hsCRP[1,3]	–	0.33(0.97)§	0.10(1.00)	-0.14(0.87)
HDLc / ApoA1[1,3]	–	-0.09(0.92)	-0.05(1.04)	0.08(1.01)
Inflammatory Markers[1,3]	–	-0.10(0.95)	0.09(0.86)	0.15(0.82)

[1] Mean (standard deviation) is shown, [2] Median (inter quartile range) is shown, [3] Bonferroni corrected

*p<0.001 compared with controls, †p<0.001 compared with SGA-CU, ‡p<0.01 compared with SGA-CU, §p<0.01 compared with controls, ||p<0.05 compared with SGA-CU, #p<0.05 compared with controls

Si: insulin sensitivity, cIMT: carotid intima media thickness

Table 2. Component loadings defining combinations of biomarkers, results of principal component analysis in the total group

	Components		
	Adverse Lipids and hsCRP	**HDLc and ApoA1**	**Inflammatory Markers**
ApoB	**0.942**	0.015	0.032
LDLc	**0.871**	-0.054	-0.054
TG	**0.640**	-0.051	0.118
hsCRP	**0.410**	0.178	0.097
HDLc	-0.070	**0.930**	-0.006
ApoA1	0.126	**0.919**	0.050
MCP-1	0.089	0.053	**0.664**
sICAM-1	0.165	0.113	**0.644**
IL-8	0.038	-0.041	**0.563**
sVCAM-1	-0.376	-0.086	**0.498**
% of variance	24.5	17.7	14.2

All variables have been log transformed (natural logarithm) for PCA; Component loadings >0.40 and <-0.40 are bolded, these variables are considered to characterize components.

Total study population

Table 3 shows the final multiple regression Model 3 with the three components as dependent variables. Details of Models 1 and 2 are shown in the supplement (S3).

Table 3. Regression analysis (final model 3) for three principal components in the total study population

	Adverse Lipids and hsCRP		**HDLc and ApoA1**		**Inflammatory Markers**	
	β	**p-value**	**β**	**p-value**	**β**	**p-value**
Age	0.043	0.091	0.078	**0.003**	0.050	0.058
Gender	0.175	0.413	0.215	0.326	-0.030	0.893
Birth size SDS*	-0.042	0.139	0.051	0.079	-0.046	0.114
Gestational age (wks)	0.005	0.738	0.010	0.542	0.007	0.663
Adult height SDS	-0.125	**0.009**	0.085	0.081	0.085	0.082
OC-use	0.744	**<0.001**	0.259	0.055	-0.012	0.930
Smoking	0.159	0.095	-0.193	**0.047**	0.193	**0.049**
Alcohol use			0.276	**0.010**		
Fat mass (kg)	0.026	**<0.001**	-0.008	0.125	0.012	**0.020**
LBM (kg)	0.016	0.109	-0.027	**0.008**	0.012	0.251
Adj. R^2		0.267		0.225		0.205

Adjusted for and the storage duration of the samples; OC-use= oral contraceptive use, LBM= Lean body mass, Gender: male=1, female=2; β= unstandardized regression coefficients, One column represents one model; *The lowest of birth weight SDS or birth length SDS; Adverse Lipids: LDLc, ApoB, TG; Inflammatory markers: MCP-1, sICAM-1, IL-8, sVCAM-1

There was no association of birth length SDS, birth weight SDS or gestational age with any of the components.

In the multiple regression analysis with component Adverse Lipids and hsCRP as dependent variable, adult height SDS showed an inverse association. Furthermore, fat mass and OC-use showed positive associations with component Adverse Lipids and hsCRP.

There was no significant association of component HDLc and ApoA1 with adult height SDS or fat mass, but an inverse association with lean body mass. Also, component HDLc and ApoA1 showed positive associations with age, OC-use, and alcohol use, and an inverse association with smoking.

In the final model, there was no association of adult height SDS with component Inflammatory Markers. However, the component was positively associated with fat mass. Furthermore, a positive association was found with smoking.

Other determinants of cardiovascular diseases

To determine which of the components was associated with other determinants of CVD, we assessed associations of the components with insulin sensitivity, systolic blood pressure, and cIMT, using MR analysis (Table 4). The components Adverse Lipids and hsCRP and Inflammatory Markers were both inversely related to insulin sensitivity. The association of Inflammatory Markers with insulin sensitivity, however, disappeared after correction for adult body size in model 2, while the significant effect of Adverse Lipids and hsCRP remained. Component HDLc and ApoA1 had a positive association with insulin sensitivity.

The component Adverse Lipids and hsCRP was positively associated with systolic blood pressure, but this association disappeared after correction for adult body size measures. There was no effect of any of the components on cIMT at this young age.

Subgroup comparisons

The clinical characteristics and levels of biomarkers of the subgroups are shown in Table 1. Figure 1A shows comparisons between the subgroups with regard to the three components, after adjustments. In Figure 1B we additionally adjusted for adult height SDS to investigate whether the differences between subgroups might be ascribed to their stature. SGA-S subjects had higher adjusted scores for component Adverse Lipids and hsCRP than controls (Figure 1A, p-value=0.026). However, after adjustment for adult height SDS, the difference in component Adverse Lipids and hsCRP between SGA-S subjects and controls disappeared (Figure 1B). There were no significant differences between the subgroups in scores for components HDLc andApoA1 and Inflammatory Markers (Figure 1A+B).

Table 4. Effect of the principal components of biomarkers on other determinants of cardiovascular diseases

	Insulin sensitivity[1]				Systolic blood pressure[1]				cIMT[1]*			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2*	
Component	B	p-value	B	p-value	B	p-value	B	p-value	B	p-value	B	p-value
Adverse Lipids and hsCRP	-0.227	**<0.001**	-0.100	**0.013**	0.008	**0.046**	0.000	0.951	0.006	0.328	0.003	0.528
HDLc and ApoA1	0.138	**0.003**	0.086	**0.033**	-0.002	0.715	0.004	0.334	-0.006	0.352	-0.005	0.419
Inflammatory Markers	-0.091	**0.024**	-0.038	0.279	0.005	0.353	-0.002	0.697	-0.004	0.541	-0.004	0.541

[1]log transformed; Model 1: adjusted for age, gender, gestational age, Oc-use, smoking, and in the case of ApoA1 / HDLc analyses also alcohol use

Model 2: additionally adjusted for adult height SDS, fat mass (kg), and lean body mass (kg); * Additionally adjusted for artery diameter, and systolic blood pressure; Insulin sensitivity was determined in 295 participants, cIMT was determined in 392 participants, systolic blood pressure determined in 381 participants

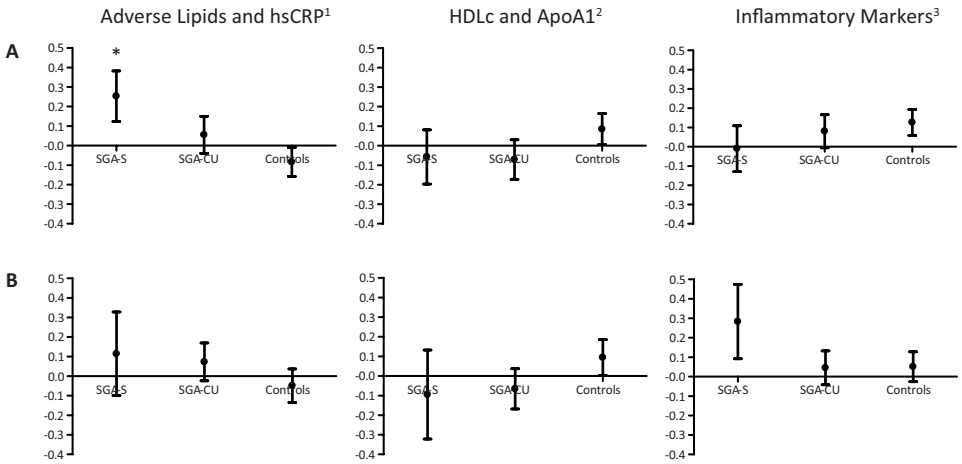

Figure 1. Adjusted mean component scores and differences between subgroups

Component scores are displayed as means (standard error); A. Adjusted for gender, age, smoking, and [1]OC-use, percentage fat mass, [2]OC-use, alcohol use, percentage lean body mass, [3]percentage fat mass; B. Additionally adjusted for adult height SDS; * p=0.026 compared with controls

Discussion

In this study we investigated the effect of small birth size (either due to SGA birth or low gestational age) and adult body size on serum biomarkers related to CVD-risk in young adulthood. By performing PCA, we distinguished three combinations of biomarkers known to be associated with early stage atherosclerosis. The first component included ApoB, LDLc, TG, and hsCRP (component Adverse Lipids and hsCRP), the second included HDLc and ApoA1 (component HDLc and ApoA1), and the third included the chemokines and adhesion molecules MCP-1, sICAM-1, IL-8, and sVCAM-1 (component Inflammatory Markers). Our results showed no association of birth size, and gestational age with these three components. Fat mass was, however, positively associated with components Adverse Lipids and hsCRP and Inflammatory Markers. Furthermore, short stature was associated with higher scores for component Adverse Lipids and hsCRP.

To our knowledge, we are the first to investigate the relationship of small birth size with combinations of biomarkers for CVD-risk by performing PCA, a method used to study combined parameters.[21-23] Recently, Bhuiyan et al. reported decreasing hsCRP levels across quartiles of increasing birth weight.[24] Other studies also showed an inverse association between birth weight and hsCRP,[25-26] and in adult rats an association was found between early undernutrition (resulting in intrauterine growth retardation) and increasing basal inflammation.[27] Our study shows that hsCRP forms a combination with lipoproteins (LDLc, ApoB, TG). The component Adverse Lipids and hsCRP was, however, not associated with birth weight SDS and birth length SDS. The differences in results might be explained by the fact that the reported association between birth weight and hsCRP was not adjusted for adult height, which in our study proved to be an important confounder in this association.

Adult weight SDS was an important determinant of each component, being positively associated with Adverse Lipids and hsCRP, and Inflammatory Markers, and inversely associated with HDLc and ApoA1. This is in line with previous research.[28-29] We were also able to specify adult weight in fat mass and lean body mass, by using DXA measurements. Higher fat mass was associated with increased component scores for Adverse Lipids and hsCRP, and Inflammatory Markers, also after adjustment for adult height SDS. Thus, increased fat mass is associated with increased cardiovascular risk, already in young adulthood.[30]

Multiple regression analyses also showed significant positive associations of age and smoking with combinations of cardiovascular risk biomarkers, which is in agreement with other studies.[28-29,31] In addition, oral contraceptive (OC) use was positively associated with Adverse Lipids and hsCRP. This is in line with previous findings of increased hsCRP levels and adverse lipid profile in OC-users.[32-35]

Adult height SDS was inversely associated with Adverse Lipids and hsCRP and had a trend towards a positive association with Inflammatory Markers. Up until now, the association of adult height with lipoprotein levels and inflammatory determinants of atherosclerosis has not been thoroughly investigated. Previously, researchers studied the effect of BMI on various lipoproteins

and inflammatory markers,[26,36] but not the independent effects of adult weight SDS and height SDS on a combination of markers. It has been suggested that restricted diet and infectious disease could generate suboptimal growth in childhood as well as higher risk for CVD in later life, via increased inflammatory markers.[37]

Our study shows that higher scores for Adverse Lipids and hsCRP were associated with lower insulin sensitivity, also after correction for confounders including fat mass, while HDLc and ApoA1 had a positive effect on insulin sensitivity. This is in line with previous studies,[38-39] and confirms that component Adverse Lipids and hsCRP has adverse effects on health risk and that component HDLc and ApoA1 has protective effects. We previously demonstrated an association of adult body size with insulin sensitivity in our study population, whereas there was no significant association of small size at birth or preterm birth with insulin sensitivity.[14,40]

The component Inflammatory Markers also showed an inverse association with insulin sensitivity, but this association disappeared after correction for adult height, fat mass and lean body mass. After adjustments there was no association of any of the components with systolic blood pressure and cIMT. Because of the young age of our study population it might however be that the latter association will be revealed at an older age.

To interpret our results for clinical practice, we divided the total study population into three subgroups. The component scores of Adverse Lipids and hsCRP were significantly higher in young adults born SGA with a short stature than in controls, also after adjustment for confounders including fat mass. This difference disappeared after additional correction for adult height SDS, indicating that the higher scores in SGA-S subjects are unlikely due to their being born SGA, but are rather due to their short stature. The effect of short stature on Adverse Lipids and hsCRP is in agreement with a previous report showing that short stature is associated with increased risk of cardiovascular disease.[41] That twin-study also showed that a genetic factor might be underlying.

We previously demonstrated an inverse association of adult height SDS with fat mass,[3] and we demonstrated that higher fat mass in early adulthood leads to higher blood pressure and cIMT.[30] Our research warrants future prospective studies investigating causal pathways of atherosclerosis in subjects with short stature, including genetic factors, fat mass, adverse lipids and hsCRP.

We introduce PCA to study the association of small size at birth and adult body size with a broad range of serum biomarkers associated with early stage atherosclerosis. In a public health perspective, our data indicate that small birth size is not associated with increased biomarkers of atherosclerosis risk in young adulthood. Adult body size, however, has a significant association with the components identified in the present study, with increased adult weight SDS having an adverse effect via increased fat mass. Because the prevalence of fat accumulation in childhood and adulthood is increasing rapidly, this is likely to induce future public health problems. The association of short stature with biomarkers of atherosclerosis is more complex, as adult height SDS was inversely associated with component Adverse Lipids and hsCRP but tended to a positive

association with component Inflammatory Markers. These findings were confirmed in subgroup comparisons and warrant further research in subjects with short stature.

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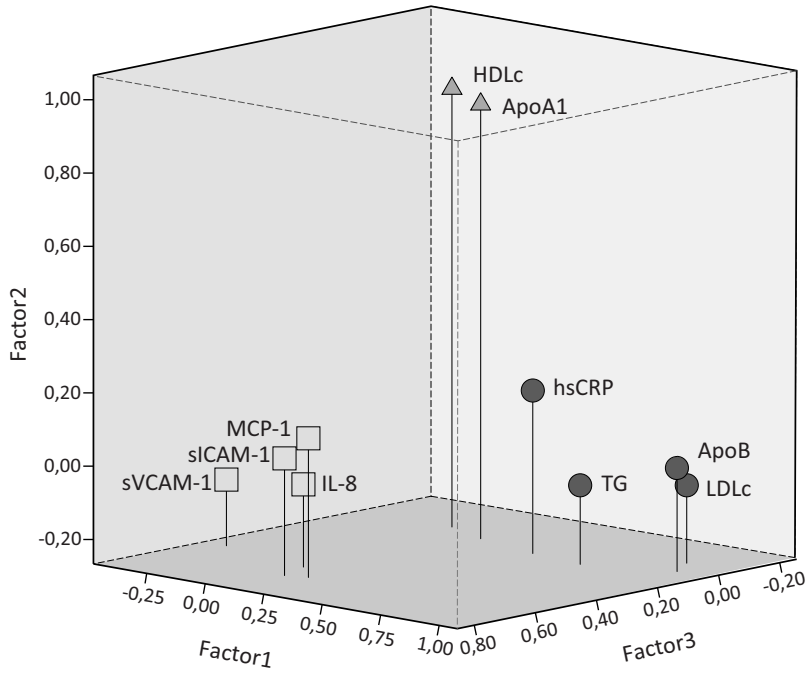
Supplement



S1. 3D plot of rotated component loadings

Circle: Component Adverse Lipids and hsCRP
Triangle: Component HDLc and ApoA1
Square: Component Inflammatory Markers
hsCRP: high sensitivity C-reactive protein, MCP-1: monocyte chemotactic protein-1, IL-8: interleukin-8, sVCAM-1: soluble vascular adhesion molecule 1, sICAM-1: soluble intracellular adhesion molecule 1, HDLc: high-density lipoprotein, LDLc: low density lipoprotein, ApoB: apolipoprotein B, ApoA1: apolipoprotein A1, TG: triglycerides

S2. Spearman correlation analyses of DM2 risk factors in early adulthood

	hsCRP	**MCP-1**	**IL-8**	**sVCAM-1**	**sICAM-1**	**HDLc**	**LDLc**	**ApoB**	**ApoA1**	**TG**
hsCRP		-0.022	0.031	-0.089	**0.172***	0.035	**0.171***	**0.318***	**0.207***	**0.246***
MCP-1			**0.191***	**0.211***	**0.223***	-0.079	**0.096***	**0.095***	-0.009	0.051
IL-8				0.214*	**0.133***	0.026	0.002	0.006	0.028	0.014
sVCAM-1					**0.175***	-0.080	**-0.189***	**-0.188***	-0.010	**-0.140***
sICAM-1						0.033	0.050	**0.137***	**0.120***	0.107*
HDLc							-0.028	-0.010	**0.704***	**-0.150***
LDLc								**0.893***	0.055	**0.303***
ApoB									**0.160***	**0.462***
ApoA1										**0.113***
TG										

*p<0.05; hsCRP: high sensitive C-reactive protein, MCP-1: monocyte chomotactic protein, IL-8: interleukin-8, sVCAM-1: soluble vascular adhesion molecule 1sICAM-I: soluble intracellular adhesion molecule 1, HDLc: high density lipoprotein, LDLc: low density lipoprotein, ApoB: apolipoprotein, ApoA1:apoliporotein A1, TG: triglycerides.

S3. Multiple regression analysis for three components in the total study population

	Adverse Lipids and hsCRP						HDLc and ApoA1						Inflammatory Markers					
	Model 1		Model 2		Model 3		Model 1		Model 2		Model 3		Model 1		Model 2		Model 3	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Age	0.066	**0.012**	0.061	**0.016**	0.043	0.091	0.064	**0.014**	0.066	**0.010**	0.078	**0.003**	0.062	**0.018**	0.059	**0.022**	0.050	0.058
Gender	0.064	0.636	0.093	0.480	0.175	0.413	0.607	**<0.001**	0.593	**<0.001**	0.215	0.326	-0.150	0.268	-0.136	0.313	-0.030	0.893
Birth size SDS*	-0.048	0.099	-0.046	0.103	-0.042	0.139	0.048	0.103	0.047	0.109	0.051	0.079	-0.048	0.100	-0.047	0.105	-0.046	0.114
Gestational age	0.002	0.890	0.002	0.892	0.005	0.738	0.008	0.598	0.009	0.590	0.010	0.542	0.006	0.708	0.006	0.709	0.007	0.663
Adult height SDS	-0.057	0.119	-0.162	**<0.001**	-0.125	**0.009**	-0.008	0.827	0.049	0.228	0.085	0.081	0.131	**<0.001**	0.078	0.059	0.085	0.082
Adult weight SDS			0.196	**<0.001**					-0.107	**0.003**					0.099	**0.006**		
OC-use	0.782	**<0.001**	0.743	**<0.001**	0.744	**<0.001**	0.239	0.079	0.259	0.055	0.259	0.055	0.007	0.956	-0.012	0.929	-0.012	0.930
Smoking	0.130	0.188	0.164	0.087	0.159	0.095	-0.172	0.079	-0.190	0.050	-0.193	**0.047**	0.177	0.072	0.194	**0.048**	0.193	**0.049**
Alcohol use							0.285	**0.009**	0.289	**0.007**	0.276	**0.010**						
Fat mass (kg)					0.026	**<0.001**					-0.008	0.125					0.012	**0.020**
LBM (kg)					0.016	0.109					-0.027	**0.008**					0.012	0.251
Adj. R^2	0.211		0.263		0.267		0.209		0.223		0.225		0.194		0.206		0.205	

Adjusted for the interaction term birth length SDS * adult height SDS and the storage duration of the samples; β= unstandardized regression coefficients, p= p-value; *The lowest of birth weight SDS or birth length SDS; OC-use= oral contraceptive use, LBM= Lean body mass, Gender: male=1, female=2. Adverse Lipids: LDLc, ApoB, TG; Inflammatory markers: MCP-1, sICAM-1, IL-8, sVCAM-1

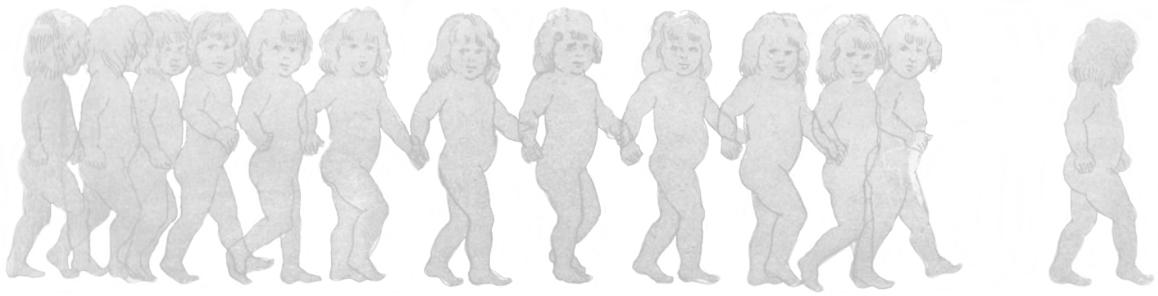
References

1. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA and Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lance*t 1993,341:938-941.
2. Levy-Marchal C and Jaquet, D. Long-term metabolic consequences of being born small for gestational age. *Pediatr Diabetes* 2004, 5:147-153.
3. Leunissen RW, Stijnen T and Hokken-Koelega AC. Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol* (Oxf), 2009, 70:245-251.
4. Singhal A, Kennedy K, Lanigan J, Fewtrell M, Cole TJ, Stephenson T, Elias-Jones A, Weaver LT, Ibhanesebhor S, MacDonald PD, Bindels J and Lucas A. Nutrition in infancy and long-term risk of obesity: evidence from 2 randomized controlled trials. *Am J Clin Nutr* 2010 92:1133-1144.
5. Fagerberg B, Bondjer L and Nilsson P. Low birth weight in combination with catch-up growth predicts the occurrence of the metabolic syndrome in men at late middle age: the Atherosclerosis and Insulin Resistance study. *J Intern Med* 2004, 256:254-259.
6. Leunissen RW, Kerkhof GF, Stijnen T and Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA* 2009, 301: 2234-2242.
7. Hansson GK, Atherosclerosis--an immune disease: The Anitschkov Lecture 2007. *Atherosclerosis* 2009, 202:2-10.
8. Ballantyne CM and Hoogeveen RC. Role of lipid and lipoprotein profiles in risk assessment and therapy. *Am Heart J* 2003, 146:227-233.
9. de Ferranti SD and Rifai N. C-reactive protein: a nontraditional serum marker of cardiovascular risk. *Cardiovasc Pathol* 2007, 16:14-21.
10. Braunersreuther V, Mach F and Steffen, S. The specific role of chemokines in atherosclerosis. *Thromb Haemost* 2007, 97:714-721.
11. Libby P. Inflammation in atherosclerosis. *Nature* 2002, 420:868-874.
12. Galkina, E. and Ley, K., Vascular adhesion molecules in atherosclerosis, *Arterioscler Thromb Vasc Biol*, 2007, 27: 2292-2301.
13. Meigs JB. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am J Epidemiol* 2000, 152:908-911; discussion 912.
14. Willemsen RH, Leunissen RW, Stijnen T and Hokken-Koelega AC. Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab* 2009, 94:1695-1700.
15. Pacini G and Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 1986, 23:113-122.
16. Anwar Y A, Tendler BE, McCabe EJ. Mansoor, G. A. and White, W. B., Evaluation of the Datascope Accutorr Plus according to the recommendations of the Association for the Advancement of Medical Instrumentation. *Blood Press Monit* 1997, 2:105-110.
17. Bots ML, Hoes AW, Koudstaal PJ, Hofman A and Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 1997, 96:1432-1437.
18. Usher R and McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr*, 1969, 74:901-910.
19. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP and Wit JM. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res*, 2000, 47:316-323.
20. DiStefano C, Zhu M and Mîndila D. Understanding and Using Factor Scores: Considerations for the Applied Researcher, Practical Assessment. *Research & Evaluation* 2009, 14.

21. Salmenniemi U, Ruotsalainen E, Pihlajamaki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E and Laakso M. Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation* 2004, 110:3842-3848.
22. Goodman E, Dolan LM, Morrison JA and Daniels SR. Factor analysis of clustered cardiovascular risks in adolescence: obesity is the predominant correlate of risk among youth. *Circulation* 2005, 111:1970-1977.
23. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J and Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 2002, 288:2709-2716.
24. Bhuiyan AR, Srinivasan SR, Chen W, Azevedo MJ and Berenson GS. Influence of low birth weight on C-reactive protein in asymptomatic younger adults: the bogalusa heart study. *BMC Res Notes* 2011, 4:71.
25. Sattar N, McConnachie A, O'Reilly D, Upton MN, Greer IA, Davey Smith G and Watt G. Inverse association between birth weight and C-reactive protein concentrations in the MIDSPAN Family Study. *Arterioscler Thromb Vasc Biol* 2004, 24:583-587.
26. Tzoulaki I, Jarvelin MR, Hartikainen AL, Leinonen M, Pouta A, Paldanius M, Ruokonen A, Canoy D., Sovio, U, Saikku P and Elliott P. Size at birth, weight gain over the life course, and low-grade inflammation in young adulthood: northern Finland 1966 Birth Cohort study. *Eur Heart J* 2008, 29:1049-1056.
27. Desai M, Gayle DA, Casillas E, Boles J and Ross MG. Early undernutrition attenuates the inflammatory response in adult rat offspring. *J Matern Fetal Neonatal Med* 2009, 22:571-575.
28. Van Gaal LF, Mertens IL and De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature*, 2006, 444:875-880.
29. Pischon T. Use of obesity biomarkers in cardiovascular epidemiology. *Dis Markers* 2009, 26:247-263.
30. Kerkhof GF, Duivenvoorden HJ, Leunissen RW and Hokken-Koelega AC. Pathways leading to atherosclerosis: a structural equation modeling approach in young adults. *Hypertension* 2011, 57: 255-260.
31. McMahan CA, Gidding SS and McGill HC Jr. Coronary heart disease risk factors and atherosclerosis in young people. *J Clin Lipidol* 2008, 2:118-126.
32. Krintus M, Sypniewska G and Kuligowska-Prusinska M. Effect of second and third generation oral contraceptives on C-reactive protein, lipids and apolipoproteins in young, non-obese, non-smoking apparently healthy women. *Clin Biochem* 2010, 43:626-628.
33. Haarala A, Eklund C, Pessi T, Lehtimaki T, Huupponen R, Jula A, Viikari J, Raitakari O and Hurme M. Use of combined oral contraceptives alters metabolic determinants and genetic regulation of C-reactive protein. The Cardiovascular Risk in Young Finns Study. *Scand J Clin Lab Invest* 2009, 69:168-174.
34. Cauci S, Di Santolo M, Culhane JF, Stel G, Gonano F and Guaschino S. Effects of third-generation oral contraceptives on high-sensitivity C-reactive protein and homocysteine in young women. *Obstet Gynecol* 2008, 111:857-864.
35. Mantel-Teeuwisse AK, Kloosterman JM, Maitland-van der Zee AH, Klungel OH, Porsius AJ and de Boer A. Drug-Induced lipid changes: a review of the unintended effects of some commonly used drugs on serum lipid levels. *Drug Saf* 2001, 24:443-456.
36. Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, Kwon BS, Erickson KL and Yu R. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *Int J Obes* (Lond) 2006, 30:1347-1355.
37. Crimmins, E. M. and Finch, C. E., Infection, inflammation, height, and longevity. *Proc Natl Acad Sci U S A*, 2006, 103: 498-503.
38. Lopez S, Bermudez B, Abia R and Muriana FJ. The influence of major dietary fatty acids on insulin secretion and action. *Curr Opin Lipidol* 2010, 21:15-20.

39. Leinonen E, Hurt-Camejo E, Wiklund O, Hulten LM, Hiukka A and Taskinen MR. Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. *Atherosclerosis* 2003, 166:387-394.
40. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T and Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab* 2008, 93:445-451.
41. Silventoinen K, Kaprio J, Koskenvuo M and Lahelma E. The association between body height and coronary heart disease among Finnish twins and singletons. *Int J Epidemiol* 2003, 32:78-82.

Chapter 6



Early Origins of Type 2 Diabetes: Growth Trajectories in Early Childhood and Risk for Type 2 Diabetes in Young Adults

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Submitted

Abstract

Accelerated early life weight gain has been associated with individual risk factors for type 2 diabetes. The pathophysiology of type 2 diabetes is complex and comprises a wide spectrum of risk factors. Our objective was to identify combinations of known risk factors in early adulthood preceding type 2 diabetes, and to investigate associations of early growth trajectories with the identified combinations of risk factors.

Our study consists of 217 participants, aged 21 years. We identified four combinations (principal components) of type 2 diabetes risk factors by using Principal Component Analysis. Gain in weight for length in the first three months of life was positively associated with the component characterized by insulin resistance, acute insulin response, and serum levels of C-reactive protein and triglycerides. Furthermore, subjects with catch-up in weight in the first year of life had higher adjusted scores for that component than those without catch-up growth, also after additional adjustment for birth weight SDS. There were no significant associations of early weight gain with any of the other components.

In conclusion, accelerated gain in weight compared to length in the first three months of life should be avoided to reduce the risk for type 2 diabetes in later life.

Introduction

The prevalence of type 2 diabetes is rapidly increasing, not only in the elderly, but also at younger ages.[1] It is therefore of major public health importance to identify prevention targets to reduce development of type 2 diabetes in early adulthood.

One of the theories on the development of type 2 diabetes comprises the common soil hypothesis, which states that type 2 diabetes and cardiovascular disease (CVD) have common genetic and environmental antecedents, rather than atherosclerosis being a complication of diabetes.[2] That implies that prevention targets of CVD and type 2 diabetes are similar. The role of early life growth trajectories in development of CVD has been acknowledged, but its role in development of type 2 diabetes remains controversial.[3-4]

We reported an association of accelerated weight gain in the first three months of life with decreased insulin sensitivity in young adulthood.[5] However, the pathophysiology of type 2 diabetes is complex and comprises a wide spectrum of parameters and risk factors,[1] and it remained difficult to investigate several risk factors of type 2 diabetes simultaneously. Principal Component Analysis (PCA) is a multivariate correlation technique that enables solving this issue by reducing a large number of intercorrelated variables to a smaller set of independent components.[6-7] Thus, it enables investigating combinations of known risk factors of type 2 diabetes. Several publications have reported PCA of metabolic syndrome variables,[7-11] but only a limited number has focused on specific risk factors of type 2 diabetes.[12-13] We use PCA as a novel approach to investigate early origins of type 2 diabetes.

The objective of this study was to identify combinations of known risk factors in early adulthood preceding type 2 diabetes,[14-17] using PCA. Subsequently, we studied associations of early life growth trajectories with the identified principal components, in order to determine whether growth in early life could be a prevention target to reduce development of type 2 diabetes in early adulthood.

In our study population of healthy young adults, many parameters were measured to determine type 2 diabetes risk status, including frequently sampled intravenous glucose tolerance (FSIGT)-tests (insulin resistance (IR), acute insulin response (AIR), disposition index (DI)), and fasting glucose. Furthermore, body composition by dual-energy X-ray absorptiometry, and serum levels of several known predictors of type 2 diabetes, such as lipid levels, C-reactive protein (CRP), and adiponectin, were determined.

Methods

Subjects

The study population consists of 217 young adults of the PROGRAM study cohort, 18 to 24 years of age, of whom data of first year growth were available. Participants, who were registered in several hospitals because of their small size at birth (birth length <-2 SDS), were randomly selected for this study. In addition, young adults with short stature were included. Healthy young adults (neither small at birth nor having short adult stature) from schools with different educational levels were also asked to participate by using advertisement. This design was purposely chosen because it increased the contrast in growth patterns and thus the statistical power to find a relationship between early growth patterns and risk factors of type 2 diabetes.

Figure 1 shows how many young adults were invited and how many were included in the study. The participation rate was 84.1%. Only whites born singleton at 36 weeks of gestation or longer were invited to participate to exclude a potential influence of ethnicity, parity, and prematurity. All included young adults had an uncomplicated neonatal period without severe asphyxia (defined as an Apgar score below three after five minutes) and did not have sepsis or long-term complications of respiratory ventilation, such as bronchopulmonary dysplasia. Individuals were excluded if they 1) had any serious condition or disorder, 2) were receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy), or 3) had endocrine or metabolic disorders, chromosomal defects, syndromes, or serious dysmorphic symptoms suggestive of a yet unknown syndrome. Of the 323 study participants in the PROGRAM study, data on first-year growth were available for 217 young adults. Weight and length at birth, 3, 6, 9, and 12 months had been prospectively measured at primary health care centers or hospitals. Growth data were collected during the study period between August 2004 and September 2007 from the records. Some centers and hospitals did not store records for 25 years, therefore early growth data were missing for 106 young adults. Due to logistical reasons, FSIGT tests were not performed from 2006 to 2007, and of the 217 participants with early growth data, complete data on type 2 diabetes risk factors for PCA was available for 86 participants. Data on educational level were obtained using questionnaires to determine socioeconomic status (SES). The Medical Ethics Committee of Erasmus Medical Center approved the study. Written informed consent was obtained from all participants.

Measurements

Participants were invited to visit Erasmus Medical Center and were reimbursed for travel expenses. Prior to the taking of measurements, participants fasted for 12 hours and abstained from smoking and drinking alcohol for 16 hours. All anthropometric measurements were performed twice and the mean value was used for the analysis.

Fat mass and trunk fat mass were measured on one Dual-Energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, England). Insulin sensitivity index and acute insulin response to glucose were determined using the Bergman minimal model (MINMOD Millennium version 6.01, MINMOD Inc, Los Angeles, California), which calculated the paired glucose and insulin data obtained by frequent measurements during and intravenous glucose tolerance test with Tolbutamide.

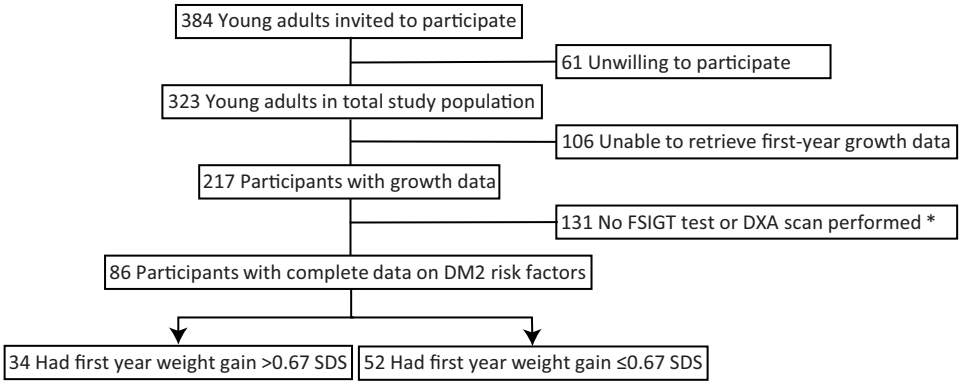

Figure 1. PROGRAM-study flow chart

* Randomly, due to logistical reasons

Laboratory Methods

Plasma glucose levels were determined on a VITROS analyzer 750 (Ortho-Clinical Diagnostics, Johnson & Johnson Company, Beerse, Belgium) and plasma insulin levels were measured using an immunoradiometric assay (Medgenix Diagnositcs, Fluenes, Belgium). Triglycerides were measured using an enzymetric colometric method (WAKO Chemicals), and automated enzymetic method, with the GPO-PAP reagent kit (Roche Diagnostics). High-density lipoprotein cholesterol level (HDLc) was measured using a homogeneous enzymatric colorimetric assay (Roche Diagnostics). LDL cholesterol (LDLc) was calculated using the Friedewald formula: LDL cholesterol (mmol/L)=total cholesterol-HDLc-0.45xTG. Apolipoprotein A-I (ApoA1) and apolipoprotein B (ApoB) were determined by rate nephelometry on the Image Immunochemistry System according to manufacturer instructions (Beckman Coulter). For C-reactive protein (CRP) an in-house high-sensitivity ELISA with polyclonal rat CRP antibodies for catching and tagging (DAKO, Denmark) was used. Serum adiponectin levels were assayed using ELISA (R&D Systems Inc., Minneapolis, MN), in duplicate and the mean of those two measures used for analysis.

Statistical Analysis

The standard deviation (SD) scores for birth length, birth weight, and first-year growth were calculated to correct for gestational age and sex.[18] The SD-scores for adult height and adult weight were calculated to correct for sex and age.[19] All SD-scores were calculated using the growth analyser program (http://www.growthanalyser.org).

Spearman correlations were determined to estimate intercorrelations of type 2 diabetes risk factors. To identify combinations of type 2 diabetes risk factors, we performed Principal Component Analysis (PCA) on metric data.[6] Not-normally distributed parameters were log-transformed (natural logarithm) before added in the PCA. This applied to LDLc, ApoB, CRP, acute insulin response, insulin resistance, triglyceride, HDLc, fasting glucose, Waist/Hip ratio, and adiponectin. The principal components with eigenvalues (the variance in all variables which is accounted for by that component) greater than 1.0 were retained.[6] Varimax (orthogonal) rotation was used for sake of interpretation; Varimax rotation is orthogonal, thus the components were uncorrelated. Component scores (a score for each participant) were calculated for each principal component to use in multiple regression analyses and subgroup comparisons. Component loadings >0.40 and <-0.40 were considered to characterize principal components. Multiple linear regression analyses were performed to investigate the association between weight gain per each three months in the first year of life and the principal components of type 2 diabetes risk factors. The four three-months periods were analyzed separately from each other. Adjustments were made for gestational age, sex, age, and SES. To investigate the association between weight gain and the components independently of height, adjustments were also made for height growth during the same three-months period.

To determine whether catch-up in weight in the first year of life might be a high risk growth trajectory for type 2 diabetes, we subdivided the total study population in two subgroups, irrespective of birth length or birth weight, one with catch-up in weight (weight gain >0.67 SDS) and one without catch-up in weight in the first year of life (Figure 1). Gain in SD-scores of 0.67 represent the width of each percentile band on standard growth charts (second to ninth percentile, ninth to 25th percentile etc).[20] Differences between the two subgroups were determined using Estimated Marginal Means (EMM) adjusted for gestational age, sex, age, socioeconomic status, and height growth in the first year.

The SPSS statistical package version 17 (SPSS Inc, Chicago, Illinois) was used for the analysis. All statistical tests were performed two-sided and results were regarded as statistically significant if the P value was less than 0.05.

Results

The clinical characteristics of the study population (n=217) are shown in Table 1. The mean (SD) age in early adulthood was 20.8 (1.66) years, 40.1% of all participants were male.

Table 1. Clinical Characteristics of the Study Population

		Mean (SD)
Characteristic	**Actual**	**SD Score**
	At Birth	
Gestational age, wks	39.2(1.66)	
Length, cm	47.6(3.17)	-1.53(1.45)
Weight, kg	2.78(0.67)	-1.21(1.35)
	First year of life	
Height growth, cm		
Birth-3 mo	10.1(2.65)	0.41(1.05)
>3-6 mo	6.88(1.23)	0.10(0.53)
>6-9 mo	4.74(0.86)	0.08(0.35)
>9-12 mo	3.75(0.80)	-0.03(0.30)
Weight gain, kg		
Birth-3 mo	2.38(0.69)	0.22(1.16)
>3-6 mo	1.71(0.42)	0.08(0.58)
>6-9 mo	1.22(0.37)	0.06(0.40)
>9-12 mo	0.99(0.34)	0.08(0.34)
	Early Adulthood	
Age, y	20.8(1.66)	
Height, cm	168.1(10.9)	-1.13(1.38)
Weight, kg	63.9(12.6)	-0.63(1.43)
Waist/Hip ratio	0.89(0.07)	
Trunk fat / total body fat ratio	0.48(0.06)	
Fasting glucose, mmol/L	4.88(0.43)	
Insulin sensitivity, µU/mL	6.82(4.33)	
Acute insulin response, mU/L	570.6(559.7)	
Triglycerides, mmol/L	1.03(0.49)	
HDLc, mmol/L	1.39(0.38)	
ApoA1, g/L	1.30(0.22)	
LDLc, mmol/L	2.67(0.83)	
ApoB, g/L	0.82(0.24)	
Total Cholesterol, mmol/L	4.53(0.94)	
Adiponectin, ng/mL	7658.8(3618.7)	
C-reactive protein, mg/L	4.05(7.00)	

Table 2. Spearman correlation analyses of type 2 diabetes risk factors in early adulthood

	Glucose	IR	AIR	TG	HDLc	ApoA1	LDLc	ApoB	Chol	W/H	TF/TBF	ApN	CRP
Glucose		0.105	-0.099	-0.095	-0.118	**-0.223***	0.043	-0.068	-0.040	0.137	**0.268***	**-0.215***	**-0.324***
IR			**0.356***	**0.268***	-0.104	0.000	**0.229***	**0.287***	**0.258***	**0.237***	0.186	-0.062	**0.372***
AIR				0.146	0.058	0.075	0.189	0.184	**0.253***	0.087	0.030	0.152	**0.459***
TG					**-0.154***	0.132	**0.280***	**0.449***	**0.435***	0.019	0.121	-0.174	**0.246***
HDLc						**0.789***	-0.104	-0.047	**0.250***	-0.066	**-0.235***	**0.400***	0.066
ApoA1							0.000	0.135	**0.338***	-0.037	**-0.202***	**0.343***	**0.221***
LDLc								**0.901***	**0.875***	-0.077	0.034	-0.030	0.114
ApoB									**0.873***	-0.018	-0.007	-0.003	**0.256***
Chol										-0.124	-0.047	0.074	**0.167***
W/H											**0.363***	**-0.356***	**0.205***
TF/TBF												**-0.421***	-0.021
ApN													**0.217***
CRP													

*p<0.05; IR: insulin resistance (1/Si), AIR: acute insulin response, TG: triglycerides, Chol: cholesterol, W/H: waist/hip ratio, TF/TBF: Trunk/total body fat ratio, ApN: adiponectin, CRP: C-reactive protein; See Table 1 for units

Combinations of type 2 diabetes risk factors

The results of Spearman correlation analyses of the type 2 diabetes risk factors used in PCA are presented in Table 2.

PCA resulted in four principal components of type 2 diabetes risk factors in early adulthood (Table 3). The four components explained 72.2% of the total variance in the original set of variables. The first was characterized by LDLc, ApoB, and total cholesterol (in the following text referred to as component Adverse Lipids). The second principal component was characterized by C-reactive protein, acute insulin response, insulin resistance, and triglycerides (referred to as component CRP / AIR / IR / TG). The third component was characterized by HDLc, ApoA1, and waist/hip ratio (referred to as component HDL / ApoA1 / WHR). The fourth component was characterized by trunk/total body fat ratio, fasting glucose, waist/hip ratio, and adiponectin (referred to as component Central Obesity / glucose / ApN).

First year weight gain and risk for type 2 diabetes in early adulthood

Associations between first-year weight gain and principal components of type 2 diabetes risk factors in early adulthood are shown in Table 4. Adjustments were made for gestational age, sex, age, SES, and SD score for height growth in the same three-months period. Adjustments for height growth were performed to investigate the association between weight gain and the components independently of height growth.

Weight gain in the first three months of life had a significant positive association with component CRP / AIR / IR / TG, and was not associated with any of the other components. Furthermore, no significant associations were found between weight gain in the other three-months periods and any of the components of type 2 diabetes risk factors.

To investigate whether the association of weight gain in the first three months and component CRP / AIR / IR / TG was explained by small birth size, an additional adjustment was performed for birth weight SDS. The association between weight gain in the first three months and component CRP / AIR / IR / TG remained significant (regression coefficient: 0.353, p-value: 0.007), and birth weight SDS had no significant association with that component (regression coefficient: -0.124, p-value: 0.291).

Next, to determine whether catch-up in weight in the first year of life might be a high-risk growth trajectory for type 2 diabetes, we subdivided the study population of 86 participants in two subgroups, one with catch-up in weight (>0.67 SDS) in the first year of life (n=34) and one without. Comparisons of component CRP / AIR / IR / TG between the two subgroups are shown in Figure 1. Subjects with catch-up in weight in the first year had significantly higher scores for component CRP / AIR / IR / TG (p-value=0.002), which remained significant (p-value=0.004) after additional adjustment for birth weight SDS. Birth weight SDS itself was not significantly associated with the component.

Table 3. Component loadings defining components of type 2 diabetes risk factors, results of Principal Component Analysis

	Component 1	Component 2	Component 3	Component 4
LDLc*	**0.960**	0.110	-0.051	-0.007
Apolipoprotein B*	**0.958**	0.180	-0.094	-0.014
Cholesterol	**0.932**	0.158	0.242	-0.011
C-reactive protein*	0.003	**0.817**	-0.051	-0.302
Acute insulin response*	0.122	**0.732**	-0.005	-0.063
Insulin resistance*	0.221	**0.645**	0.009	0.240
Triglycerides*	0.340	**0.446**	-0.060	0.263
HDLc*	-0.051	-0.105	**0.925**	-0.142
Apolipoprotein A1	0.080	0.033	**0.902**	-0.156
Ratio Trunk/Total fat	-0.169	0.233	-0.178	**0.765**
Fasting glucose*	0.153	-0.189	0.031	**0.668**
Ratio waist/hip*	-0.272	0.304	**-0.439**	**0.651**
Adiponectin*	-0.121	0.232	0.397	**-0.595**
Eigenvalue	**3.379**	**3.063**	**1.770**	**1.168**
% of Variance	**25.99**	**23.56**	**13.62**	**8.98**

*Log transformed (natural logarithm) for Principal Component Analysis.
Component Loadings >0.40 and <-0.40 are bolded, these variables are considered to characterize components.

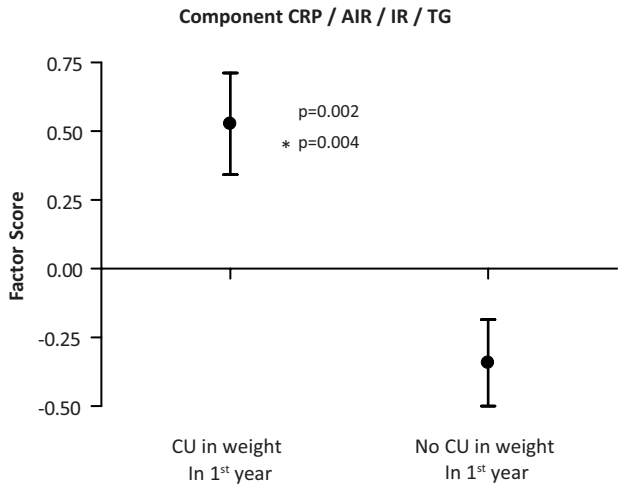

Figure 1. Component score comparison in subjects with and without catch-up in weight in the first year of life

CRP: C-reactive protein, AIR: Acute Insulin Response, IR: insulin resistance, TG: triglycerides, CU: catch-up in weight (weight gain >0.67SDS); Comparisons are adjusted for gestational age, sex, age, socioeconomic status, and height growth in the first year of life; * additionally adjusted for birth weight SDS

Table 4. Associations between standard deviations in weight gain in the first year of life and principal components of type 2 diabetes risk factors in early adulthood

	Component 1			Component 2			Component 3			Component 4		
	Adverse Lipids			CRP / AIR / IR / TG			HDL / ApoA1 / WHR			Central Obesity / glucose / ApN		
	β	P	Model R^2	β	P	Model R^2	β	P	Model R^2	β	P	Model R^2
Weight gain SDS 0-3 mo	0.055	0.647	0.097	**0.415**	**<0.001**	0.212	-0.203	0.099	0.170	0.002	0.983	0.349
Weight gain SDS 3-6 mo	-0.028	0.899	0.079	0.281	0.199	0.115	0.305	0.175	0.087	0.027	0.886	0.355
Weight gain SDS 6-9 mo	-0.058	0.855	0.063	0.256	0.421	0.093	-0.216	0.500	0.087	-0.173	0.525	0.352
Weight gain SDS 9-12 mo	-0.113	0.760	0.029	0.023	0.949	0.088	0.220	0.550	0.071	0.189	0.528	0.346

CRP: C-reactive protein, AIR: acute insulin response, IR: insulin resistance, TG: triglycerides, WHR: waist/hip ratio, ApN: adiponectin, All associations were adjusted for gestational age, sex, age, socioeconomic status, and height growth in the same period.

There were no differences between the subgroups in component Adverse Lipids (p-value=0.251), component HDL / ApoA1 / WHR (p-value=0.861), and component Central Obesity / glucose / ApN (p-value=0.833), adjusted for gestational age, sex, age, socioeconomic status and gain in length in the first year of life.

Discussion

In this study we investigated combinations of known risk factors preceding type 2 diabetes mellitus to identify type 2 diabetes risk profiles in early adulthood, using principal component analysis. This resulted in four principal components of type 2 diabetes risk factors. Subsequently, we studied associations of early life growth trajectories with the identified risk profiles, in order to determine whether growth in early life could be a prevention target to reduce development of type 2 diabetes in early adulthood. Our results demonstrate that gain in weight for length in the first three months of life is associated with a combination of specific risk factors for type 2 diabetes independently of birth weight, namely with the component characterized by insulin resistance, acute insulin response (AIR), and serum levels of C-reactive protein and triglycerides Gain in weight for length in the first three months of life was not associated with the other components of type 2 diabetes risk factors. Subgroup analyses confirmed our findings showing that of all young adults, those with catch-up in weight in the first year of life had the highest scores for the component characterized by insulin resistance, AIR, and serum levels of C-reactive protein and triglycerides, regardless of birth weight.

Low birth weight has previously been associated with an increased risk of cardiovascular diseases.[21-22] Although this was initially thought to be due to an unfavorable fetal environment,[23] other studies reported that postnatal catch-up growth influenced the risk as well.[24-27] To our knowledge, our study is the first study investigating the relationship of early life growth trajectories with combined type 2 diabetes risk factors in early adulthood. We previously showed an inverse association of accelerated early weight gain with insulin sensitivity,[5] but type 2 diabetes is a complex disease with many risk factors, and in the present study PCA enabled us to study combinations of determinants rather than individual risk factors. Remarkably, only certain risk factors of type 2 diabetes in young adults were affected by early growth, as we only found an association of first three-months accelerated weight gain with one (component CRP / AIR / IR / TG) of the four components identified. This implies that particularly the risk factors that characterize this principal component are involved in the mechanism linking early accelerated weight gain with risk for type 2 diabetes. The promising results of the present study motivate future research to focus on mechanisms affecting insulin resistance, AIR, and levels of C-reactive protein and triglycerides in adulthood when studying early origins of type 2 diabetes.

Our study population consisted of a relatively large number of subjects born small for gestational age (SGA), which enabled us to study the associations with more statistical power because of more contrast in early growth between the subjects. When we compared the subjects with and without catch-up in weight in the first year of life, we found that the subjects with catch-up in weight had the highest scores for component CRP / AIR / IR / TG in early adulthood, which was independent of size at birth. Early nutrition might be involved in the association between early life weight gain and risk for type 2 diabetes in young adulthood. Generally, nutrient-enriched diets lead to rapid weight gain in early life, and subsequently have adverse effects on cardiovascular risk factors in later life.[28-29] Infants born SGA often receive nutrient enriched feeding in the early postnatal period, which leads to rapid weight gain. Thus, subjects born SGA are likely to have increased risk for type 2 diabetes risk factors in early adulthood due to accelerated postnatal weight gain, rather than due to their small size at birth.

Formula-fed infants grow at a faster rate than breast-fed infants and have a higher risk of being overweight later in life.[30-31] Our study did not have nutritional data to investigate the relationship between early nutrition, growth in infancy, and type 2 diabetes risk factors in later life, but our findings suggest that the use of nutrient-enriched formulas, which induce rapid weight gain in early life, might increase risk for type 2 diabetes later in life. In contrast, breastfeeding during the first three months of life might decrease the prevalence of type 2 diabetes in adulthood. The findings in this study need to be confirmed in population-based cohort studies with standardized early life measurements. These studies would enable to investigate the optimal target of gain in weight for length after birth. Furthermore, it would be of additional value to include family history, as a risk factor of type 2 diabetes, in our analyses. Unfortunately, we did not have sufficient information to assess family history in our cohort of young adults. However, none of the subjects who fully completed the questionnaires mentioned a family history of type 2 diabetes.

In conclusion, our study indicates that accelerated gain in weight compared to length the first three months of life should be avoided to reduce the risk for type 2 diabetes in later life. Thus same pace in weight and length gain in early postnatal life should be a prevention target to avoid development of type 2 diabetes in early adulthood. This may be achieved by nutritional intervention according to weight for length trajectories. Our findings also point to the need for new prospective data to investigate the optimal target of gain in weight for length after birth.

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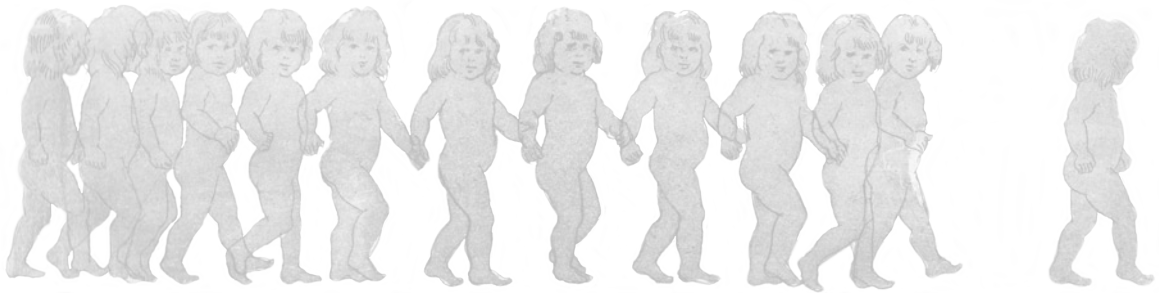
References

1. Desai J, Geiss L, Mukhtar Q, Harwell T, Benjamin S, Bell R, Tierney E. Public health surveillance of diabetes in the United States. *J Public Health Manag Pract.* 2003;Suppl:S44-51.
2. Stern MP. Diabetes and cardiovascular disease. The "common soil" hypothesis. *Diabetes.* 1995;44:369-374.
3. Lammi N, Moltchanova E, Blomstedt PA, Tuomilehto J, Eriksson JG, Karvonen M. Childhood BMI trajectories and the risk of developing young adult-onset diabetes. *Diabetologia.* 2009;52:408-414.
4. Eriksson JG, Forsen TJ, Osmond C, Barker DJ. Pathways of infant and childhood growth that lead to type 2 diabetes. *Diabetes Care.* 2003;26:3006-3010.
5. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301:2234-2242.
6. DiStefano C, Zhu M, Mîndila D. Understanding and Using Factor Scores: Considerations for the Applied Researcher. *Practical Assessment, Research & Evaluation.* 2009;14.
7. Meigs JB. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am J Epidemiol.* 2000;152:908-911; discussion 912.
8. Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? *J Pediatr.* 2008;152:160-164.
9. Meigs JB, D'Agostino RB, Sr., Wilson PW, Cupples LA, Nathan DM, Singer DE. Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. *Diabetes.* 1997;46:1594-1600.
10. Chen W, Srinivasan SR, Elkasabany A, Berenson GS. Cardiovascular risk factors clustering features of insulin resistance syndrome (Syndrome X) in a biracial (Black-White) population of children, adolescents, and young adults: the Bogalusa Heart Study. *Am J Epidemiol.* 1999;150:667-674.
11. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA.* 2002;288:2709-2716.
12. Kekalainen P, Sarlund H, Pyorala K, Laakso M. Hyperinsulinemia cluster predicts the development of type 2 diabetes independently of family history of diabetes. *Diabetes Care.* 1999;22:86-92.
13. Hanley AJ, Festa A, D'Agostino RB, Jr., Wagenknecht LE, Savage PJ, Tracy RP, Saad MF, Haffner SM. Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. *Diabetes.* 2004;53:1773-1781.
14. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. *Lancet.* 2003;361:226-228.
15. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet.* 2009;373:2215-2221.
16. Olafsdottir E, Aspelund T, Sigurdsson G, Thorsson B, Benediktsson R, Harris TB, Launer LJ, Eiriksdottir G, Gudnason V. Unfavourable risk factors for type 2 diabetes mellitus are already apparent more than a decade before onset in a population-based study of older persons: from the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik). *Eur J Epidemiol.* 2009;24:307-314.
17. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA.* 1990;263:2893-2898.
18. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* 1969;74:901-910.
19. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47:316-323.

20. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ.* 2000;320:967-971.
21. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* 1989;2:577-580.
22. Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD. Birthweight, body-mass index in middle age, and incident coronary heart disease. *Lancet.* 1996;348:1478-1480.
23. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993;341:938-941.
24. Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A. Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation.* 2007;115:213-220.
25. Chomtho S, Wells JC, Williams JE, Davies PS, Lucas A, Fewtrell MS. Infant growth and later body composition: evidence from the 4-component model. *Am J Clin Nutr.* 2008;87:1776-1784.
26. Ekelund U, Ong KK, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S. Association of weight gain in infancy and early childhood with metabolic risk in young adults. *J Clin Endocrinol Metab.* 2007;92:98-103.
27. Ekelund U, Ong K, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). *Am J Clin Nutr.* 2006;83:324-330.
28. Fewtrell MS, Morley R, Abbott RA, Singhal A, Stephenson T, MacFadyen UM, Clements H, Lucas A. Catch-up growth in small-for-gestational-age term infants: a randomized trial. *Am J Clin Nutr.* 2001;74:516-523.
29. Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet.* 2004;363:1571-1578.
30. Armstrong J, Reilly JJ, Child Health Information T. Breastfeeding and lowering the risk of childhood obesity. *Lancet.* 2002;359:2003-2004.
31. Gillman MW, Rifas-Shiman SL, Camargo CA, Jr., Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA. Risk of overweight among adolescents who were breastfed as infants. *JAMA.* 2001;285:2461-2467.

Chapter 7



Pathways Leading to Atherosclerosis: A Structural Equation Modeling Approach in Young Adults

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Abstract

Several risk factors of cardiovascular diseases have been studied using direct association measures. Because the incidence of obesity and cardiovascular diseases is rising, it is important to correctly model these risk factors involved in development of cardiovascular diseases. Until now, statistical methods lacked to achieve this goal because of complex interrelationships involved. Structural Equation Modeling (SEM) is an advanced statistical technique that enables solving this issue. The aims of this study were to investigate whether SEM could unravel pathways involved in cardiovascular diseases and to visualize these pathways in a model. In 322 healthy participants of the PROGRAM (PROgramming factors for GRowth And Metabolism) study, 18 to 24 years of age, we explored pathways leading to atherosclerosis measured by carotid intima-media thickness. Using SEM, we were able to model these pathways for males and females using body fat percentage, serum lipid levels, and blood pressure. We are the first to present a model of complex direct and indirect effects of fat mass leading to atherosclerosis using SEM. Both male and female path-model had an excellent fit. Fat mass had a significant effect on carotid intima-media thickness through various pathways, with the largest effect size on carotid intima-media thickness via blood pressure. SEM showed that the pathways differed between males and females, with a larger effect of serum lipids on carotid intima-media thickness in males. In conclusion, SEM is suitable in identifying models to unravel potential causal pathways in complex origins of diseases. We present a model involving several pathways, showing that fat mass has an influence on risk factors for atherosclerosis, already at 21 years of age.

Introduction

The World Health Organization estimates a rise in mortality of cardiovascular diseases (CVD) from 17.1 million in 2004 to 23.4 million in 2030.[1] These statistics explain the increasing interest of clinical researchers to determine risk factors for CVD. Atherosclerosis is an important etiologic element of CVD, and although causes of atherosclerosis have been explored previously, it remained difficult to investigate several atherosclerosis risk factors simultaneously. Cohort studies have been used to determine associations between risk factors of atherosclerosis.[2,3] However, these studies did not take into account indirect effects of risk factors because only direct effects between two variables were analyzed. Thus, such studies lacked to provide a statistical method that could unravel the pathways simultaneously in one path analysis.

Structural Equation Modeling (SEM) is an advanced statistical technique that enables solving these issues. SEM has been applied in several research fields but is still rarely used in clinical research, despite its ability to identify, test, and estimate pathways in a non-hypothesis-driven manner.[4]

We hypothesized that SEM is a suitable method to unravel multidirectional associations and potential causal pathways in complex origins of diseases such as CVD. This approach, in which the interdependency of risk factors is unraveled simultaneously, is innovative in this field. Our objective was to explore several pathways, leading to vascular changes in early adulthood, using SEM. We examined direct and indirect effects of fat mass in particular because fat mass accumulation during childhood is an important risk factor for CVD in adulthood.[5-7] Prevalence of obesity in children and young adults is rising, and this is likely to induce future problems in public health.[8,9] We aimed to study pathways through several determinants of atherosclerosis, including lipid levels and blood pressure. As far as we know, this is the first study using SEM to explore the pathways between fat mass and vascular changes in early adulthood.

Our study population consisted of 322 healthy subjects 18 to 24 years of age who participated in the PROgramming factors for GRowth And Metabolism (PROGRAM) study cohort. Several parameters were measured to determine metabolic and cardiovascular status of the participants.

Methods

Study Participants

The PROGRAM study cohort comprises 322 healthy subjects, 18 to 24 years of age. The PROGRAM study was performed in one medical center in The Netherlands between August 2004 and September 2007. Participants were recruited randomly from several hospitals in The Netherlands, where they had been registered because of small size at birth or short stature. Also randomly, healthy subjects from schools of various educational levels were asked to participate.

Only those born singleton, at ≥36 weeks of gestation and white, were invited to participate. The study population has been described previously in detail.[5]

The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, The Netherlands, approved the study. Signed informed consent was obtained from all participants.

Measurements

All participants were invited to visit Erasmus Medical Centre and were reimbursed for travel expenses. Before the taking of measurements, participants fasted for 12 hours and had abstained from smoking and alcohol for 16 hours. Fasting blood samples were drawn and centrifuged between 8 AM and 1 PM and were kept frozen until assayed (-80°C).

Fat mass was measured on a Dual-Energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy; GE Healthcare). The intra-assay coefficient of variation for fat tissue was 0.41% to 0.88%.[10] Brachial blood pressure was measured after 10 minutes at rest, in the supine position, using the nondominant arm with an automatic device (Accutorr Plus; Datascope Corp.) every five minutes for one hour, and the mean values of these 13 measurements were taken to reflect resting blood pressure. A standard cuff size was used unless a large cuff was necessary.

Carotid intima-media thickness (cIMT) was measured by recording ultrasonographic images of both left and right carotid artery, when subjects were supine, using a 7.5-MHz linear array transducer (ATL Ultramark IV; Advanced Tech Laboratories). On the R wave of the ECG, 3 longitudinal images of the near and far wall of the common carotid artery were frozen and stored on videotape. These images were digitized and displayed on the screen of a computer using a frame grabber (VP 1400-KIT-512-E-AT; Imaging Technology). The common cIMT was determined as the mean of the mean near-wall and far-wall measurements of both the left and right side common carotid artery.

Laboratory measurements

Lipid concentrations were analyzed in the same laboratory. Free fatty acids (FFA) and triglycerides (TG) were measured using an enzymatic colometric method (WAKO Chemicals), an automated enzymatic method, with the GPO-PAP reagent kit (Roche Diagnostics). HDL cholesterol (HDLc) was measured using a homogenous enzymatic colorimetric assay (Roche Diagnostics). LDL cholesterol (LDLc) was calculated using the Friedewald formula: LDL cholesterol level in mmol/L = total cholesterol level – HDL cholesterol level – 0.45 x level of triglycerides. Apolipoprotein A-I (apoA-1) and apolipoprotein B (apoB) were determined by rate nephelometry on the Image Immunochemistry System according to manufacturer instructions (Beckman Coulter). Plasma acylation stimulating protein (ASP)[11,12] concentrations were measured using a sandwich ELISA. The intra-assay variations of measurements of TG, HDL cholesterol, and ASP were 2.9, 3.9%, and <4%, respectively. Between-run coefficients of variation for apoA-1 and apoB were 4.2% and 2.8% at levels of 0.94 and 0.53 g/L, respectively.

Statistical Analysis

Body fat percentage was calculated as: [body fat (kg)/weight (kg)]x100%. Differences between males and females and between oral contraceptive (OC) users and non-OC users were determined using ANOVA. The difference between males and females regarding the percentage of smokers was determined using a Pearson χ^2 test. We used the Pearson correlations to estimate intercorrelations, and the Fisher Z-transformation was used to explore differences between male and female correlation coefficients.

To unravel the interrelationships among atherosclerosis risk factors, we used SEM,[13] a powerful statistical tool for path analysis using maximum likelihood estimation. SEM has been used in psychological, social, educational, and management fields[14] and is applicable in clinical research, specifically to visualize pathways and calculate the magnitudes of direct and indirect effects on human diseases. Using SEM, we explored several path models to identify, test, and estimate models.

Although there are no absolute standards for the relationship between sample size and model complexity, a desirable goal is to have a minimal subject/parameter ratio of 10:1.[13] The models generated in this study consist of eight parameters. Because the female model was based on data from 197 subjects and the male model on those from 125 subjects, both models had a subject/parameter ratio clearly larger than 10:1, indicating a sufficient sample size.

Because of statistical collinearity of the variables HDLc and ApoA1 (males, r=0.83; females, r=0.70), LDLc and ApoB (males, r=0.92; females, r=0.87), and diastolic and systolic blood pressure (males, r=0.78; females, r=0.78), each of these pairs of variables was combined as one variable using Z scores for standardization. These variables are strongly related because ApoA1 and ApoB are structural proteins for HDLc and LDLc, respectively. The variable with the most unfavorable Z score of the two was used in analysis. The combined variables were called HDL&apoA1, LDL&apoB, and blood pressure.

Using the model-generating approach in SEM, we first explored relationships between exogenous (independent) and endogenous (dependent) variables in a model starting with fat mass percentage and ending with cIMT. Secondly, for each nonsignificant path, we determined whether it was acceptable to remove the path while maintaining an acceptable fit. Models were tested until no meaningful improvements were found on models that had been tested previously. All models were constructed for males and females, separately. Regression-based imputation was used for missing data using full information matrix. The number of missing data for blood pressure was 103, and for cIMT, 79; for all other parameters, the number of missing data were <20. The generated SEM model was also tested using a complete case analysis without imputed data. Bootstrapping was applied for internal validation. The 95% confidence intervals after bootstrapping are shown in Supplement 1 (Table S1). Because not all variables were characterized by a normal distribution, robust maximum likelihood was used to test the generated model. This showed similar results.

We used standardized path coefficients as effect estimate (range, -1.0 to 1.0). The effect size of these coefficients can be determined using this classification: <0.10 as a small effect, 0.30 as a medium effect, and >0.50 as a large effect. These values are recommendations. Effect sizes can be reasonably estimated in combination with tests of significance, which also take account of sample size and intercorrelations among variables.[13] Supplemental Figure 2 provides more information regarding path diagrams.

Model Fit

For each model, we evaluated the fit by measures of overall fit and detailed assessment of fit (fitted and standardized residuals and modification indices) and by examining the individual parameter estimates. The following performance measures were used: (1) χ^2 for model fit (low and nonsignificant values of the two are desired);[13] (2) χ^2/degrees of freedom ratio (a value <2.0 was considered acceptable); (3) Comparative Fit Index; (4) Tucker-Lewis Index (Comparative Fit Index and Tucker-Lewis Index, where values of 1.0 suggest a perfect fit, and high values are desired, but where values >1.0 indicate an overidentification);[15,16] (5) root mean square error of approximation (a value <0.05 indicates a close fit);[17] and (6) standardized root mean squares of residuals (where a value of 0.08 indicates a good fit).[18]

Statistical package SPSS version 15.0 (SPSS, Inc.) was used for the Pearson χ^2 test and ANOVA. M-plus version 5.2.1 (Muthén and Muthén) was used for SEM. Results were regarded as statistically significant if two-sided *P* was <0.05.

Results

Study Population

Table 1 shows unadjusted clinical characteristics of the 322 participants and males and females separately. There were no differences between males and females regarding age and proportion of smokers. Males had higher mean waist/hip ratio, systolic blood pressure, and cIMT. Females had a higher mean percentage of fat mass and serum FFA, TG, ASP, LDL, HDL, apoB, and apoA1 levels (all P<0.001).

In Table 2, linear correlation coefficients of parameters used in SEM analyses are shown for comparison with previous studies. Correlation coefficients that differed significantly between males and females were the correlations between FFA and fat mass (P=0.008), TG and fat mass (P<0.001), HDL&apoA1 and fat mass (P=0.007), HDL&apoA1 and TG (P=0.002), and HDL&apoA1 and cIMT (P=0.018).

Table 1. Clinical Characteristics of Males and Females

Characteristic	Male (n=125)	Female (n=197)	P Value
Age (y)	20.9 (1.66)	20.9 (1.69)	0.756
%FM	16.3 (7.91)	29.5 (8.55)	<0.001
Waist/hip ratio	0.91 (0.07)	0.87 (0.07)	<0.001
Smokers (%)	27.0	27.5	0.863
OC use(%)	—	76.7	—
Systolic BP (mm Hg)	114.1 (7.96)	107.6 (7.16)	<0.001
Diastolic BP (mm Hg)	66.6 (5.39)	65.8 (5.26)	0.231
cIMT (mm)	0.53 (0.05)	0.51 (0.04)	<0.001
FFA (mmol/L)	0.55 (0.24)	0.66 (0.23)	<0.001
TG (mmol/L)	0.90 (0.44)	1.11 (0.52)	<0.001
ASP (nmol/L)	14.3 (7.17)	19.6 (11.13)	<0.001
LDL cholesterol (mmol/L)	2.49 (0.66)	2.83 (0.86)	<0.001
HDL cholesterol (mmol/L)	1.24 (0.29)	1.47 (0.37)	<0.001
ApoB (g/L)	0.73 (0.18)	0.88 (0.25)	<0.001
ApoA1 (g/L)	1.19 (0.17)	1.38 (0.22)	<0.001

Values given are mean (SD). %FM indicates percentage of body fat; BP, blood pressure.

Table 2. Linear correlation coefficients between atherosclerosis parameters used in SEM, for males and females separately

Parameter	%FM	FFA	TG	ASP	HDL&apoA1	LDL&apoB	BP	cIMT	Mean	SD
%FM	–	0.00	0.02	**0.16**[3]	0.06	0.13	**0.35**[1]	-0.06	29.5	8.55
FFA	**0.30**[1,5]	–	0.09	0.02	-0.02	0.10	-0.04	0.09	0.66	0.23
TG	**0.49**[1,4]	0.04	–	**-0.19**[2]	0.06	**0.29**[1]	0.14	-0.05	1.11	0.52
ASP	0.01	-0.08	**0.21**[3]	–	-0.02	0.10	**0.15**[3]	0.00	19.6	11.1
HDL&apoA1	**-0.24**[2,5]	-0.14	**-0.30**[1,5]	0.10	–	**-0.16**[3]	0.10	-0.03	0.19	0.98
LDL&apoB	**0.24**[2]	0.01	**0.31**[1]	**0.18**[3]	-0.01	–	-0.12	0.03	-0.02	1.00
BP	**0.34**[1]	0.10	**0.26**[2]	-0.07	-0.07	**0.18**[3]	–	**-0.17**[3]	0.15	0.83
cIMT	0.07	0.10	0.14	0.03	**-0.30**[1,6]	**0.24**[2]	**0.22**[3]	–	-0.51	0.04
Mean	16.3	0.55	0.90	14.3	-0.53	-0.51	0.82	0.53		
SD	1.66	0.24	0.44	7.17	0.78	0.77	0.93	0.05		

Dark grey: males, light grey: females Significant Pearson correlation coefficients are given in bold, p-value of the correlation coefficients: [1]:p<0.001, [2]:p<0.01, [3]:p<0.05. Significant difference between correlations of males and females: [4]:p<0.001, [5]:p<0.01, [6]:p<0.05, %FM= percentage body-fat, FFA= free fatty acids, TG= triglycerides, ASP= acyl stimulation protein, HDL= high-density lipoprotein cholesterol, apoA1= apolipoprotein A-I, LDL= low-density lipoprotein cholesterol, apoB= apolipoprotein B, BP = blood-pressure, cIMT= carotid intima-media thickness

Male and female model by SEM

The implementation of SEM as statistical approach to identify pathways from fat mass leading to changes in cIMT resulted in a model for males and females with an adequate model fit (Figures 1 and 2): χ^2 was 41.1 with 34 degrees of freedom (ratio 1.2) and a P value of 0.188. Low, nonsignificant values of the χ^2 are desired, and the ratio has to be <2.0.[13] The Comparative Fit Index was 0.96 and the Tucker-Lewis Index was 0.93. Both Comparative Fit Index and Tucker-Lewis Index need to be high for a good fit, but values >1.0 indicate an overfit.[15,16] The root mean square error of approximation and standardized root mean squares of residuals had values of 0.036 and 0.050, respectively. Root mean square error of approximation and standardized root mean squares of residuals need to be <0.05 and <0.08, respectively, for a good fit.[17,18] Also after using bootstrapping for internal validation, the model fit remained good. The complete case analysis resulted in a similar model with a good fit. The directionality and magnitude of the path coefficients also resembled those of the original model.

SEM analyses resulted in a good model for both males (Figure 1) and females (Figure 2). The largest effect in both males and females was that of fat mass on cIMT, via blood pressure. In contrast, the pathways regarding the serum lipid levels differed between males and females. The effect of LDL&apoB on cIMT was present in the male model but absent in the female model, whereas the effect of FFA on TG in the female model was absent in the male model. Further, the effect sizes of the pathways via serum lipids were higher in males than in females.

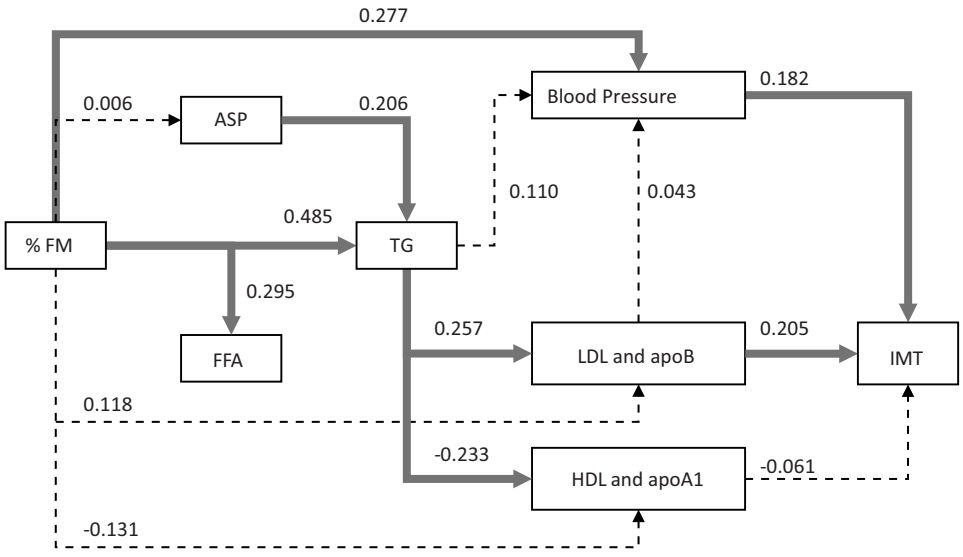

Figure 1. Path diagram for males with standardized coefficients of direct effects

Bold arrows indicate p<0.05, and dashed arrows indicate p>0.10. Standardized path coefficients (presented next to all arrows) are used as effect estimate (range: -1.0 to +1.0): <0.10 is considered as a small effect, 0.30 as a medium effect and >0.50 as a large effect.

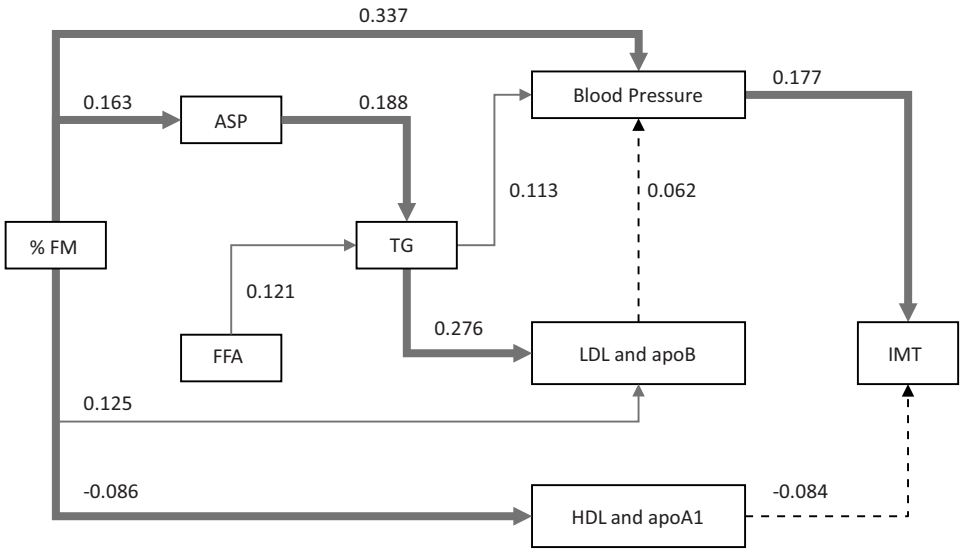

Figure 2. Path diagram for females with standardized coefficients of direct effects
Bold arrows indicate p<0.05, not-bolded arrows indicate p<0.10, and dashed arrows indicate p>0.10. Standardized path coefficients (presented next to all arrows) are used as effect estimate (range: -1.0 to +1.0): <0.10 is considered as a small effect, 0.30 as a medium effect and >0.50 as a large effect.

Table 3 shows the direct, indirect, and total effects of fat mass on endogenous variables used in path analyses. Both models show a relatively large effect of fat mass on blood pressure. For males, the total effect of fat mass on blood pressure was 0.34 (P<0.001; range, -1.0 to 1.0). For females, this effect was 0.35 (P<0.001). The total effect of fat mass on cIMT was significant for both males (P=0.002) and females (P=0.013).

Of the females, 76.7% used OCs. The parameters (means) that differed between OC users and non-OC users were, respectively, TG (1.21 versus 0.81; P0.001), FFA (0.70 versus 0.54; P<0.001), LDL (2.92 versus 2.58; P=0.024), apoB (0.92 versus 0.76; P<0.001), and systolic blood pressure (108.2 versus 105.0; P=0.008). The body fat percentage did not differ between OC users and non-OC users (29.7 versus 28.4; P=0.39). We tested the same female model in OC users and non-OC users separately to test whether the model was applicable to both groups. Despite the small number of participants without OC use (n=44), the female models both showed a good fit, thus, we decided to combine the data for OC users and non-OC users in the final model.

Table 3. Direct, indirect, and total effects of fat mass on endogenous variables in males and females

	Male Model						Female Model					
	Direct Effect		Indirect Effect		Total Effect		Direct Effect		Indirect Effect		Total Effect	
Endogenous Variables	**Coeff**	**P Value**	**Coeff**	**P Value**	**Coeff**	**P Value**	**Coeff**	**P Value**	**Coeff**	**P Value**	**Coeff**	**P Value**
ASP	0.006	n.s.	—	—	0.006	n.s.	0.163	0.019	—	—	0.163	**0.019**
FFA	0.295	**0.008**	—	—	0.295	**0.008**	—	—	—	—	—	—
TG	0.485	**<0.001**	0.001	n.s.	0.486	**<0.001**	—	—	0.031	0.076	0.031	0.076
LDL&apoB	0.118	n.s.	0.125	**0.012**	0.242	**0.004**	0.125	0.067	0.008	n.s.	0.134	**0.047**
HDL&apoA1	0.131	n.s.	0.113	**0.022**	0.244	**0.004**	0.086	**<0.001**	—	—	0.086	**<0.001**
BP	0.277	**0.003**	0.064	n.s.	0.341	**<0.001**	0.337	**<0.001**	0.012	n.s.	0.349	**<0.001**
cIMT	—	—	0.127	**0.002**	0.127	**0.002**	—	—	0.069	**0.013**	0.069	**0.013**

The direct, indirect, and total effects of fat mass on the endogenous variables included in the models. We used standardized path coefficients as effect estimate (range, -1.0 to 1.0): <0.10 is considered a small effect, 0.30 a medium effect, and >0.50 a large effect. Coeff indicates path coefficient; BP, blood pressure. Significant P values (P<0.05) are given in bold; n.s., not shown (P>0.10).

Discussion

This is the first study to show that SEM is an innovative statistical method to unravel multidirectional associations and potential causal pathways in complex origins of diseases like CVD. SEM can analyze complex interrelationships among variables in a non-hypothesis-driven manner. By using SEM, we could visualize the direct and indirect effects of fat mass on cIMT via various pathways such as blood pressure and serum lipids.

SEM has been used in other fields, such as genetic epidemiology and psychology,[19,20] but remains very rarely used in medical research. Path analysis is an appropriate method to assess the causal contribution of one variable to another.[14] It assumes that causality is not a 1-to-1 correspondence between cause and effect but that each dependent variable has an unexplained variance. However, to determine causal relationships with certainty, there has to be a time course.[13] The promising results of the present study, applying path analysis in clinical research, might motivate the use of SEM in complex origins of disease to assess causal relationships.

Many of the estimated effects in the present study were substantial and statistically significant, which is remarkable, especially when taking into account the young age of the healthy study population. The effect sizes on cIMT remained low, but we can conclude that even at such a young age, a higher fat mass already has a negative influence on the cardiovascular status. This finding is alarming because the effects are likely to be larger in subjects of an older age.

As was expected, the pathways differed between males and females. The relatively large effect of fat mass on ASP in females might be attributable to their higher percentage of fat mass compared with males. Fat mass had an indirect effect on TG via ASP in the female model, in contrast to the direct effect shown in the male model. It was shown previously that females have higher lipolytic rates than males, independently of the percentage of fat mass.[21] This induces higher levels of free fatty acids (FFA), and consequently, the effect of FFA on TG might mask the relatively small direct effect of fat mass on TG in the female model. The sexual dimorphism in effects of serum lipid levels on cIMT is likely to be affected by sex hormones as well. It is well established that premenopausal females have a lower risk of developing atherosclerosis than age-matched males.[22] Because estrogens have hypolipidemic properties, these are also likely to attribute to the differences between the male and female models.[23]

In contrast to the female model, the male model showed a direct effect of LDL&apoB on cIMT, a measure of atherosclerosis.[24] An explanation of this finding might be that LDLc particles in males are smaller than those in females, which was shown previously to be predictive of increased CVD risk.[25]

Both models show a significant total effect of fat mass on cIMT. The indirect effects are, for a considerable part, ascribed to the effect of fat mass on blood pressure, both in males and females. This indicates that the effect of fat mass on blood pressure and cIMT is not exerted only via serum lipids. Other pathophysiological processes might also play a role, such as inflammatory

effects. The present results warrant further investigations using SEM to expand the models, including more variables.

When interpreting the female model for clinical practice, it should be taken into account that a large percentage of the females used OC. Because the lipid profile of OC users differs from that of non-OC users,[26] we tested the model fit of the female model in OC users and non-OC users separately. Despite the small number of participants without OC use, the same female model did show a good fit in both OC users and non-OC users. Thus, it is very likely that the pathways from fat mass to cIMT are similar for OC users and non-OC users.

A desirable goal of SEM is to have a minimal subject/parameter ratio of 10:1 to achieve sufficient power.[13] Although the present study meets this requirement, this rule remains arbitrary. The present study used a model-generating rather than a confirmatory approach.[14] The strength of this approach is that it is non-hypothesis driven, although the measured variables have to be preselected. However, one weakness is that this is inevitably accompanied by multiple testing. In addition, this approach is exploratory, and for definitive conclusions, external validation of the models in another large group of young adults is desirable, using SEM in a confirmatory approach taking into account multiple testing.

In conclusion, this is the first study using SEM to present a model of complex direct and indirect effects of fat mass leading to changes in cIMT. This study resulted in a path model with a good fit. It showed that vascular status, measured by cIMT, is influenced by fat mass via blood pressure and serum lipids, even in young healthy adults. Further, the model showed that the lipid profile has a larger effect in the development of atherosclerosis in males than in premenopausal females.

Perspectives

We introduce an accessible method for analyzing complex origins of diseases. The promising results of the present study, applying path analysis in clinical research, might motivate the use of SEM to assess causal relationships in future research. In a public health perspective, our data indicate that higher fat mass in young adulthood should be prevented because it is associated with vascular changes through various pathways, even at such a young age. Because the prevalence of fat accumulation in childhood and adulthood is increasing rapidly, this is likely to induce future problems in public health.

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Supplement

Table S1. Standardized path coefficients of the males and females, respectively

	Males			Females		
Pathway	**Coeff**	**95% CI**	**Bootstrapped 95% CI**	**Coeff**	**95% CI**	**Bootstrapped 95% CI**
%FM→ASP*	0.006	-0.169 to 0.181	-0.172 to 0.184	0.163	0.027 to 0.299	0.038 to 0.288
%FM→FFA	0.295	0.135 to 0.455	0.115 to 0.475	–	–	–
%FM→TG	0.485	0.353 to 0.616	0.295 to 0.672	–	–	–
%FM→LDL&apoB	0.118	-0.071 to 0.306	-0.105 to 0.341	0.125	-0.007 to 0.257	-0.004 to 0.254
%FM→HDL&apoA	-0.131	-0.321 to 0.058	-0.320 to 0.058	-0.086	-0.098 to -0.074	†
%FM→BP	0.277	0.095 to 0.459	0.094 to 0.460	0.337	0.214 to 0.460	0.203 to 0.471
ASP→TG	0.206	0.059 to 0.353	0.071 to 0.341	0.188	0.055 to 0.322	0.048 to 0.328
FFA→TG	–	–	–	-0.121	-0.014 to 0.256	-0.012 to 0.254
TG→LDL&apoB	0.257	0.073 to 0.442	-0.006 to 0.520	0.276	0.148 to 0.403	0.135 to 0.417
TG→HDL&apoA	-0.233	-0.419 to -0.046	-0.428 to -0.038	–	–	–
TG→BP	0.110	-0.078 to 0.297	-0.110 to 0.330	0.113	-0.019 to 0.246	-0.012 to 0.238
LDL&apoB→BP	0.043	-0.040 to 0.126	-0.053 to 0.139	0.062	-0.058 to 0.181	-0.076 to 0.200
LDL&apoB→IMT	0.205	0.039 to 0.372	0.033 to 0.377	–	–	–
HDL&apoA→IMT	-0.061	-0.148 to 0.027	-0.161 to 0.039	-0.084	-0.202 to 0.035	-0.222 to 0.054
BP→IMT	0.182	0.015 to 0.349	0.005 to 0.359	0.177	0.041 to 0.312	0.037 to 0.317

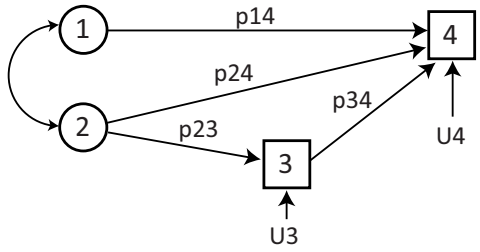
Coeff= Standardized path coefficients, 95% CI= 95% confidence interval, %FM= percentage body-fat, FFA= free fatty acids, TG= triglycerides, ASP= acyl stimulation protein, HDL= high-density lipoprotein cholesterol, apoA1= apolipoprotein A-I, LDL= low-density lipoprotein cholesterol, apoB= apolipoprotein B, BP= blood-pressure, cIMT= carotid intima media thickness * %FM → ASP stands for: effect of fat mass percentage on ASP. † Because the effect of fat mass percentage on HDL&apoA was fixed at -0.10, it was not possible to calculate the 95% confidence interval for this effect after bootstrapping.

Figure S1 Path Analysis

Path analysis is used to study interrelationships between variables. The pathways that are determined using path analysis, can be displayed using a path diagram.

Path Diagrams

The following diagram is an example of a path analysis.



In this diagram;

- Latent, unmeasured, or unobserved variables are denoted in path analysis by a circle. Manifest, measured or observed variables enclosed in squares.
- Variables 1 and 2 are exogenous variables. Exogenous variables are variables whose causes are not represented in the model. These variables are causally prior to all dependent variables in the model. Any variable without a single-headed arrow going into it is termed an exogenous variable. One exogenous variable can be joined to another by a double headed arrow; this denotes a correlation between the two exogenous variables.
- Variables 3 and 4 are endogenous variables. The causes of endogenous variables are specified in the model.
- Exogenous variables must always be independent variables. Endogenous variables can be either dependent or independent.
- U3 and U4 are disturbances/residual terms.
- The arrows represent direct causal effects of the model, also known as the structural effects.
- Path coefficients are represented by p12, p23, p24, and p34 in the model

References supplement

http://ibgwww.colorado.edu/~carey/p4102dir/handouts/path_analysis/pathnew.htm

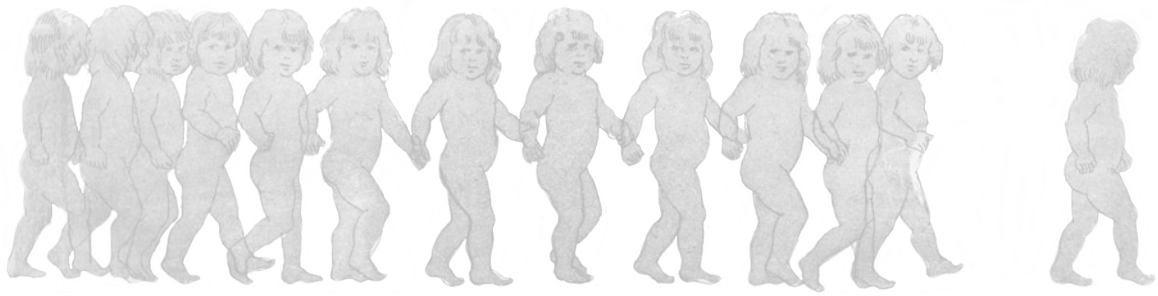
http://www.nd.edu/~rwilliam/stats2/l62.pdf

References

1. WHO. World Health Statistics. World Health Organization. 2008.
2. Hofman A, Breteler MM, van Duijn CM, Krestin GP, Pols HA, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, Witteman JC. The Rotterdam Study: objectives and design update. *Eur J Epidemiol. 2007; 22:819-829.*
3. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB Sr, Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *Am J Epidemiol*. 2007;165: 1328-1335.
4. Rabe-Hesketh S, Skrondal A. Classical latent variable models for medical research. *Stat Methods Med Res*. 2008;17:5-32.
5. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *J Am Med Assoc*. 2009;301: 2234-2242.
6. Krassas GE, Tzotzas T. Do obese children become obese adults: childhood predictors of adult disease. *Pediatr Endocrinol Rev*. 2004; 1(suppl 3):455-459.
7. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet*. 2004;363:1642-1645.
8. Baker JL, Olsen LW, Sorensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. *N Engl J Med*. 2007;357: 2329 2337.
9. Berghofer A, Pischon T, Reinhold T, Apovian CM, Sharma AM, Willich SN. Obesity prevalence from a European perspective: a systematic review. *BMC Public Health*. 2008;8:200.
10. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P. The intra-and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res*. 2004;12:1925-1929.
11. Yang Y, Lu HL, Zhang J, Yu HY, Wang HW, Zhang MX, Cianflone K. Relationships among acylation stimulating protein, adiponectin and complement C3 in lean vs obese type 2 diabetes. *Int J Obes* (Lond). 2006; 30:439-446.
12. Leunissen RW, Gao Y, Cianflone K, Stijnen T, Hokken-Koelega AC. Growth patterns during childhood and the relationship with acylationstimulating protein. *Clin Endocrinol (Oxf).* 2010;72:775–780.
13. Kline RB. Structural models with observed variables and path analysis: I. Fundamentals, recursive models. Structural Equation Modeling. Principals and Practice of Structural Equation Modeling. New York, NY: *The Guilford Press*; 1998:112.
14. Jöreskog KG, Sörbom D. In: Stam L, Darrell Bock R, eds. LISREL 8: Structural Equation Modeling With the SIMPLIS Command Language. Chicago, Ill: Scientific Software International, Inc; 1993.
15. Bentler PM. Comparative fit indexes in structural models. *Psychol Bull*. 1990;107:238-246.
16. Tucker LR, Lewis C. A reliability coefficient for maximum likelihood factor analysis. *Psychometrika*. 1973;38:1-10.
17. Browne MW, Cudeck R. Alternative ways of assessing model fit. In: Bollen KA, Long JS, eds. Testing Structural Equation Models. Beverly Hills, Calif: Sage Publications; 1992:136-162.
18. Hu LT, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Structural Equation Modeling*. 1999;6:1-55.
19. Pieterse K, van Dooren S, Seynaeve C, Bartels CC, Rijnsburger AJ, de Koning HJ, Klijn JG, van Elderen T, Tibben A, Duivenvoorden HJ. Passive coping and psychological distress in women adhering to regular breast cancer surveillance. *Psychooncology*. 2007;16:851-858.
20. Schur EA, Noonan C, Buchwald D, Goldberg J, Afari N. A twin study of depression and migraine: evidence for a shared genetic vulnerability. *Headache*. 2009;49:1493-1502.
21. Mittendorfer B. Sexual dimorphism in human lipid metabolism. *J Nutr.* 2005;135:681-686.
22. Maxwell SR. Women and heart disease. *Basic Res Cardiol*. 1998;93; (suppl 2):79-84.

23. De Marinis E, Martini C, Trentalance A, Pallottini V. Sex differences in hepatic regulation of cholesterol homeostasis. *J Endocrinol*. 2008;198: 635-643.
24. Paul TK, Srinivasan SR, Wei C, Li S, Bhuiyan AR, Bond MG, Tang R, Berenson GS. Cardiovascular risk profile of asymptomatic healthy young adults with increased femoral artery intima-media thickness: the Bogalusa Heart Study. *Am J Med Sci.* 2005;330:105-110.
25. Lemieux I, Pascot A, Lamarche B, Prud'homme D, Nadeau A, Bergeron J, Despres JP. Is the gender difference in LDL size explained by the metabolic complications of visceral obesity? *Eur J Clin Invest.* 2002;32: 909-917.
26. Berenson AB, Rahman M, Wilkinson G. Effect of injectable and oral contraceptives on serum lipids. *Obstet Gynecol.* 2009;114:786-794.

Chapter 8



Influence of preterm birth and birth size on gonadal function in young men

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Abstracts

Background/Objectives: Preterm birth has been associated with reduced reproduction rates and being born small for gestational age (SGA) with reduced gonadal function. We hypothesized that alterations concerning gonadal function in young men are not due to preterm birth or being born SGA, but are due to other (environmental) factors.

Methods: In 207 young men of the PROGRAM/PREMS cohort study, aged 18-24 yr, the influence of preterm birth, birth length, and birth weight on serum levels of anti-Mullerian hormone, inhibin B, testosterone, SHBG, non-SHBG-bound testosterone, LH, and FSH was analyzed with multiple regression modeling. In addition, markers of male gonadal function were analyzed in four subgroups: men born SGA with either short stature or catch-up growth, or men born appropriate for gestational age with idiopathic short stature or with normal stature (control).

Results: Preterm birth and SGA did not affect gonadal function. After adjustment for age, birth size, adult height, fat mass, and socioeconomic status (SES), preterm birth even showed a positive relation with inhibin B. Higher SES was associated with higher inhibin B levels. Higher fat mass was associated with decreased testosterone and SHBG levels and maternal smoking with increased LH and non-SHBG-bound testosterone levels. After adjustment for confounders, there were no significant differences in gonadal function between the subgroups.

Conclusion: Preterm birth and SGA did not affect gonadal function in young men. Factors that affected gonadal function were lower SES, a higher fat mass, and maternal smoking during pregnancy.

Introduction

Preterm birth has been associated with chronic diseases in later life, such as type 2 diabetes, and hypertension.[1] Only limited research has been performed regarding the influence of preterm birth on gonadal function. Recently, a longitudinal study in Norway showed a reduced reproduction rate in men born preterm; this diminished reproduction improved with increasing gestational age.[2] It is unknown whether this effect is related to marital status, lower social class, or reduced gonadal function.

Controversies exist concerning the relation between birth size and gonadal function. Alterations indicating a reduced gonadal function in males born small for gestational age (SGA) were found, such as a smaller testicular size and a decreased testosterone level in post pubertal boys and increased serum FSH in infancy.[3,4] In contrast, others did not find significant differences in gonadal function between boys born SGA and those born appropriate for gestational age (AGA).[5,6] Even elevated serum levels of inhibin B in men born SGA were reported.[7]

We hypothesized that both preterm birth and small birth size for gestational age do not reduce male gonadal function in young adulthood. Differences concerning gonadal function between subgroups in previous studies might be caused by lack of adjustment for confounders such as fat mass and socioeconomic status (SES). To test our hypothesis, we investigated the influence of preterm birth, birth weight, birth length, adult height, fat mass, SES, and maternal smoking during gestation on several markers of gonadal function in a large group of male subjects aged 18-24 yr. We determined LH, FSH, inhibin B, testosterone, anti-Müllerian hormone (AMH), and SHBG.[8-12]

In addition, we investigated whether gonadal function differed among four clinically relevant subgroups of young adult men: men with a short stature born SGA (SGA-S), men with a normal stature born SGA (SGA-CU), men with an idiopathic short stature (ISS) and a control group of men born AGA (control).

Subjects and Methods

Subjects

The PROGRAM/PREMS study cohort consists of 207 healthy men with an age range between 18 and 24 yr. Participants were recruited from hospitals in The Netherlands, where they had been registered because of being born prematurely (<36 wk gestational age), being small at birth (SGA with a birth length <-2 SD)[13] or showing short stature (with an adult height <-2 SD after being born SGA or AGA).[14] In addition, healthy subjects (neither small at birth nor having short stature) from schools with different educational levels were randomly asked to participate as controls. This design was purposely chosen to increase the contrast within the study population regarding birth size and adult stature. Figure 1 shows a flow chart; the participation rate of the PROGRAM/

PREMS study cohort was 79.5%. All participants fulfilled the same inclusion criteria: 1) age 18-24 yr; 2) born singleton; 3) Caucasian; 4) uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below three after five min), without sepsis or long-term complications of respiratory ventilation, such as bronchopulmonary dysplasia; and 5) maximum duration of respiratory ventilation and/or oxygen supply in the neonatal period of two weeks. Subjects were excluded if they had been suffering from any serious complication or condition (including necrotizing enterocolitis, intraventricular hemorrhage with a degree of three or more, spastic hemiplegia, or quadriplegia), from any disease or had received any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, GH treatment, treatment with glu cocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes, or serious dysmorphic symptoms suggestive for a yet unknown syndrome. Participants were excluded from the present study if they had disorders (e.g. hypopituitarism) or used medication that might affect gonadal function or when they had undergone orchiopexy.

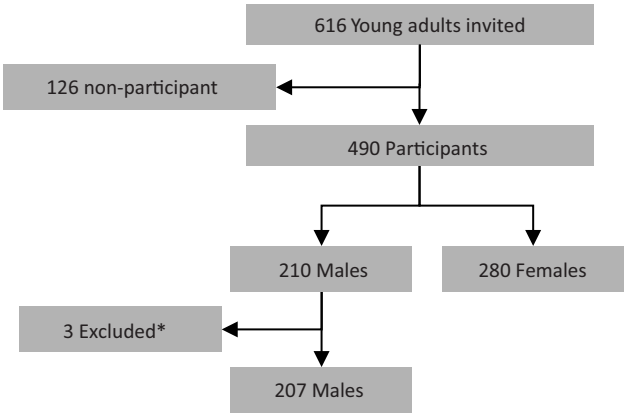

Figure 1. Flowchart of the PROGRAM/PREMS study cohort.
* Excluded from present study due to orchiopexy, hypopituitarism or medication use affecting gonadal function.

Birth data regarding gestational age and birth size were taken from records of hospitals, community health services, and general practitioners. Information regarding SES, smoking, alcohol use, and gestational smoking was obtained using questionnaires, which were answered by the participant and his mother (including questions about gestation). Education level of the participant was used as a socioeconomic indicator to determine SES.[15]

The Medical Ethics Committee of Erasmus Medical Centre (Rotterdam, The Netherlands), approved the study. Written informed consent was obtained from all participants.

Based on the SD-scores (SDS) of birth length and adult height, the participants were assigned to one of four subgroups. To increase statistical power for subgroup comparisons, the cutoff values of normal birth length and adult height were set at above -1 SDS (± 0.1 SDS). Of 207 participants, 140 were included in one of the four subgroups: 1) subjects born SGA (<-2 SDS) with a short adult height (<-2 SDS) (SGA-S) (n=18); 2) subjects born SGA (<-2 SDS) with catch-up growth resulting in a normal adult height (>-1 SDS) (SGA-CU) (n=38); 3) subjects born at term and AGA (birth length >-1 SDS) with growth retardation resulting in a short adult height (<-2 SDS)(ISS) (n=16); and 4) subjects born AGA (birth length >-1 SDS) and a normal adult height (>-1 SDS) (controls) (n=68).

Measurements

All participants visited the Erasmus Medical Centre in Rotterdam. They had been fasting for at least 12 h and had abstained from smoking and alcohol for at least 16 h. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer, and weight was measured to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice, and the mean value was used for analysis. All fasting blood samples were drawn between 0800 and 1300 h and centrifuged after clotting.

Fat mass was measured on one DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was performed daily. The intra-assay coefficient of variation for lean tissue and fat tissue was 1.57-4.49% and 0.41-0.88%, respectively.[16]

Assays

All samples were kept frozen until assayed (-80ºC). Per subject, all hormone concentrations were analyzed in the same blood sample in the same laboratory. Inhibin B was measured using ELISA, intra and interassay coefficients of variation were less than 9% and 15%, respectively. AMH was measured using an in-house double-antibody ELISA.[17] Of 37 men, the AMH levels were measured using the Immunotech-Coulter assay. The values from the Immunotech-Coulter assay were adjusted (*2.147) for comparison with the in-house ELISA. The intra and interassay coefficients of variation were less than 5% and 10% in the in-house ELISA and less than 5% and 8% in the Immunotech-Coulter assay, respectively. LH, FSH, and SHBG were measured using immunometric assays (Immulite 2000, Siemens DPC), the intra and interassay coefficients of variation were less than 5% and 12% for LH, less than 3% and 8% for FSH, and less than 7% and 9% for SHBG. Testosterone levels were measured by coated tube RIA (Siemens DPC); intra and interassay coefficients of variation were less than 7% and 9%. Non-SHBG-bound testosterone (T non-SHBG) was calculated using the method described by Sodergard et al.[18] using a fixed albumin level of 40 g/liter. The formulas for these calculations have been described earlier.[19]

Statistical analysis

SDS for birth length, birth weight, adult height, and adult weight were calculated to correct for gestational age and age.[13,14] Due to a skewed distribution, AMH, testosterone, SHBG, LH, and FSH concentrations were log-transformed.

The associations between birth size and various gonadal parameters were analyzed with multiple regression modeling. Birth length SDS, adult height SDS, and an interaction term for birth length SDS and adult height SDS were added to all models because the study cohort had been selected on the basis of birth length and adult height.[20] This ensured that the effect of these variables was modeled correctly. For the first models, we entered age, preterm birth (born <36 wk gestation) and birth weight SDS (model 1). In the second models, we added adult weight SDS (model 2). Thirdly, we replaced weight SDS for fat mass (model 3). Finally, we added SES to the models (model 4). Smoking and alcohol use were added to the last models, but these factors had no significant effects.

We also performed multiple regression analysis to determine the effect of maternal smoking during gestation on gonadal parameters, corrected for age, preterm birth, birth length SDS, birth weight SDS, adult height SDS, fat mass, and SES.

In all regression models, the variance inflation factor was determined to evaluate collinearity among covariates. The variance inflation factor was less than 4.0 for each covariate in each model, indicating that collinearity did not substantially influence the regression estimates.[21]

ANOVA was used to determine whether there were differences between subgroups with regard to group characteristics. Bonferroni correction was used for pairwise group comparisons. A Kruskal-Wallis test was used to determine associations between preterm birth/subgroups and SES. To determine which subgroups differed significantly regarding SES, the Kruskal-Wallis test was performed pairwise. To be able to compare the gonadal parameter levels of the present study to those of previous studies, unadjusted parameter levels of SGA-S and SGA-CU were compared with those of controls, selecting the subgroups on birth weight SDS instead of birth length SDS (using the same cutoff values).

To determine differences in gonadal function between the four subgroups, an analysis of covariance model was used with controls as reference group and SGA-S, SGA-CU, and ISS as dummy variables, adjusted for age (model A). Stepwise additional adjustment was performed for adult height SDS (model B), fat mass (model C), preterm birth (model D), and SES (model E). Adjustment for adult height SDS was done to determine differences in subgroups independent of adult height. In the final model (model F), adjustment for height SDS was removed to compare subgroups in a more clinically relevant manner.

Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if P was <0.05.

Results

The clinical characteristics of the total study population and subgroups are shown in Table 1. The mean age (SD) of the study population was 20.9 (1.7) yr.

Table 1. Clinical characteristics of men in the PROGRAM/PREMS study

			Subgroups			
	Preterm	**Term**	**SGA-S**	**SGA-CU**	**ISS**	**Controls**
n	85	122	18	38	16	68
Birth length SDS)	1.07 (1.9)	1.50 (1.4)	3.2 (1.0)[c,d]	2.8 (0.7)[c,d]	0.5 (0.44)[f]	0.3 (0.8)
Birth weight (SDS)	0.14 (1.9)[a]	1.27 (1.4)	2.15 (1.0)[c,d]	2.2 (0.9)[c,d]	0.4 (0.8)[e]	0.5 (1.3)
Gestational age (wk)	32.0 (2.1)[a]	39.3 (1.6)	37.4 (3.1)	36.3 (3.4)[g]	39.7 (1.3)[c]	35.5 (4.1)
Age (yr)	21.0 (1.7)	20.9 (1.6)	21.0 (1.9)	21.1 (1.7)	20.7 (1.7)	20.9 (1.7)
Height (cm)	180.9 (6.5)[a]	175.4 (8.8)	165.7 (4.3)[c,h]	182.4 (4.4)[d]	166.9 (2.8)[c]	184.0 (4.8)
Height (SDS)	0.40 (0.9)[a]	1.15 (1.2)	2.5 (0.6)[c,h]	0.2 (0.6)[d]	2.4 (0.4)[c]	0.05 (0.7)
Weight (kg)	72.3 (11.0)	69.1 (12.8)	61.3 (8.8)[c,h]	76.2 (11.6)[d]	60.4 (11.3)[c]	76.3 (11.4)
Weight (SDS)	0.30 (1.1)[b]	0.68 (1.4)	1.6 (1.0)[c,h]	0.1 (1.1)[d]	1.7 (1.3)[c]	0.1 (1.0)
Fat mass (kg)	12.9 (9.2)	11.8 (7.8)	11.0 (5.5)	14.5 (8.6)	11.8 (9.0)	13.8 (10.4)
Fat mass (%)	16.8 (8.5)	16.3 (7.9)	17.4 (6.5)	18.2 (8.3)	18.1 (10.5)	17.0 (9.0)
SES (%)						
1	14.1	12.7	20.0	27.3	7.1	3.4
2	28.1	25.5	40.0	21.2	42.9	20.3
3	57.8	61.8	40.0	51.5	50.0	76.3

Data are expressed as mean (SD) or percentage. [a] P<0.001 compared with term. [b] P<0.05 compared with term. [c] P<0.001 compared with controls. [d] P<0.001 compared with ISS. [e] P<0.05 compared with controls. [f] P<0.01 compared with controls. [g] P<0.05 compared with ISS. [h] P<0.001 compared with SGA-CU.

Preterm born men had a higher birth weight SDS and weight SDS and were taller than men born at term. The ISS group had a higher mean gestational age than the controls because this group consisted of only men born at term (39.7 wk vs. 35.5 wk; P<0.001). There was no significant difference in SES between preterm and term born men. However, there was a significant difference between subgroups with regard to SES (P=0.007), with SGA-S and SGA-CU having a lower SES than controls (P=0.004 and P=0.005, respectively). Unadjusted serum levels of gonadal parameters for men born preterm vs. term are shown in Table 2. The preterm born men had higher serum inhibin B levels (P=0.002). Furthermore, there were no differences between preterm and term born men regarding gonadal function.

Table 2. Unadjusted gonadal parameters in preterm vs. term subjects and in the various subgroups

			Subgroup selection					
	Prematurity		Birth length SDS				Birth weight SDS[b]	
	Preterm	Term	SGA-S	SGA-CU	ISS	Controls	SGA-S	SGA-CU
n	85	122	18	38	16	68	13	34
Inhibin B (ng/liter)	230.0 (182.5-282.5)[c]	195.0 (148.0-249.5)	169.5 (130.5-254.5)	175.5 (141.0-234.3)[d,e]	256.0 (161.0-311.0)	230.5 (187.3-275.0)	172.0 (138.5-262.0)	182.0 (148.0-242.0)[f]
AMH (g/liter)[a]	15.2 (11.5-20.4)	16.8 (3.7-21.6)	17.3 (14.4-24.3)	16.5 (12.1-24.2)	15.7 (14.3-17.9)	16.0 (12.3-22.9)	14.5 (11.0-17.6)	14.8 (10.0-17.4)
T (nmol/liter)[a]	19.2 (16.5-23.3)	20.0 (16.9-23.3)	19.8 (15.0-23.3)	18.7 (16.1-22.2)	21.8 (19.1-24.6)	20.5 (17.1-22.9)	19.7 (15.7-23.2)	18.4 (16.0-20.2)
SHBG (nmol/liter)[a]	27.6 (20.8-33.4)	27.1 (22.7-34.9)	28.0 (18.8-39.1)	27.2 (20.6-35.0)	27.5 (23.5-34.3)	25.7 (21.7-32.3)	25.8 (20.3-29.8) 2	5.4 (18.8-30.2)
T non-SHBG (mol/liter)	13.0 (11.3-14.9)	13.1 (11.5-15.0)	13.9 (10.5-15.8)	12.5 (10.6-13.9)	14.0 (12.7-17.1)	12.9 (11.6-15.3)	14.4 (10.9-16.6)	12.7 (10.5-14.0)
LH (U/liter)[a]	4.2 (2.9 -6.4)	4.2 (2.9-5.7)	4.1 (3.0-5.7)	4.3 (3.2-6.1)	5.3 (3.6 -8.0)	4.0 (3.2-5.4)	4.6 (3.5-5.5)	3.9 (2.7-6.3)
FSH (U/liter)[a]	4.4 (3.2-6.7)	4.5 (3.0-7.2)	4.6 (3.8 -8.0)	5.3 (2.9-7.7)	5.3 (3.6 -8.0)	4.1 (2.9-5.8)	5.7 (3.9 -8.2)	4.5 (2.7-7.6)

Values are given as median (interquartile range). Number of missing data: inhibin B, n=1; AMH, n=1; T, n=1; SHBG, n=3; LH, n=2; and FSH, n=3. [a]Log-transformed for ANOVA. [b] SGA-S and SGA-CU subgroups selected on birth weight SDS instead of birth length SDS for comparison with previous studies. [c]P = 0.002 compared with term. [d]P = 0.015 compared with controls. [e]P = 0.032 compared with ISS. [f]P = 0.018 compared with controls.

Table 3. Multiple regression analysis: factors of influence on gonadal parameters

		Preterm birth		Birth length SDS		Birth weight SDS		Adult height SDS		Adult weight SDS		Fat mass (kg)		SES		Adjusted R^2
	Model	β	P	β	P	β	P	β	P	β	P	β	P	β	P	
Inhibin B (ng/liter)[a]	2	23.3	n.s.	1.61	n.s.	8.54	n.s.	-7.5	n.s.	-6.89	n.s.					0.092
	3	25.7	0.072	2.61	n.s.	7.31	n.s.	-10.8	n.s.			-1.23	n.s.			0.099
	4	28.7	**0.044**	1.98	n.s.	6.29	n.s.	-12.8	0.080			-0.89	n.s.	18.3	**0.042**	0.117
T (nmol/liter)[a,b]	2	-0.04	n.s.	-0.01	n.s.	0.01	n.s.	0.03	n.s.	-0.08	**<0.001**					0.064
	3	-0.02	n.s.	<0.01	n.s.	-0.01	n.s.	-0.02	n.s.			-0.01	**<0.001**			0.099
	4	-0.01	n.s.	<0.01	n.s.	-0.01	n.s.	-0.02	n.s.			-0.01	**<0.001**	0.02	n.s.	0.095
SHBG (nmol/liter)[a,b]	2	-0.05	n.s.	-0.02	n.s.	0.03	n.s.	0.11	**0.003**	-0.16	**<0.001**					0.173
	3	-0.01	n.s.	-0.01	n.s.	0.01	n.s.	0.02	n.s.			-0.02	**<0.001**			0.144
	4	-0.02	n.s.	-0.01	n.s.	0.01	n.s.	0.02	n.s.			-0.02	**<0.001**	0.02	n.s.	0.141
FSH (U/liter)[a,b]	2	0.14	n.s.	-0.02	n.s.	-0.02	n.s.	0.07	n.s.	-0.07	n.s.					0.015
	3	0.16	n.s.	-0.01	n.s.	-0.03	n.s.	0.03	n.s.			-0.01	n.s.			0.012
	4	0.13	n.s.	0.01	n.s.	-0.02	n.s.	0.05	n.s.			-0.01	**0.044**	0.16	**0.009**	0.048

Table shows only the significant models. Number of missing data: fat mass, n=3; SES, n=33. Significant P values are given in bold. n.s.= not significant (P>0.10); β = regression coefficient. All models include an adjustment for age (n.s.) and the interaction term for birth length SDS and adult height SDS. [a]Log-transformed. [b]Model 1 not shown (all regression coefficients were n.s.).

The associations of preterm birth and birth size with various gonadal parameters were analyzed using multiple regression modeling (Table 3). This indicated that in the total study population preterm birth might have an effect on Sertoli cell function; in the final model (4), preterm birth was positively related with serum inhibin B levels (P=0.044) after adjustment for age, birth length SDS, birth weight SDS, adult height SDS, fat mass, and SES. Birth weight and birth length did not have a significant influence on any of the gonadal parameters. In the final model, a higher SES was positively related with inhibin B levels (P=0.042) and negatively with FSH levels (P=0.009). Fat mass, adjusted for adult height SDS, had an inverse relation with serum testosterone and SHBG levels, also after adjustment for age, preterm birth, birth length SDS, birth weight SDS, and SES (P<0.001). Adult height SDS showed a relation with SHBG levels; however, this relation disappeared after entering fat mass instead of adult weight SDS. The models with AMH, non-SHBG-bound testosterone, and LH as dependent factor were not significant. Smoking and alcohol use of the participants were added to the last models, but these factors had no significant effects (data not shown).

Multiple regression analysis showed that maternal smoking during gestation was significantly associated with higher serum LH levels (P<0.001), also after adjustment for age, preterm birth, birth length SDS, birth weight SDS, adult height SDS, and fat mass (Table 4). This significant effect remained after additional adjustment for SES. Although maternal smoking did not have an effect on testosterone and SHBG levels, it was significantly associated with higher serum level of non-SHBG-bound testosterone. Furthermore, maternal smoking during gestation did not influence inhibin B, AMH, and FSH levels.

There were no differences among the subgroups in unadjusted serum levels of gonadal parameters, except for inhibin B (Table 2). SGA-S and SGA-CU had lower inhibin B levels than ISS and controls, which reached significance in SGA-CU (P=0.032 and P=0.015, respectively). Inhibin B levels also differed between SGA-CU and controls after selection for birth weight SDS instead of birth length SDS (P=0.018). However, all median gonadal parameters were within the normal range for adult men.

Table 5 shows the results of the analysis of covariance to compare subgroups after adjustment for age, adult height SDS, fat mass, preterm birth, and SES. SGA-CU had lower serum inhibin B levels than controls when corrected for preterm birth, adult height SDS, and fat mass (P=0.026), but this significant difference disappeared after additional adjustment for SES (model E). In model A, SGA-S also showed lower inhibin B levels than controls (P=0.011), but after adjustment this was no longer significant. ISS subjects had higher inhibin B levels than controls after adjustment for age, fat mass, preterm birth, and SES (model F). However, this difference was not significant after additional adjustment for height SDS (model E). There were no significant differences between the subgroups regarding AMH, testosterone, SHBG, non-SHBG-bound testosterone, LH, and FSH in any of the models.

Table 4. Multiple regression analysis: the effect of maternal smoking on gonadal parameters adjusted for fat mass, SES, and other confounders

	Fat mass (kg)		Maternal smoking		SES		Adjusted R^2
Dependent variables	**β**	**P**	**β**	**P**	**β**	**P**	
LH (U/liter)[a]	-0.01	n.s.	0.44	**<0.001**			0.076
	-0.01	n.s.	0.45	**<0.001**	0.04	n.s.	0.071
T[a]	-0.01	**<0.001**	0.09	n.s.			0.163
	-0.01	**<0.001**	0.10	0.091	0.032	n.s.	0.162
SHBG[a]	-0.02	**<0.001**	-0.06	n.s.			0.163
	-0.02	**<0.001**	-0.07	n.s.	-0.01	n.s.	0.157
T non-SHBG	-0.07	**0.012**	1.49	**0.016**			0.048
	-0.06	**0.029**	1.63	**0.009**	0.516	n.s.	0.057

Table shows only the significant models. Non-significant dependent variables: Inhibin B, AMH, FSH. All models include an adjustment for age, preterm birth, birth weight SDS, birth length SDS, adult height SDS, and the interaction term for birth length SDS and adult height SDS (all n.s.). Number of missing data: fat mass, n=3; maternal smoking, n=60; SES, n=33. Significant P values are given in bold. n.s., Not significant (P>0.10); , regression coefficient; T, testosterone. [a] Log-transformed.

Table 5. Differences in inhibin B levels between subgroups after adjustment for confounders

	Inhibin B (ng/liter)											
	Model A		**Model B**		**Model C**		**Model D**		**Model E**		**Model F**	
Variables	**β**	**P**	**β**	**P**	**β**	**P**	**β**	**P**	**β**	**P**	**β**	**P**
SGA-S	-60.7	**0.011**	-62.1	n.s.	-61.7	n.s	-55.5	n.s	-45.3	n.s.	-43.0	0.077
SGA-CU	-46.7	**0.010**	-46.8	**0.011**	-43.9	**0.015**	-39.5	**0.026**	-32.1	0.081	-31.9	0.078
ISS	44.7	0.086	43.4	n.s.	45.7	n.s.	60.2	n.s.	66.8	0.086	68.9	**0.010**
Adjusted R^2		0.107		0.099		0.134		0.170		0.177		0.185

Significant P values are given in bold. n.s. = not significant (P>0.10); β = regression coefficient. Model A is corrected for age. Model B is corrected for age, adult height. Model C is corrected for age, adult height, fat mass (kg). Model D is corrected for age, adult height, fat mass (kg), preterm birth. Model E is corrected for age, adult height, fat mass (kg), preterm birth, SES. Model F is corrected for age, fat mass (kg), preterm birth, SES.

Discussion

In this cohort study, we investigated the influence of preterm birth and birth size on gonadal function of men in early adulthood. There were no adverse effects of preterm birth and small birth size for gestational age on gonadal function. Preterm birth even had a positive association with serum levels of inhibin B.

Other factors showing a significant relation with gonadal function in young men were SES, fat mass, and maternal smoking during gestation. Higher SES was associated with higher inhibin

B levels and lower FSH levels; fat mass was inversely related with testosterone and SHBG levels; and maternal smoking during gestation was associated with higher LH-and non-SHBG-bound testosterone levels. Thus, preterm birth and SES influenced Sertoli cell function, and fat mass as well as maternal smoking during gestation influenced Leydig cell function.

From our results we can conclude that preterm birth does not lead to a worse gonadal function in young men. Until now only limited research has been performed regarding the influence of preterm birth on gonadal function. It was reported that gestational age had an effect on FSH and LH levels in infancy.[22,23] However, this relation has not been studied in adulthood. Previous research showed diminished reproduction rate in subjects born preterm.[2] Our results imply that it is very unlikely that this reduced reproduction rate is due to a reduced gonadal function, in particular because we found that men born preterm had higher inhibin B levels than those born at term, which remained after adjustment for various other parameters. The cause of this positive association is unclear, and we can only speculate about the clinical relevance of these findings. In fact, median inhibin B levels of both preterm and term born men were within the normal range (150-400 ng/liter). Future sperm analysis in young men might further unravel the relationship between preterm birth and Sertoli cell function.

Low birth weight or low birth length for gestational age did not influence gonadal function of men in early adulthood. These results are in line with previous studies.[5,6,24] We found lower serum inhibin B levels in normal statured men born SGA (SGA-CU), but these levels were within the normal range, and the difference disappeared after adjustment for SES. Some studies demonstrated an influence of birth size on gonadal function or subfertility in subgroup comparisons or a relationship between birth size and testis function.[4,25,26] However, none of these studies adjusted for SES, which might explain the differences in results, especially concerning inhibin B levels.

This is the first study showing a relationship between SES and Sertoli cell function. Lower SES was related with lower inhibin B levels and higher FSH levels. This relationship even remained significant after adjustment for smoking and alcohol use. An explanation of this finding could be that there are nutritional and environmental differences due to SES. Endocrine disrupters have been shown to affect pubertal development, and subjects with a lower SES might be more exposed to endocrine disrupters, for example by inadequate eating habits.[27,28] Although SES did have an effect on inhibin B, it had no effect on AMH, which is also produced by the Sertoli cells. This might be explained by the relationship between spermatogenesis and inhibin B, which is not found for AMH (de Jong, F. H., unpublished observation).

Using DXA measurements, we were able to measure fat mass. Both weight and fat mass, adjusted for adult height, showed a strongly negative association with testosterone and SHBG. These findings are in line with several studies.[29-31] One study showed lower testosterone levels in SGA males compared with males born AGA.[4] This might suggest a decreased Leydig cell function in males born SGA. However, in that study weight or fat mass was not taken into account. Our data show that one should always adjust for weight or fat mass when evaluating differences in

testosterone levels between men born SGA and men born AGA, or when assessing the influence of birth size on testosterone levels. This is the case particularly because it was previously reported that SGA subjects with catch-up growth have a higher percentage of fat mass than AGA controls,[32,33] although this difference did not reach significance in our study population.

Maternal smoking during gestation showed a relation with the pituitary-Leydig cell axis. It was positively associated with serum LH and non-SHBG-bound testosterone levels, even after adjustment for SES. Because LH and human chorionic gonadotropin (hCG) bind to the same receptor (LH receptor), a possible explanation of this finding might be the negative effect of maternal smoking on fetal hCG levels.[34,35] The LH receptor is critical for Leydig cell differentiation, and if hCG levels are decreased, compensatory increased LH levels might follow to maintain normal testicular development. Although we did not have data on maternal smoking of the total group and the R^2 remained low, we postulate that maternal smoking could be associated with altered programming of the pituitary-Leydig cell axis. Because it is known that mothers tend to underreport smoking during gestation when they had an adverse event during pregnancy,[36] it might well be that maternal smoking is also underreported in our group, which might have caused underestimation of the effect of maternal smoking during gestation on Leydig cell function. This was supported by the finding that the SGA-S and ISS groups had more missing data concerning maternal smoking than the controls (P=0.001 and P=0.026, respectively). Previous studies reported an influence of gestational smoking on the semen quality and testis size of young men.[37,38] We did not find an effect of gestational smoking on Sertoli cell function, which could imply that the reported effect on semen quality is caused by impairment of other factors, such as germinative epithelium and accessory sex glands.[38] To our knowledge, a relation between maternal gestational smoking and LH levels and non-SHBG-bound testosterone has never been reported.

Subgroup comparisons showed that ISS subjects had higher inhibin B levels than controls after adjustment for age, fat mass, preterm birth, and SES, even in the relatively small number of ISS subjects. This difference was not significant after additional adjustment for height SDS, which implies that height might be of influence on inhibin B levels. Also, in multiple regression modeling, the effect of height on inhibin B levels was almost significant (P=0.08). There are no previous studies on gonadal function and in particular on the inhibin B levels in (untreated) males with ISS. However, previous studies showed no influence of GH therapy on testicular size in boys with ISS and no influence of GH therapy on inhibin B levels in boys born SGA.[39] This indicates that the influence of height on inhibin B levels is unlikely due to altered GH levels.

We had the opportunity to perform comparisons between subgroups with regard to gonadal function in the largest study of young men so far. We have chosen a study population that consisted of a relatively large percentage of subjects born preterm and/or SGA compared with the normal population. This results in a better statistical model because there was more contrast in the study population, so relationships between various factors could be detected with

more statistical power. Another advantage of this study population is that it allows comparison between clinically relevant subgroups.

In conclusion, our study in 207 young men showed that preterm birth and small birth size for gestational age did not have a negative effect on gonadal function. The factors that did affect gonadal function were: SES, fat mass, and maternal smoking during gestation. However, in our population all median values of gonadal parameters remained within normal ranges for adult men and the R^2 remained low. Comparison of clinically relevant subgroups showed that after adjustment for age, adult height, fat mass, preterm birth, and SES, there were no differences with regard to serum inhibin B, testosterone, SHBG, non-SHBG-bound testosterone, LH, and FSH levels. Our findings should be confirmed by other studies. However, in public health perspective our data show that exaggerated fat accumulation during childhood and maternal smoking during gestation should be prevented in order not to disturb future testicular function.

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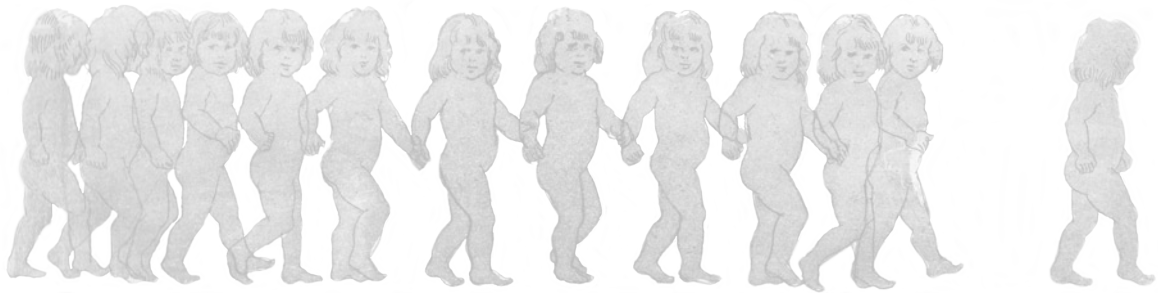
References

1. de Boo HA, Harding JE 2006 The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol* 46:4-14
2. Swamy GK, Ostbye T, Skjaerven R 2008 Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. *JAMA* 299:1429-1436
3. Ibáñez L,Valls C, ColsM,Ferrer A, Marcos MV, De Zegher F 2002 Hypersecretion of FSH in infant boys and girls born small for gestational age. *J Clin Endocrinol Metab* 87:1986-1988
4. Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E 2002 Low birth weight for gestational age and subsequent male gonadal function. *J Pediatr* 141:376-379
5. Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A 2007 Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. *J Clin Endocrinol Metab* 92:1353-1357
6. Boonstra VH, Weber RF, de Jong FH, Hokken-Koelega AC 2008 Testis function in prepubertal boys and young men born small for gestational age. *Horm Res* 70:357-363
7. Allvin K, Ankarberg-Lindgren C, Fors H, Dahlgren J 2008 Elevated serum levels of estradiol, dihydrotestosterone, and inhibin B in adult males born small for gestational age. *J Clin Endocrinol Metab* 93: 1464-1469
8. Young J, Rey R, Couzinet B, Chanson P, Josso N, Schaison G 1999 Antimullerian hormone in patients with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 84:2696-2699
9. Andersson AM, Jørgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE 2004 Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. *J Clin Endocrinol Metab* 89:3161-3167
10. Jensen TK, Andersson AM, Hjollund NH, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J, McNeilly A, Groome NP, Skakkebaek NE 1997 Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* 82:4059-4063
11. Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, Bremner WJ 1996 Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. *J Clin Endocrinol Metab* 81:3341-3345
12. Klingmüller D, Haidl G 1997 Inhibin B in men with normal and disturbed spermatogenesis. Hum Reprod 12:2376–2378
13. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-910
14. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955–1997. *Pediatr* Res 47:316-323
15. 2006 Dutch standard classification of education. Heerlen, The Netherlands: Centraal Bureau van de Statistiek, Statistics Netherlands
16. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P 2004 The intra-and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res* 12:1925-1929
17. Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, Groome NP, Themmen AP, Visser JA 2006 Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. *Endocrinology* 147:3228-3234
18. Södergård R, Bäckström T, Shanbhag V, Carstensen H 1982 Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem* 16:801-810

19. de Ronde W, van der Schouw YT, Muller M, Grobbee DE, Gooren LJ, Pols HA, de Jong FH 2005 Associations of sex-hormone-binding globulin (SHBG) with non-SHBG-bound levels of testosterone and estradiol in independently living men. *J Clin Endocrinol Metab* 90:157-162
20. Willemsen RH, Leunissen RW, Stijnen T, Hokken-Koelega AC 2009 Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab* 94:1695-1700
21. Kleinbaum DG, Kupper LL, Muller KE, Nizam A 1988 Applied regression analysis and other multivariate methods. 2nd ed. Boston: PWS-Kent
22. Massa G, de Zegher F, Vanderschueren-Lodeweyckx M 1992 Serum levels of immunoreactive inhibin, FSH, and LH in human infants at preterm and term birth. *Biol Neonate* 61:150-155
23. Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H, Tachibana Y 1983 Changes of serum gonadotropin levels and sex differences in premature and mature infant during neonatal life. *J Clin Endocrinol Metab* 56:1327-1331
24. Olsen J, Bonde JP, Basso O, Hjøllund NH, Sørensen HT, Abell A 2000 Birthweight and semen characteristics. *Int J Androl* 23:230-235
25. Allvin K, Ankarberg-Lindgren C, Fors H, Dahlgren J 2008 Elevated serum levels of estradiol, dihydrotestosterone and inhibin B in adult males born small for gestational age. *J Clin Endocrinol Metab* 93: 1464-1469
26. Francois I, de Zegher F, Spiessens C, D'Hooghe T, Vanderschueren D 1997 Low birth weight and subsequent male subfertility. *Pediatr Res* 42:899-901
27. El-Seweidy MM, Hashem RM, Abo-El-matty DM, Mohamed RH 2008 Frequent inadequate supply of micronutrients in fast food induces oxidative stress and inflammation in testicular tissues of weanling rats. *J Pharm Pharmacol* 60:1237-1242
28. Schoeters G, Den Hond E, Dhooge W, van Larebeke N, Leijs M 2008 Endocrine disruptors and abnormalities of pubertal development. *Basic Clin Pharmacol Toxicol* 102:168-175
29. Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C, Liu K 2002 Serum androgen concentrations in young men: a longitudinal analysis of associations with age, obesity, and race. The CARDIA male hormone study. *Cancer Epidemiol Biomarkers Prev* 11:1041-1047
30. Blouin K, Boivin A, Tchernof A 2008 Androgens and body fat distribution. *J Steroid Biochem Mol Biol* 108:272-280
31. van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW 2000 Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. *J Clin Endocrinol Metab* 85:3276-3282
32. Leunissen RW, Stijnen T, Hokken-Koelega AC 2009 Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol (Oxf)* 70:245-251
33. Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C 2008 Consequences of being born small for gestational age on body composition: an 8-year follow-up study. *J Clin Endocrinol Metab* 93:3804-3809
34. Varvarigou AA, Liatsis SG, Vassilakos P, Decavalas G, Beratis NG 2009 Effect of maternal smoking on cord blood estriol, placental lactogen, chorionic gonadotropin, FSH, LH, and cortisol. *J Perinat Med* 37:364-369
35. Cole LA 2009 New discoveries on the biology and detection of human chorionic gonadotropin. *Reprod Biol Endocrinol* 7:8
36. Wong M, Koren G 2001 Bias in maternal reports of smoking during pregnancy associated with fetal distress. *Can J Public Health* 92: 109-112
37. Jensen TK, Jørgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus Ø, Matulevicius V, Nermoen I, Vierula M, Keiding N, Toppari J, Skakkebaek NE 2004 Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. *Am J Epidemiol* 159:49-58

38. Ramlau-Hansen CH, Thulstrup AM, Storgaard L, Toft G, Olsen J, Bonde JP 2007 Is prenatal exposure to tobacco smoking a cause of poor semen quality? A follow-up study. *Am J Epidemiol* 165:1372-1379
39. Lindgren AC, Chatelain P, Lindberg A, Price DA, Ranke MB, Reiter EO, Wilton P 2002 Normal progression of testicular size in boys with idiopathic short stature and isolated growth hormone deficiency treated with growth hormone: experience from the KIGS. *Horm Res* 58:83-87

Chapter 9



Influence of Preterm Birth and Small Birth Size on Serum Anti-Müllerian Hormone Levels in Young Adult Women

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Abstract

Background/objectives: Preterm birth has been associated with reduced reproduction rates, and controversies remain regarding the effect of being born small for gestational age (SGA) on ovarian function. Recent findings in young men showed no effect of preterm and SGA birth on testis function. We hypothesised that follicle pool size in young adult women is also not affected by preterm and SGA birth.

Design/methods: In 279 young women of the PROGRAM/PREMS study, aged 18–24 years, the influence of gestational age, birth length and birth weight on serum levels of anti-Müllerian hormone (AMH) was analysed with multiple regression modelling. Additionally, AMH levels were analysed in preterm versus term born females and in three subgroups: females born SGA with either short stature or catch-up growth (SGA-CU), and females born term and appropriate for gestational age with normal stature (AGA controls).

Results: Preterm and SGA birth did not affect AMH and other hormone levels. Older age at menarche and oral contraceptive pill use (OC-use) were related to lower AMH levels, and maternal smoking during gestation was related to higher AMH levels. After correction for maternal smoking, lower socioeconomic status (SES) was associated with lower AMH levels. In subgroup comparisons, SGA-CU women showed higher AMH levels than AGA controls, also after adjustment for several factors.

Conclusion: Preterm and SGA birth did not affect AMH levels. Factors associated with serum AMH levels were OC-use, age at menarche, maternal smoking during gestation and SES. We conclude that preterm and/or SGA born females are not likely to have a reduced follicle pool size.

Introduction

Controversies exist regarding the association between preterm and small for gestational age (SGA) birth and abnormal ovarian function in adulthood. Some studies showed smaller ovaries and uterus in infant and adolescent women born SGA.[1,2] Others found no differences in ultrasonic measurements of the uterus and ovaries in girls born SGA;[3] neither did intrauterine growth retardation (IUGR) affect the ovarian volume of fetuses nor the volume percentage of follicles in the ovaries.[4]

With regard to the relationship between preterm birth and ovarian function, less research has been performed. Preterm birth might affect gonadal function, as reduced reproduction rates have been reported in men and women who had been born preterm.[5] However, this lower reproduction rate in women could be a consequence of altered environmental factors related to socioeconomic status (SES), rather than reduced gonadal function, as has been demonstrated in men.[6] As a consequence of undernutrition causing altered programming of the fetus and SGA birth, one might expect a negative effect of SGA birth on gonadal function.[7] However, we previously demonstrated that preterm and SGA birth did not affect inhibin B levels and other gonadal parameters in young men.[6] In young women, the association between preterm and SGA birth and ovarian function has not been studied. Because we did not expect the influence of SGA and preterm birth in women to be different from that in men, we hypothesised that the size of the follicle pool in young adult women is not affected by preterm and/or SGA birth.

To test our hypothesis, we investigated the effects of gestational age at birth, birth weight (BW) SDS, birth length (BL) SDS and adult size on serum anti-Müllerian hormone (AMH), in a large group of 279 women aged 18-24 years. AMH is correlated with the number of growing follicles and indirectly correlated with the primordial follicle pool.[8] It is a marker of the follicle pool size, which decreases with age and is undetectable after menopause.[8,9] Most studies showed that AMH levels are independent of menstrual cycle,[10-12] but controversies still exist.[13]

In addition, serum AMH levels were investigated after dividing the total study population into women born either preterm or term and into three clinically relevant subgroups of young adult women: women with either a short or a normal stature born SGA, and a group of women with a normal stature born term and appropriate for gestational age (AGA controls).

Subjects and methods

Subjects

The PROGRAM/PREMS study cohort consists of 279 healthy female participants aged 18-24 years. The subjects who were asked to participate were selected using registration data from academic hospitals in the Netherlands. They were selected because of being born preterm (gestational age <36 weeks), being small at birth (SGA with a BL <-2 SDS)[14] or showing short stature (after being

born SGA or AGA with an adult height <-2 SDS).[15] In addition, healthy subjects were randomly asked to participate as controls. They were randomly recruited by advertising in newspapers, public transport, schools with different educational levels, festivals, etc. to avoid selection bias. The participation rate of the PROGRAM/PREMS study cohort was 79.5%.[6] All subjects fulfilled the same inclusion criteria age 18-24 years, born singleton, Caucasian, an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below three after five min), without sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia, maximum duration of respiratory ventilation and/or oxygen supply in the neonatal period of two weeks. Subjects were excluded if they suffered or had suffered from any serious condition (including heart, lung, neurological, gastrointestinal and kidney disease), serious complication (including necrotizing enterocolitis, intraventricular haemorrhage with a degree of three or more, spastic hemiplegia or quadriplegia) or had been subjected to any condition or treatment known to interfere with growth (e.g. growth hormone treatment, treatment with glucocorticosteroids, radiotherapy, GH deficiency, severe chronic illness, emotional deprivation), or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive of a yet unknown syndrome.

Birth data were collected from medical records of hospitals, community health services and general practitioners. Information regarding SES, smoking, alcohol use, maternal smoking during gestation and presence of irregular menstrual cycle (before the start of oral contraceptive pill use (OC-use)) was obtained using questionnaires, which were answered by the participant and her mother (including questions about gestation). Menstrual regularity was defined as a menstrual cycle length of 21-35 days. Age at menarche was also determined using questionnaires. Previous research showed that recalled and actual age at menarche correlate well in young women.[16] Educational level of the participant was used as socioeconomic indicator to determine SES (categorised as low, medium and high; range 1-3).[17] The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, the Netherlands, approved the study. Written informed consent was obtained from all the participants.

Based on the SDS of BL and adult height, the total study group of 279 women was also divided into three subgroups. To increase the statistical power for subgroup comparison, the cut-off values for small birth size and short adult height were set at <-2 SDS, and the cut-off values for normal birth size and normal adult height were set at >-1 SDS. Based on these cut-off values, a total of 137 participants were included in one of the three subgroups:

- Subjects born SGA (BL <-2 SDS), either preterm or term, with a short adult height (<-2 SDS) (SGA-S): n=31,
- Subjects born SGA (BL <-2 SDS), either preterm or term, with catch-up growth resulting in a normal adult height (>-1 SDS) (SGA-CU): n=56,
- Subjects born AGA (BL >-1 SDS), term, with a normal adult height (>-1 SDS) (AGA controls): n=50.

To study the effect of preterm birth, women were also divided based on preterm or term birth. The preterm group comprised of women with a gestational age <36 weeks. The term group comprised women with a gestational age ≥36 weeks.

Measurements

All the participants visited the Erasmus Medical Centre in Rotterdam. They had been fasting for at least 12 h and had abstained from smoking and alcohol for at least 16 h. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer and weight to the nearest 0.1 kg using scales (Servo Balance KA-20-150S). All anthropometric measurements were performed twice, and the mean value was used for analysis. All fasting blood samples were drawn between 0800 and 1300 h and centrifuged after clotting. Fat mass (FM) was measured on a single DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was performed daily. The intra-assay coefficients of variation (CV) for lean tissue and fat tissue was 1.57-4.49 and 0.41-0.88% respectively.[18]

Assays

All samples were kept frozen (-80ºC) until assayed. Per subject, all hormone concentrations were analysed in the same blood sample at the same laboratory. The AMH levels were measured using the Diagnostic System Laboratories (DSL) ELISA (Inc., Webster, TX, USA).[19] For 33 women, the values were adjusted (∗3) because another DSL kit was used. The intra and inter-assay CV were <5 and 10% respectively. In 156 of 279 subjects, serum sex hormone-binding globulin (SHBG), testosterone, non-SHBG-bound testosterone and androstenedione were additionally determined. This group consisted of 93 OC-users and 62 non-OC-users. To ensure that LH and FSH were determined during the same phase of the menstrual cycle in all participants, LH and FSH were only determined in women who visited the hospital during day 3-6 of the menstrual cycle (n=21), or in the pill-free period in the case of OC-use (n=78).

LH, FSH, SHBG and androstenedione were measured using immunometric assays (Immulite 2000, Siemens DPC, Los Angeles, CA, USA); the intra and inter-assay CV were <5 and 12% for LH, <3 and 8% for FSH and <7 and 9% for SHBG. Testosterone was measured by coated tube RIA (Siemens DPC); the intra and inter-assay CV were <7 and 9%. Non-SHBG-bound testosterone was calculated using the method described by Sodergard et al.[20] using a fixed albumin level of 40 g/l. The formulas for these calculations have been previously described.[21]

Statistical analysis

SDS for BL, BW, adult height and adult weight were calculated, to correct for gestational age and age.[14,15] Owing to a skewed distribution, AMH, LH, FSH, SHBG, testosterone and non-SHBG-bound testosterone were log transformed; for all log transformations, natural log was used.

The associations of birth size and gestational age with AMH levels were analysed with multiple regression modelling. BL SDS, adult height SDS and an interaction term for BL SDS and adult height

SDS were added to all models because the study cohort had been selected on the basis of BL and adult height.[22] This ensured that the effect of these variables was modelled correctly. For the first model, we entered age, gestational age, BW SDS (model 1). Because recent findings suggested an influence of OC-use on AMH levels, we adjusted for OC-use in model 1.[23] In the second model, we additionally corrected for FM (kg) (model 2) because obesity has been previously related to serum AMH levels.[24] Thirdly, we added age at menarche to the model (model 3), and finally SES was added (model 4). We also performed multiple regression analysis to determine the effect of maternal smoking during gestation on AMH after correction for age, gestational age, BW SDS, OC-use, FM, age at menarche and SES. Because maternal smoking was only known for 231 of the 279 subjects in the total group, we performed a separate analysis (model 5). Unstandardised coefficients (β) are shown in the tables.

Smoking and alcohol use of the participants were added to the last models, but these factors had no significant effects and did not influence the results (data not shown). In addition, we performed similar stepwise regression modelling (models 1-4) using LH, FSH, SHBG, testosterone, non-SHBG-bound testosterone and androstenedione as dependent variables.

ANOVA was used to determine if there were differences between subgroups, and preterm- versus term-born women with regard to group characteristics. Post hoc Bonferroni's correction was used for pairwise group comparisons. A Kruskal-Wallis test was used to determine associations between preterm birth/ subgroup and OC-use, proportion of mothers smoking during pregnancy and SES, and to determine the association between maternal smoking during gestation and SES. To determine which subgroups differed significantly regarding SES, the Kruskal-Wallis test was performed pairwise. Subgroup comparisons were not done for LH and FSH because the sizes of the subgroups were too small. To determine differences in AMH, SHBG, testosterone, non-SHBG-bound testosterone and androstenedione levels between the subgroups, an ANCOVA model was used with AGA controls as reference group and SGA-S and SGA-CU as dummy variables, adjusted for age, OC-use and gestational age (model 1). To avoid over adjustment for gestational age in ANCOVA analysis, the AGA women born preterm were included in the AGA control group. This resulted in a total number of 76 subjects in the reference group. Stepwise additional adjustment was performed for FM (model 2), age at menarche (model 3) and SES (model 4). Maternal smoking during gestation was studied in a separate analysis (model 5). Smoking and alcohol use were added to the last models, but these factors did not influence the results of the subgroup comparisons.

Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA) was used for analysis. Results were regarded statistically significant if P was <0.05.

Results

Total study population

Table 1 shows the clinical characteristics of the total study population. Table 2 shows unadjusted median (interquartile range) hormone levels of the total study population. The results of multiple regression analysis are shown in Table 3. Gestational age, BW SDS and BL SDS were not significantly associated with AMH levels. OC-use had an inverse association with AMH in each model (P<0.001). In model 3, age at menarche was added, which showed a significant inverse association with AMH levels (P=0.004). Smoking and alcohol use of the participants were added to the last models, but these factors had no significant effects and did not influence the results. Although several parameters showed a significant influence on AMH, the adjusted R^2 remained low (R^2=0.15 in model 4).

Table 1. Clinical characteristics of women in the PROGRAM/PREMS study and unadjusted differences between subgroups. Values are given as mean (SD)

	Study group					
	Total (n=279)	**Preterm** (n=84)	**Term** (n=195)	**SGA-S** (n=31)	**SGA-CU** (n=56)	**AGA controls** (n=50)
BL (SDS)	-1.5 (1.6)	-1.4 (1.9)‡	-1.5 (1.5)	-2.9 (0.7)‡	-3.0 (0.9)‡	0.06 (0.8)
BW (SDS)	-1.0 (1.5)	-0.77 (1.7)*,§	-1.2 (1.4)	-2.0 (1.0)‡	-2.4 (0.8)‡	-0.15 (1.2)
GA (weeks)	37.0 (3.8)	32.0 (2.3)†,‡	39.1 (1.7)	38.6 (3.2)¶	35.9 (3.3)‡	39.4 (1.7)
Age (years)	20.7 (1.7)	20.5 (1.6)	20.8 (1.7)	20.8 (1.8)	20.6 (1.6)	20.7 (1.8)
Height (cm)	165.0 (8.7)	167.7 (6.5)†,‡	163.8 (9.2)	153.6 (3.3)‡,¶	169.9 (4.6)$^{||}$	173.0 (6.2)
Height (SDS)	-0.84 (1.3)	-0.43 (1.0)†,‡	-1.0 (1.4)	-2.6 (0.5)‡,¶	-0.12 (0.7)$^{||}$	0.41 (1.0)
Weight (kg)	61.4 (11.3)	63.3 (11.3)	60.6 (11.2)	56.2 (11.9)$^{a,||}$	64.5 (13.6)	65.0 (8.6)
Weight (SDS)	-0.46 (1.4)	-0.21 (1.3)*	-0.57 (1.4)	-1.2 (1.6)^{‡,a}	-0.10 (1.3)‡	0.10 (0.94)
BMI (kg/m^2)	22.6 (4.0)	22.5 (3.9)	22.6 (4.0)	22.5 (3.3)	23.8 (5.0)	21.7 (2.7)
Fat mass (kg)	18.9 (8.5)	20.5 (8.5)*	18.2 (8.5)	18.8 (8.8)	20.0 (10.8)	18.3 (7.5)
Fat mass (%)	30.2 (8.5)	31.8 (7.9)$^{*,||}$	29.4 (8.6)	32.7 (8.1)	30.3 (8.6)	27.6 (8.2)
Age menarche (years)	13.0 (1.4)	13.1 (1.6)	12.9 (1.4)	12.8 (1.6)	12.9 (1.4)	13.0 (1.3)
Maternal smoking (% smokers)	33.3	24.7	37.3	50.0§	45.2§	25.0
OC-use (% users)	77.0	72.6	78.9	64.5	81.8	74.0
SES (%)						
1	10.4	12.3	9.5	14.8	6.7	6.4
2	25.3	32.9^{s}	22.0	33.3^{s}	37.8$^{||}$	8.5
3	64.3	54.8	68.5	51.9	55.6	85.1

BL, birth length; BW, birth weight; GA, gestational age; OC-use, oral contraceptive pill use; SES, socioeconomic status. *P<0.05 compared with term, †P<0.001 compared with term, ‡P<0.001 compared with AGA controls, §P<0.05 compared with AGA controls, ||P<0.01 compared with AGA controls, ¶P<0.001 compared with SGA-CU, ^{a}P<0.05 compared with SGA-CU.

Because maternal smoking was only known for 231 of the 279 subjects in the total group, we performed separate analyses. Multiple regression analyses showed that maternal smoking during gestation was significantly associated with higher serum AMH levels after adjustment for age, gestational age, BW SDS, OC-use, BL SDS, adult height SDS, FM, age at menarche and SES (P=0.022) (Table 3, model 5). Only after correction for maternal smoking during gestation in model 5, SES was positively associated with AMH levels (P=0.044).

Table 4 summarises the results of regression analyses, using LH, FSH, SHBG, testosterone, non-SHBG-bound testosterone and androstenedione as dependent variables. Adult height SDS was positively associated with androstenedione levels (P=0.044), after correction for several factors including BL SDS and OC-use. The only other significant effect was OC-use, which had a significant effect on all parameters. Maternal smoking during gestation did not influence any of the additional parameters.

Subgroup comparisons

Clinical characteristics of the subgroups are shown in Table 1. Women born preterm had a higher BW SDS (P=0.043) and adult height SDS (P<0.001) than those born term. Furthermore, their mean weight SDS (P<0.046), percentage FM (P=0.030) and FM (kg) (P=0.038) were higher. The difference in SES between preterm and term born women was borderline significant (P=0.054). Women born preterm had a lower adult height (height (cm): P<0.001 and height SDS: P<0.001), a higher percentage FM than AGA controls (FM (%): P=0.004) and a lower BW SDS (P=0.037). The SES of AGA controls was higher than that of women born preterm (P=0.002).

In agreement with selection criteria, significant differences were observed between SGA-S, SGA-CU women and AGA controls in gestational age, BL SDS, BW SDS, height and height SDS. Mean gestational age of SGA-CU women was lower than that of SGA-S (P<0.001) women. SGA-S (P=0.003) and SGA-CU (P=0.004) women had a lower SES than AGA controls. There were no significant differences between subgroups regarding age at menarche and proportion of OC-use. In both SGA subgroups (SGA-S and SGA-CU), the prevalence of maternal smoking during gestation was higher than in the AGA control group (P=0.035 and P=0.050 respectively). Furthermore, lower SES was associated with a higher proportion of mothers smoking during gestation (P<0.001).

Table 2 shows median (interquartile range) serum hormone levels of the women in the PROGRAM/PREMS study divided in subgroups. Preterm and term born subjects had similar AMH levels, also after adjustment for BL SDS, adult height SDS, BW SDS, age, OC-use, FM, age at menarche and SES. Preterm born women had higher non-SHBG-bound testosterone levels (P=0.030) and androstenedione levels (P=0.012), and lower SHBG levels (P=0.013) than term-born women. However, there were no significant differences in serum hormone levels between women born preterm and AGA controls.

Table 2. Unadjusted hormone levels in the total group, preterm-versus term-born subjects and in the various subgroups. Subgroup comparisons were not done for LH and FSH because of small group sizes

	Study group					
	Total (n=279)	**Preterm** (n=84)	**Term** (n=195)	**SGA-S** (n=31)	**SGA-CU** (n=56)	**AGA controls** (n=50)
AMH (mg/l)[a,b]	7.6 (4.7-11.5)	7.8 (5.2-11.4)	7.5 (4.3-11.5)	5.9 (4.4-9.7)	9.4 (6.5-13.5)	7.9 (4.8-11.4)
LH (U/l)[a,b,c]	2.5 (0.50-4.9)	4.7 (1.9-14.6)	2.4 (0.40-4.7)	2.8 (0.43-4.6)	1.6 (0.10-3.1)	2.7 (0.20-6.0)
FSH (U/l)[a,b,c]	5.4 (1.5-7.2)	7.3 (6.1-11.5)	5.3 (1.4-7.1)	6.3 (3.9-7.4)	3.9 (0.80-6.2)	4.9 (0.70-6.6)
SHBG (nmol/l)[a,b,d]	69.5 (45.0-132.0)	54.0 (38.2-80.7)*	81.1 (50.4-152.0)	66.4 (47.7-141.0)	78.7 (52.7-136.5)	71.3 (43.4-168.0)
Testosterone (nmol/l)[a,b,d]	0.90 (0.50-1.4)	1.1 (0.80-1.5)	0.80 (0.50-1.3)	0.80 (0.50-1.3)	0.90 (0.40-1.4)	0.80 (0.50-1.4)
Non-SHBG-bound testosterone (nmol/l)[a,b,d]	0.29 (0.11-1.4)	0.46 (0.22-0.61)*	0.25 (0.10-0.50)	0.35 (0.12-0.48)	0.20 (0.09-0.48)	0.29 (0.13-0.51)
Androstenedione (nmol/l)[a,d]	8.2 (5.8-10.8)	10.2 (7.5-11.9)*	8.0 (5.3-10.3)	8.1 (6.4-10.5)	7.7 (5.0-11.3)	8.3 (5.3-10.5)

AMH, anti-Müllerian hormone; SHBG, sex hormone-binding globulin. *P<0.05 compared with term. [a]Values are given as median (interquartile range). [b]Log transformed for ANOVA. [c]Only determined during day 3-6 of the menstrual cycle (n=21) or in the pill-free period in case of OC-use (n=78). [d]SHBG, testosterone and A were determined in a subset of 157 females.

Table 3. Multiple regression analysis of factors that influence serum anti-Müllerian hormone (AMH) levels (µg/l) in young women. AMH is log transformed. A β value of 0.50, 0.10 and 0.01 equals an increase in AMH of 64.8, 10.5 and 1.01% per unit change of the independent variable respectively. Model 1 includes age, gestational age, birth weight SDS, birth length SDS, oral contraceptive pill use (OC-use), adult height SDS and an interaction term for birth length SDS and adult height SDS. Model 2 additionally includes fat mass. Model 3 additionally includes age at menarche. Model 4 additionally includes socioeconomic status (SES). Model 5 additionally includes maternal smoking during gestation.

	Model 1		**Model 2**		**Model 3**		**Model 4**		**Model 5[a]**	
	β	**P value**	**β**	**P value**	**β**	**P value**	**β**	**P value**	**β**	**P value**
GA (weeks)	-0.006	0.675	-0.004	0.805	-0.002	0.893	-0.006	0.654	-0.008	0.561
Birth weight SDS	-0.091	0.073	-0.087	0.088	-0.075	0.134	-0.083	0.098	-0.080	0.114
Birth length SDS	0.038	0.456	0.033	0.533	0.021	0.684	0.020	0.639	0.022	0.670
OC-use	-0.579	**<0.001**	-0.576	**<0.001**	-0.576	**<0.001**	-0.564	**<0.001**	-0.592	**<0.001**
Adult height SDS	-0.024	0.628	-0.026	0.594	-0.017	0.718	-0.029	0.549	-0.025	0.617
Fat mass (kg)			0.006	0.359	0.000	0.951	0.001	0.817	0.002	0.760
Age at menarche (years)					-0.105	**0.004**	-0.107	**0.003**	-0.104	**0.005**
SES							0.125	0.093	0.158	**0.044**
Maternal smoking									0.255	**0.022**
Overall P value	**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**	
Adjusted R^2	<0.11		0.11		0.14		0.15		0.18	

OC-use, oral contraceptive use (1=yes 0=no); GA, gestational age; SES, socioeconomic status. Maternal smoking: 1=yes, 0=no. [a]Maternal smoking was known in 231 of 279 women. Values in boldface indicate P<0.05.

Table 4. Multiple regression analysis of factors that influence on sex hormone-binding globulin (SHBG), testosterone, non-SHBG-bound testosterone and androstenedione. Adjusted for age and an interaction term for birth length SDS and adult height SDS.

	LH (U/l)[a,b]		SHBG (nmol/l)[a,c]		Testosterone (nmol/l)[a,c]		Non-SHBG-bound testosterone (nmol/l)[a,c]		Androstenedione (nmol/l)[c]	
	β	P value	β	P value	β	P value	β	P value	β	P value
GA (weeks)	-0.096	0.274	0.024	0.105	-0.019	0.357	-0.035	0.103	-0.103	0.323
Birth weight SDS	0.037	0.870	-0.063	0.238	0.081	0.305	0.031	0.697	-0.093	0.810
Birth length SDS	0.177	0.391	0.034	0.505	-0.047	0.521	0.033	0.654	0.096	0.790
OC-use	-1.278	**0.007**	1.025	**<0.001**	-0.720	**<0.001**	-1.386	**<0.001**	-3.899	**<0.001**
Adult height SDS	0.110	0.571	-0.058	0.258	0.097	0.177	0.139	0.067	0.750	0.044
Fat mass (kg)	0.037	0.129	0.000	0.958	0.004	0.682	0.000	0.987	-0.013	0.764
Age at menarche (years)	0.017	0.910	0.035	0.331	-0.058	0.252	-0.102	0.057	-0.251	0.335
SES	-0.227	0.361	-0.032	0.644	0.021	0.825	0.056	0.578	-0.409	0.407
Overall P value	0.054		**<0.001**		**<0.001**		**<0.001**		**<0.001**	
Adjusted R^2	0.11		0.52		0.24		0.51		0.24	

OC-use, oral contraceptive use (1=yes 0=no); GA, gestational age; SES, socioeconomic status. The model with FSH as dependent variable is not shown (overall P value was 0.220). [a]Log transformed. [b]Only determined during day 3-6 of the menses (n=21) or in the pill-free period in the case of OC-use (n=78). Subgroup comparisons were not done for LH and FSH. [c]SHBG, testosterone and A were determined in a number of 157 females. Values in boldface indicate P<0.05.

Table 5. Subgroup comparisons of anti-Müllerian hormone (AMH) levels (mg/l). Appropriate for gestational age (AGA) controls are used as reference group. AMH is log transformed. Model 1 is corrected for age, oral contraceptive pill use (OC-use) and gestational age. Model 2 is additionally corrected for fat mass (kg). Model 3 is additionally corrected for age at menarche. Model 4 is additionally corrected for socioeconomic status (SES). Model 5 additionally includes maternal smoking during gestation.

	Model 1		Model 2		Model 3		Model 4		Model 5[a]	
	β	P value	β	P value	β	P value	β	P value	β	P value
SGA-S	-0.24	0.159	-0.24	0.161	-0.26	0.123	-0.23	0.190	-0.31	0.081
SGA-CU	0.33	0.029	0.33	0.030	0.33	0.026	0.35	0.019	0.34	0.033
Overall P value	<0.001		<0.001		<0.001		<0.001		<0.001	
Adjusted R^2	0.15		0.14		0.19		0.19		0.21	

[a]Separately analysed, maternal smoking during gestation was known for 26 SGA-S, 42 SGA-CU and 68 control women. Values in boldface indicate P<0.05.

SGA-CU women had the highest median AMH levels, but this difference did not reach significance (Table 2). The women in the SGA-S and SGA-CU subgroups more often reported an irregular menstrual cycle (before the start of OC-use) than AGA controls (P=0.016 and P=0.003 respectively).

After adjustment for age, gestational age and OC-use, subgroup comparisons revealed significantly higher AMH levels in SGA-CU women than in AGA controls (Table 5: P=0.029). This difference remained significant after stepwise additional adjustment for FM, age at menarche and SES (P=0.030, P=0.026 and P=0.019 respectively). Additional adjustment for maternal smoking during gestation and smoking or alcohol use did not influence these results. Comparison of SGA-S and SGA-CU with AGA controls revealed no differences in SHBG, testosterone, non-SHBG-bound testosterone and androstenedione levels. Also after correction for age, OC-use, gestational age, FM, age at menarche, SES and maternal smoking during gestation, no significant differences could be established between subgroups.

Discussion

This is the first study to investigate the influence of preterm and SGA birth on AMH levels in young women. There were no adverse effects of preterm and SGA birth on AMH levels, a good proxy for the size of the ovarian follicle pool,[9-11] and on other gonadal function parameters. Older age at menarche and OC-use were related to lower AMH levels. Maternal smoking during gestation was related to higher AMH levels, after correction for SES, whereas lower SES was associated with lower AMH levels. Subgroup comparison showed higher AMH levels in SGA-CU women than in AGA controls, also after adjustment for several factors including gestational age.

Our study demonstrates that preterm and SGA birth did not affect serum AMH levels, and therefore the size of the ovarian follicle pool in young adulthood. Previously, an association was found between preterm birth and a reduced reproduction rate.[5] Unfortunately, that study did not investigate serum AMH levels. From the present study, we can conclude that this was unlikely caused by a reduced primordial follicle pool count. An effect of gestational age on FSH and LH levels in infants born preterm has also been reported,[25,26] but this had never been studied in adult women. Our study shows that gestational age does neither affect FSH nor LH levels in young women. This is in line with our study in young men that showed no effect of preterm birth on FSH and LH levels.[6]

Notably, OC-use was associated with lower AMH levels. Controversies exist with regard to the effect of OC-use on serum AMH levels. Some studies showed that exogenous sex steroids did not affect AMH levels,[27] whereas others did find an effect of OC-use.[23,28] In our study, the effect of OC-use on AMH levels remained significant, even after stepwise correction for age, gestational age, BL SDS, BW SDS, adult height SDS, FM, age at menarche, SES, smoking, alcohol use and maternal smoking during gestation. Recently, it has been suggested that variation in AMH levels during the

normal menstrual cycle and during OC-use is most pronounced in young women. This might also account for the obvious differences between the current study and previous reports.[13]

We found a significant inverse association between age at menarche and AMH levels in young women, also after correction for several factors. One explanation might be that women with earlier age at menarche have a larger ovarian follicle pool which might enhance the chance of achieving higher oestrogen levels earlier than those with a smaller follicular pool. Research showed that increased oestrogen levels may promote the onset of the first menstrual bleeding.[29] Maternal smoking during gestation was also associated with higher AMH levels in young women. Recently, it was shown in adolescent girls that maternal smoking during gestation is associated with a reduced uterus size. That study did not show an effect of maternal smoking during gestation on serum AMH levels.[30] However, the investigators did not correct their analyses for SES, which had significant influence in our study after correction for maternal smoking during gestation. Because a lower SES is related to maternal smoking during gestation and maternal smoking during gestation and lower SES have opposite effects on AMH, it is important to adjust for SES when studying the effect of maternal smoking during gestation on AMH levels.

After correction for maternal smoking and several other factors, lower SES was significantly associated with lower AMH levels. This might indicate that women with a lower SES have a smaller follicle pool than those with a higher SES. These data are in line with our findings in males. We previously showed that a lower SES was associated with lower inhibin B and higher FSH levels in young men.[6] An explanation of the relationship between SES and gonadal function in females and males could be that there are nutritional and environmental differences due to SES. Endocrine disrupters have been shown to affect pubertal development, and subjects with a lower SES might be more exposed to endocrine disrupters, for example by inadequate food consumption.[31,32]

Recently, it was shown that both low and high-BW infants had higher AMH levels than normal BW infants.[33] In that study, a link was suggested between IUGR and polycystic ovary syndrome (PCOS), possibly caused by intrauterine programming affecting reproduction function in low-BW infant girls. Another explanation could be the positive relationship between insulin and AMH, as high-BW infants as well as low-BW infants who show catch-up growth are likely to have high insulin levels.[33,34] Other recent studies showed no relation of BW with PCOS and AMH levels.[30,35] Unfortunately, in these studies no distinction was made for infants with and without catch-up growth.

In our study, the SGA-CU subgroup showed significantly higher AMH levels than AGA controls, also after adjustment for several factors including SES and maternal smoking during gestation. Furthermore, the women in the SGA-S and SGA-CU subgroups more often reported an irregular menstrual cycle than those in the AGA control subgroup. This finding should not be interpreted rigidly, as we used questionnaires to assess menstrual irregularity, and the participants who used oral contraceptives had to remember their menstrual cycle before the start of OC-use. However, as increased serum AMH levels and irregular menstrual cycle are both associated with PCOS,[8] our

findings in the SGA-CU group might suggest that a larger proportion of women born SGA with catch-up growth have early signs of PCOS. This is in line with a study in sheep demonstrating a PCOS-like phenotype in sheep that gained a lot of weight postnatally.[36] Furthermore, women with PCOS, as well as SGA-CU women, have decreased insulin sensitivity.[8,37] The hypothesis that SGA-CU women might have an increased chance to develop PCOS is not supported by the finding that SGA-CU women in the present study had similar testosterone and androstenedione levels as controls, after correction for confounders. As our study was not designed to investigate PCOS, we cannot draw definitive conclusions from these findings.

In conclusion, our study in 279 young women shows that preterm and SGA birth do not affect AMH levels. Catch-up growth after SGA birth might, however, be associated with increased AMH levels. Other factors associated with serum AMH levels were OC-use, age at menarche, maternal smoking during gestation and SES. Our results suggest that women born preterm and/or SGA do not have a smaller follicle pool size than women born at term and/or AGA, obviating the need for extra monitoring of ovarian function in these women.

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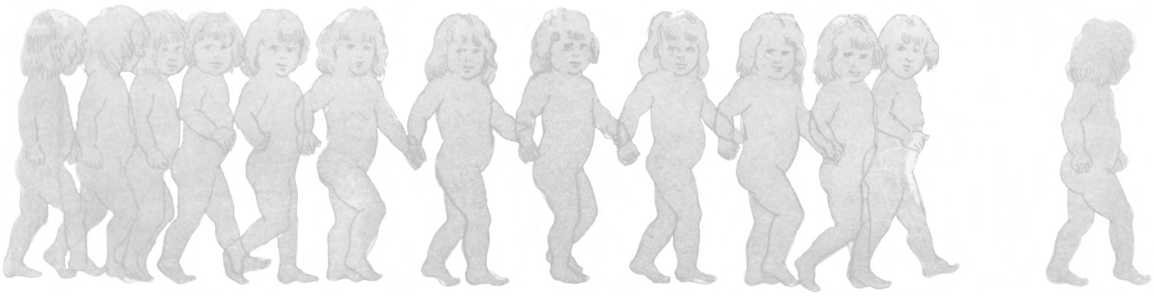
References

1. Ibanez L, Potau N, Enriquez G & de Zegher F. Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatric Research* 2000 47 575-577.
2. Ibanez L, Potau N, Enriquez G, Marcos MV & de Zegher F. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. *Human Reproduction* 2003 18 1565-1569.
3. Hernandez MI, Martinez A, Capurro T, Pena V, Trejo L, Avila A, Salazar T, Asenjo S, Iniguez G & Mericq V. Comparison of clinical, ultrasonographic, and biochemical differences at the beginning of puberty in healthy girls born either small for gestational age or appropriate for gestational age: preliminary results. Journal of Clinical Endocrinology and Metabolism 2006 91 3377-3381.
4. de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW & te Velde ER. Morphometry of human ovaries in normal and growth-restricted fetuses. *Early Human Development* 2001 60 179-192.
5. Swamy GK, Ostbye T & Skjaerven R. Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. *Journal of the American Medical Association* 2008 299 1429-1436.
6. Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T & Hokken-Koelega AC. Influence of preterm birth and birth size on gonadal function in young men. *Journal of Clinical Endocrinology and Metabolism* 2009 94 4243-4250.
7. Fowden AL, Giussani DA & Forhead AJ. Endocrine and metabolic programming during intrauterine development. *Early Human Development* 2005 81 723-734.
8. La Marca A, Broekmans FJ, Volpe A, Fauser BC, Macklon NS & Table ESIGfRE-AR. Anti-Müllerian hormone (AMH): what do we still need to know? *Human Reproduction* 2009 24 2264-2275.
9. de Vet A, Laven JS, de Jong FH, Themmen AP & Fauser BC. Anti-Müllerian hormone serum levels: a putative marker for ovarian aging. *Fertility and Sterility* 2002 77 357-362.
10. van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH & Themmen AP. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. *Human Reproduction* 2002 17 3065-3071.
11. Kwee J, Schats R, McDonnell J, Themmen A, de Jong F & Lambalk C. Evaluation of anti-Müllerian hormone as a test for the prediction of ovarian reserve. *Fertility and Sterility* 2008 90 737-743.
12. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER & Broekmans FJ. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. Journal of Clinical Endocrinology and Metabolism 2006 91 4057-4063. (doi:10.1210/jc.2006-0331)
13. Sowers M, McConnell D, Gast K, Zheng H, Nan B, McCarthy JD & Randolph JF. Anti-Mu¨ llerian hormone and inhibin B variability during normal menstrual cycles. Fertility and Sterility 2010 94 1482-1486.
14. Usher R & McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. Journal of Pediatrics 1969 74 901-910.
15. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP & Wit JM. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatric Research* 2000 47 316-323.
16. Koprowski C, Coates RJ & Bernstein L. Ability of young women to recall past body size and age at menarche. *Obesity Research* 2001 9 478-485.
17. Dutch Standard Classification of Education 2006 Centraal Bureau van de Statistiek, Statistics Netherlands. www.cbs.nl/en-GB
18. Guo Y, Franks PW, Brookshire T & Antonio Tataranni P. The intraand inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obesity Research* 2004 12 1925–1929.
19. Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, Groome NP, Themmen AP & Visser JA. Serum anti-Müllerian hormone levels reflect the size of the primordial follicle pool in mice. *Endocrinology* 2006 147 3228–3234.

20. Sodergard R, Backstrom T, Shanbhag V & Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *Journal of Steroid Biochemistry* 1982 16 801-810.
21. de Ronde W, van der Schouw YT, Muller M, Grobbee DE, Gooren LJ, Pols HA & de Jong FH. Associations of sex-hormone-binding globulin (SHBG) with non-SHBG-bound levels of testosterone and estradiol in independentlylivingmen. *Journal of Clinical Endocrinology and Metabolism* 2005 90 157-162.
22. Willemsen RH, Leunissen RW, Stijnen T & Hokken-Koelega AC. Prematurity is not associated with reduced insulin sensitivity in adulthood. *Journal of Clinical Endocrinology and Metabolism* 2009 94 1695-1700.
23. van den Berg MH, van Dulmen-den Broeder E, Overbeek A, Twisk JW, Schats R, van Leeuwen FE, Kaspers GJ & Lambalk CB. Comparison of ovarian function markers in users of hormonal contraceptives during the hormone-free interval and subsequent natural early follicular phases. *Human Reproduction* 2010 25 1520-1527.
24. Steiner AZ, Stanczyk FZ, Patel S & Edelman A. Anti-Müllerian hormone and obesity: insights in oral contraceptive users. *Contraception* 2010 81 245-248.
25. Massa G, de Zegher F & Vanderschueren-Lodeweyckx M. Serum levels of immunoreactive inhibin, FSH, and LH in human infants at preterm and term birth. *Biology of the Neonate* 1992 61 150-155.
26. Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H & Tachibana Y. Changes of serum gonadotropin levels and sex differences in premature and mature infant during neonatal life. *Journal of Clinical Endocrinology and Metabolism* 1983 56 1327-1331.
27. Streuli I, Fraisse T, Pillet C, Ibecheole V, Bischof P & de Ziegler D. Serum anti-Müllerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. *Fertility and Sterility* 2008 90 395-400.
28. Arbo E, Vetori DV, Jimenez MF, Freitas FM, Lemos N & Cunha-Filho JS. Serum anti-Müllerian hormone levels and follicular cohort characteristics after pituitary suppression in the late luteal phase with oral contraceptive pills. *Human Reproduction* 2007 22 3192-3196.
29. Emaus E, Espetvedt S, Veierod MB, Ballard-Barbash R, Furberg AS, Ellison PT, Jasienska G, Hjartaker A & Thune I. 17-b-Estradiol in relation to age at menarche and adult obesity in premenopausal women. *Human Reproduction* 2008 23 919-927.
30. Hart R, Sloboda DM, Doherty DA, Norman RJ, Atkinson HC, Newnham JP, Dickinson JE & Hickey M. Prenatal determinants of uterine volume and ovarian reserve in adolescence. *Journal of Clinical Endocrinology and Metabolism* 2009 94 4931-4937.
31. Schoeters G, Den Hond E, Dhooge W, van Larebeke N & Leijs M. Endocrine disruptors and abnormalities of pubertal development. *Basic Clinical Pharmacology & Toxicology* 2008 102 168–175.
32. Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S & Forman J. Environmental exposures and puberty in inner-city girls. *Environmental Research* 2008 107 393-400.
33. Sir-Petermann T, Marquez L, Carcamo M, Hitschfeld C, Codner E, Maliqueo M, Echiburu B, Aranda P, Crisosto N & Cassorla F. Effects of birth weight on anti-Müllerian hormone serum concentrations in infant girls. *Journal of Clinical Endocrinology and Metabolism* 2010 95 903-910.
34. Iniguez G, Ong K, Bazaes R, Avila A, Salazar T, Dunger D & Mericq V. Longitudinal changes in insulin-like growth factor-I, insulin sensitivity, and secretion from birth to age three years in small-for-gestational-age children. *Journal of Clinical Endocrinology and Metabolism* 2006 91 4645-4649.
35. Legro RS, Roller RL, Dodson WC, Stetter CM, Kunselman AR & Dunaif A. Associations of birth weight and gestational age with reproductive and metabolic phenotypes in women with polycystic ovarian syndrome and their first-degree relatives. *Journal of Clinical Endocrinology and Metabolism* 2010 95 789-799.
36. Padmanabhan V, Veiga-Lopez A, Abbott DH, Recabarren SE & Herkimer C. Developmental programming: impact of prenatal testosterone excess and postnatal weight gain on insulin sensitivity index and transfer of traits to offspring of overweight females. *Endocrinology* 2010 151 595-605.
37. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T & Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *Journal of Clinical Endocrinology and Metabolism* 2008 93 445-451.

Chapter 10



General Discussion

In the early 90's epidemiological studies revealed an association between low birth weight and increased risk for cardiovascular disease (CVD) and type 2 diabetes.[1-2] These observations led to the initiation of studies investigating Developmental Origins of Health and Disease (DOHaD).[3-21] One of these studies comprised the PROGRAM study cohort, which was started in 2002. At time of initiation, the ruling hypotheses stated that fetal condition (environmentally or genetically) was crucial for later development of health and disease,[1,22] whereas the role of postnatal factors remained unrecognized. In the PROGRAM study, our study group investigated associations of small size at birth and subsequent postnatal growth trajectories with individual determinants of CVD and type 2 diabetes in early adulthood. Those investigations have led to significant advances in our knowledge on DOHaD. Subsequently, findings from the PROGRAM study and those of other studies, led to recognition of the role of postnatal factors in DOHaD.[8,18,23-31]

In the studies presented in this thesis, we investigated early origins of individual as well as combined determinants of CVD and gonadal function, to add to the current body of knowledge on DOHaD. Furthermore, novel statistical approaches in this field were used to unravel mechanisms involved. In this chapter, the main findings of the studies described in the present thesis are discussed, also in the context of current literature. We will emphasize on clinical implications, and will also give directions for future research.

Early life factors and risk for metabolic syndrome, type 2 diabetes, and CVD

Here we discuss the results of Chapters 2, 3, and 4, describing associations of preterm birth, size at birth, and postnatal growth with individual risk factors for type 2 diabetes, prevalence of metabolic syndrome, and risk factors for CVD. Both preterm birth and poor fetal growth can lead to small size at birth. Therefore, independent effects of gestational age as well as small birth size for gestational age are important in order to unravel mechanisms involved in the association of small birth size with CVD risk factors. In **Chapter 2** we demonstrate that lower gestational age associates with higher systolic blood pressure, independent of birth weight SDS. In contrast, we report a lower diastolic blood pressure in young adults born preterm which, however, contributed to a higher pulse pressure.[32] Furthermore, we found a higher blood pressure variability and heart rate in subjects born preterm, which we hypothesized to be due to an altered sympathoadrenal function.[33] These results are in line with those of others.[33-37] In order to determine whether the associations of gestational age with CVD risk factors can be ascribed to small for gestational age (SGA) birth, we additionally performed subgroup comparisons. These comparisons showed significant differences in blood pressure, pulse pressure, and blood pressure variability, between the preterm subgroups and term appropriate for gestational age (AGA) controls, which was irrespective of SGA birth. Because the prevalence of preterm birth and the survival rate is rapidly increasing, our results are of clinical relevance for an increasing number of subjects and thus

of major importance for public health.[38] We did not find an association of preterm birth with Pulse Wave Velocity (PWV) and carotid Intima Media Thickness (cIMT), but it might well be that this effect will arise at an older age. Our data, showing that preterm birth affects several determinants of CVD, indicate that young adults born preterm might have increased risk for developing CVD, which is likely not due to being born SGA. This was confirmed by a recent study reporting increased cardiovascular mortality in subjects born preterm.[39]

Subsequently, we studied the hypothesis that the increased risk for CVD in subjects born preterm might be caused by altered early growth trajectories in infants born preterm.[40-42] Alterations in growth during the highly dynamic developmental time window after birth have programming effects on later health profile in subjects born term.[18,43] The study described in **Chapter 3** showed that in subjects born preterm, accelerated gain in weight relative to length during the period from birth to term age, as well as during the first three months after term age, has adverse effects on body composition in early adulthood. Most children born preterm lose weight for length from birth to term age, and subsequently show a marked increase in weight for length in the first three months after term age. We demonstrated that rapid weight gain after term age in subjects born preterm is an important determinant of body composition in later life, which is similar to results in subjects born term.[18] Our results indicate that accelerated weight gain immediately after preterm birth should be avoided to reduce risk for obesity and CVD. However, one should take into account that postnatal growth impairment in infants born preterm has been previously associated with adverse neurodevelopmental outcome.[44] The latter has led to concerns with regard to cognitive function after preterm birth.[45-46] In contrast, one study reported similar developmental scores in preterm infants receiving nutrient enriched formula and those receiving standard formula.[47] We did not find evidence that catch-up in weight after preterm birth is associated with educational level, but further research is warranted to determine the optimal postnatal weight trajectory after preterm birth to guarantee optimal neurodevelopment but avoid adverse effects on later body composition.

In **Chapter 4** we describe the study where we investigated mechanisms involved in the reported association of small birth size with metabolic syndrome (MetS) components and other metabolic parameters in early adulthood. Our results indicate again that the most important determinant of MetS is gain in weight relative to length SDS in the first three months of life, as this was associated with a higher number of MetS components and MetS prevalence at the age of 21 years, in contrast with birth weight SDS, weight gain during the other three months periods in the first year of life, and adult IGF-I levels. More gain in weight than length SDS in the first three months of life was also significantly associated with an increased prevalence of low HDLc, higher CRP levels and lower insulin sensitivity at the age of 21 years. Low birth weight SDS was associated with lower insulin sensitivity, but low birth weight SDS and adult IGF-I SDS were not significantly associated with any of the MetS components, or MetS prevalence at 21 years.

Conclusions, clinical implications, and directions for future research

Based on our findings presented in Chapters 2, 3, and 4, we conclude that an imbalance in neonatal gain in weight compared to length after term age as well as following preterm birth, should be avoided to reduce the risk for a less favorable body composition and MetS in adulthood. These results emphasize the need for measuring both weight and length during infancy. Furthermore, our data suggest that it might be beneficial to inform parents on the long term effects of accelerated gain in weight for length, also when infants are born preterm because the period after discharge (usually around term age) of preterm infants shows to be a critical window for programming of later body composition. Our findings point to the need for new data to investigate the optimal target of postnatal weight gain after birth in infants born either term or preterm, with regard to neurodevelopment as well as health profile in early adulthood. This could lead to public health interventions based on recommendations for infant feeding and weight gain evaluations, and thereby decreasing the risk for development of CVD, MetS, and obesity in later life, with guaranteeing optimal neurodevelopment.

Our study population consists of subjects without serious postnatal complications and did not include extreme prematurely born subjects. Thus, whether our results can be generalized to subjects with complications, such as those with broncho-pulmonary dysplasia, requires further research. Although we did not find an effect of low birth weight on CVD risk factors, prevalence of MetS or the individual components of MetS, we acknowledge that increased gain in weight for length from birth to three months of age often follows small size at birth. Therefore we consider infants born SGA as a high risk group, and would like to emphasize that monitoring of weight for length in the early postnatal period is crucial in infants born SGA. Furthermore, formula-fed infants grow at a faster rate than breast-fed infants and have a higher risk of being overweight later in life.[48-50] In our study, we did not have nutritional data to investigate the relationship between early nutrition, growth in infancy, and type 2 diabetes and CVD risk factors in later life. However, our findings suggest that the use of nutrient-enriched formulas, which induce rapid weight gain in early life, might increase risk for CVD and type 2 diabetes later in life. In contrast, breastfeeding during the first three months of life, might decrease the prevalence of CVD type 2 diabetes in adulthood. Generally, nutrient-enriched diets lead to rapid weight gain in early life, and have adverse effects on cardiovascular risk factors in later life.[8,26,51-52]

Early life factors and combinations of biomarkers and risk factors for early atherosclerosis and type 2 diabetes

Previously and in the studies described in chapters 2 to 4, we investigated associations of birth size, preterm birth and early postnatal growth with individual biomarkers and risk factors for later disease.[18, 28-31, 53-54] This has led to advances in our knowledge on DOHaD. However, the question remained whether combinations of determinants will lead to later disease such as CVD and type

2 diabetes. The pathophysiology of both atherosclerosis and type 2 diabetes is complex and comprises a wide spectrum of parameters and risk factors. Therefore, in our study population with healthy young adults, it might well be that it is more indicative to study combinations of certain biomarkers and risk factors than individual ones, as these combinations might reflect a certain risk profile.[55] In Chapter 5 and 6 we present studies in which we introduced Principal Component Analyses (PCA) to identify combinations (components) of known risk factors preceding atherosclerosis and type 2 diabetes. PCA is a multivariate correlation technique to reduce a large number of intercorrelated variables to a smaller set of independent principal components in a non-hypothesis driven manner.[55-56] Subsequently, we studied the effect of early life factors on the identified components.

Many markers of atherosclerosis are known to date, and the role of inflammation in preclinical and advanced stages of atherosclerosis has been widely acknowledged.[57] In the study presented in **Chapter 5,** we aimed to investigate the effect of small size at birth and adult body size with biomarkers of early atherosclerosis. Because of the young age of our study population, we focused on biomarkers which relate to preclinical stages of atherosclerosis, such as acute-phase proteins, pro-inflammatory cytokines and lipoproteins, namely: C-reactive protein (CRP), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), soluble vascular adhesion molecule 1 (sVCAM-1), soluble intracellular adhesion molecule 1 (sICAM-1), high-density lipoprotein (HDLc), low-density lipoprotein (LDLc), apolipoprotein B (ApoB), apolipoprotein A1 (ApoA1), and triglycerides (TG).[58-62] These biomarkers were entered in PCA, which generated three combinations of biomarkers, officially named principal components: Adverse lipids and CRP, HDLc and ApoA1, and Inflammatory Markers. Subsequently, we investigated associations of birth size, gestational age, and adult size with the principal components. Birth size and gestational age were not associated with any of the identified components. Adult weight SDS was positively associated with component Adverse Lipids and CRP, and component Inflammatory Markers, and inversely associated with component HDLc and ApoA1. These findings are in line with previous research.[63-64] We were also able to specify adult weight in fat mass and lean body mass, by using DXA measurements. Higher fat mass was associated with increased component scores for Adverse Lipids and CRP, and for Inflammatory Markers. Thus, increased fat mass is associated with increased CVD risk, already in early adulthood. Additionally, we showed that short stature was associated with higher scores for component Adverse Lipids and CRP, which is in line with a previous report showing that short stature is associated with increased risk of CVD.[65] In a public health perspective our data indicate that small birth size is not associated with increased atherosclerosis risk in young adulthood. Adult body size, and particularly body fat is however significantly associated with the components identified in the study.

In **Chapter 6** we used PCA to identify combinations of known risk factors in early adulthood preceding type 2 diabetes. Several publications have reported PCA of metabolic syndrome variables,[55,66-69] but only a limited number has focused on specific risk factors of type 2 diabetes.[70-71] In our study population, PCA resulted in four principal components of type 2 diabetes risk factors, including Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test results (insulin sensitivity, acute insulin response, disposition index). Subsequently, we studied associations of early life growth trajectories with the identified principal components, in order to determine whether growth in early life could be a prevention target to reduce development of type 2 diabetes in early adulthood. Gain in weight for length in the first three months of life was positively associated with the component characterized by insulin resistance, acute insulin response, and serum levels of CRP and triglycerides in early adulthood. Furthermore, subjects with catch-up in weight in the first year of life had higher adjusted scores for that component than those without catch-up growth. There were no significant associations of early weight gain with any of the other components. Remarkably, only certain risk factors of type 2 diabetes in young adults were affected by early weight gain, as we only found an association of first three-months accelerated weight gain with one of the four components identified (characterized by: insulin resistance, acute insulin response, and serum levels of CRP and triglycerides). Our results imply that particularly risk factors that characterize the latter principal component are involved in the mechanism linking early accelerated weight gain with risk for type 2 diabetes. This was confirmed by comparisons of the components between subjects with and without catch-up in weight in the first year of life (weight gain >0.67 SDS)[5]. Subjects with catch-up in weight had higher scores for that component, independent of size at birth. A recent study reported that excessive Body Mass Index (BMI) gain across the life span and earlier onset of overweight are associated with impaired glucose metabolism in adulthood, which is in line with our study.[72]

Conclusions, clinical implications, and directions for future research

To our knowledge, we are the first investigating the relationship of early life growth trajectories with combined risk factors of atherosclerosis and type 2 in early adulthood. We report an association of fat mass with biomarkers of early stage atherosclerosis. This is likely to induce future public health problems because the prevalence of obesity in childhood and adulthood is rapidly increasing.[73] The association of short stature with biomarkers of atherosclerosis is more complex, as adult height SDS was inversely associated with component Adverse Lipids and CRP, but tended to a positive association with component Inflammatory Markers. These findings warrant further research in subjects with short stature. We also showed that accelerated gain in weight compared to length in the first three months of life should be avoided to reduce the risk for type 2 diabetes in later life. Again, these findings point to the need for new prospective data to investigate the optimal target of gain in weight for length after birth. Furthermore, the results of our study motivate future research to focus on mechanisms affecting insulin resistance, acute

insulin response, and levels of CRP and triglycerides in adulthood when studying early origins of type 2 diabetes.

Pathways involved in the development of atherosclerosis

In Chapter 5 we demonstrated that body fat percentage is an important determinant of CVD related biomarkers, which is in line with other studies.[74-75] Several risk factors of CVD, including body fat percentage, have been studied using direct association measures. Because of the complex pathophysiology of CVD and its high occurrence rate, it is of major importance to correctly model the risk factors involved in development of CVD.[76] Standard statistical methods, however, lack to achieve that goal because of complex interrelationships involved. We used Structural Equation Modeling (SEM)[77] to explore pathways leading to increased carotid Intima-Media Thickness (cIMT), a measure of preclinical atherosclerosis.[78] Using SEM, we were able to model these pathways for males and females using body fat percentage, serum lipid levels, and blood pressure measurements. In **Chapter 7** we presented this model of complex direct and indirect effects of fat mass leading to increased cIMT, with the largest effect size via blood pressure. We also showed that the pathways differed between males and females with a larger effect of serum lipids on cIMT in males. SEM has been used in other fields, such as genetic epidemiology and psychology,[79-80] but remained very rarely used in medical research. We demonstrated that SEM is an innovative statistical method to unravel multidirectional associations and potential causal pathways in complex origins of diseases like CVD. However, one should take into account that there has to be a time course to determine causal relationships with certainty.[81]

Conclusions, clinical implications, and directions for future research

Many of the estimated effects in our study using SEM were substantial and statistically significant, which is remarkable, especially when taking into account the young age and healthy status of our study population. We conclude that even at such a young age, a higher fat mass already has a negative influence on the cardiovascular status, which is alarming because the effects are likely to be larger in subjects at an older age. Because the prevalence of fat accumulation in childhood and adulthood is increasing rapidly, this is likely to induce future problems in public health. The promising results of the present study, applying path analysis in clinical research, might motivate the use of SEM in complex origins of disease to assess causal relationships. For example, in future studies, early life growth could be added to the models identified in Chapter 7, in order to unravel pathways involved in the developmental origins of health and disease. Furthermore, SEM could be used to investigate whether the association of short stature with risk factors for CVD, as demonstrated in Chapter 5, acts via an increase in body fat percentage.

Preterm birth, birth size and later gonadal function

Next to early life influences on risk factors for CVD and type 2 diabetes, we also studied early origins of gonadal function. Decreased reproduction rates in men and women born preterm have been reported. This diminished reproduction improved with increasing gestational age.[82] It was, however, unknown whether the association between preterm birth and reduced reproduction rate was related to marital status, lower social class, or reduced gonadal function. Furthermore, controversies existed regarding the association between birth size for gestational age and gonadal function in males and females.[83-91]

In **Chapter 8** we describe our study investigating the effect of preterm birth and small birth size for gestational age on male gonadal function in young adulthood. We found that preterm and SGA birth did not affect gonadal function. Preterm birth was even associated with higher levels of Inhibin B. Furthermore, we showed that lower socioeconomic status was associated with lower inhibin B levels, and that higher fat mass was associated with lower testosterone and SHBG levels. Maternal smoking during pregnancy was associated with higher LH and non-SHBG-bound testosterone levels.

We conclude that preterm and SGA birth do not affect gonadal function in young men, but that lower SES, higher fat mass and maternal smoking during pregnancy do affect gonadal function. Our study was the first showing a relationship between SES and Sertoli cell function. Our findings are in line with a more recent study, reporting an association between SES and FSH in men aged 49-51 years.[92] Some studies demonstrated an influence of preterm birth or small birth size on male gonadal function, but none of these studies adjusted for SES[86,93-94] which might explain the differences in results, especially regarding inhibin B levels. Another recent study investigated the effect of SGA birth on fertility in young adults.[95] The results of that study were in line with ours, showing that fertility was not reduced in young men born SGA, adjusted for SES.

After concluding that preterm and SGA birth is not associated with testis function, we demonstrated that follicle pool size in young adult women is also not affected by preterm and SGA birth (study presented in **Chapter 9**). Preterm birth and SGA birth did not affect female AMH and other hormone levels. However, older age at menarche and oral contraceptive pill use were related to lower AMH levels, and maternal smoking during gestation was related to higher AMH levels. In subgroup comparisons, women born SGA with catch-up growth resulting in normal adult stature showed higher AMH levels than controls. Similar to the results in men, SES was of influence on gonadal function in young women. An explanation for the relationship between SES and gonadal function in females and males could be that there are nutritional and environmental differences due to SES. Endocrine disrupters have been shown to affect pubertal development[96-98] and subjects with a lower SES might be more exposed to endocrine disrupters, for example by consumption of less healthy food. Very recently, Sadrzadeh-Broer et al. showed

no difference in AMH and other hormone levels between women born SGA and AGA, which is in line with our results.[99] Furthermore, our research group recently demonstrated that serum AMH levels were similar in short SGA and healthy girls aged 3-10 years, and that there was also no effect of gestational age on AMH levels at that age.[100]

Clinical implications, conclusions, and directions for future research

Our data indicate that there are no adverse effects of preterm birth and small birth size for gestational age on male gonadal function and female follicle pool size. Therefore, the reported diminished reproduction rate in adults born preterm[82] is unlikely due to a reduced gonadal function. In men born preterm we even found higher Inhibin B levels than in those born term. Future sperm analysis in young men born preterm might further unravel this relationship. In the female study population, those born SGA who had shown catch-up growth had significantly higher AMH levels than controls, also after adjustment for confounders. As increased serum AMH levels are associated with Polycystic Ovary Syndrome (PCOS), our findings might suggest that a larger proportion of women born SGA with catch-up growth have early signs of PCOS. However, as our study was not designed to investigate PCOS, we cannot draw definitive conclusions from these findings and future research is warranted.

General conclusions

In the present thesis we investigated whether size at birth, preterm birth, and different growth patterns during childhood influence determinants of disease like cardiovascular disease (CVD), type 2 diabetes, and metabolic syndrome (MetS), and gonadal function in early adulthood. Furthermore, we applied novel statistical approaches in the field of DOHaD and investigated mechanisms involved in the development of early atherosclerosis. Figure 1 summarizes the most important findings described in present thesis. The figure shows the critical period in which increased weight for length should be avoided to reduce risk for obesity, MetS, type 2 diabetes, and CVD in early adulthood. The critical window comprises the first three months after birth in infants born term, and the period from birth up until three months after term age in infants born preterm. We emphasize that measuring both infant weight and length is crucial in clinical practice, also in the incubator in the case of infants born preterm. Although our results repeatedly show the importance of balanced weight gain during the early postnatal period, we recognize that there might be other critical windows later in childhood that remained unstudied in the present studies. Furthermore, we found no evidence that small size at birth influences risk for later disease, but we acknowledge that infants born SGA often receive nutrient enriched feeding in the early postnatal period, which subsequently leads to rapid weight gain. Therefore, subjects born small for gestational age who show rapid catch-up in weight are of particular interest for future studies and clinical monitoring. Figure 1 also shows that a higher fat mass in early adulthood leads to a higher carotid Intima-Media Thickness, via adverse lipids and blood pressure, and that higher fat mass in early adulthood increases biomarkers of preclinical atherosclerosis, such as inflammatory markers. The findings reported in this thesis need to be confirmed in detailed population-based cohort studies or randomized controlled trials, for example randomizing various infant diets. These studies should include detailed and standardized early life measurements including infant body composition, appetite regulating hormones and nutritional intake, which would enable to investigate the optimal target of weight gain after birth.

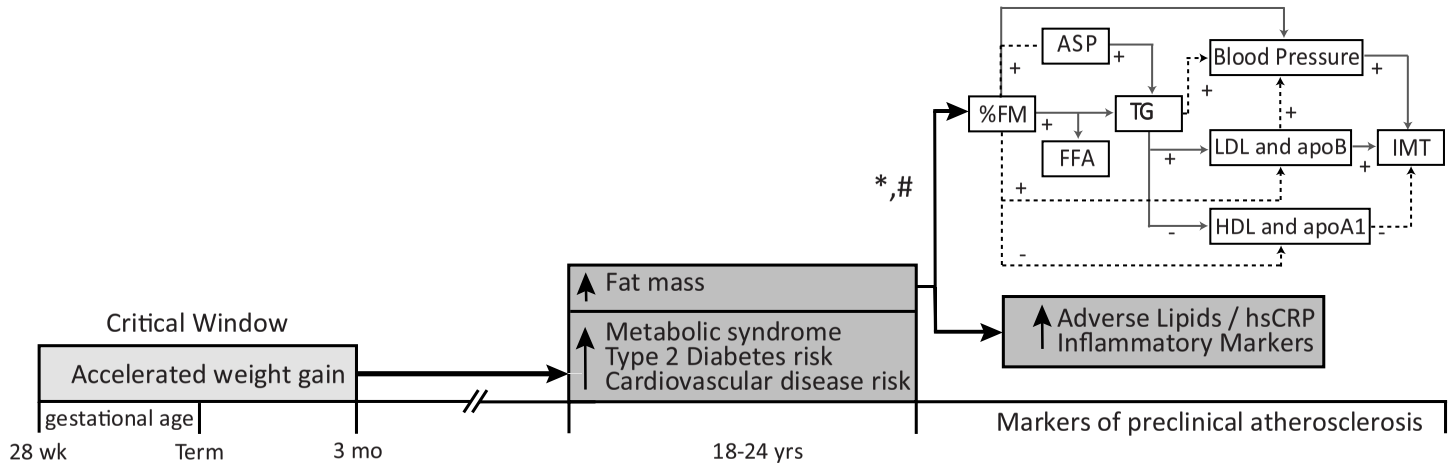


Figure 1. Summary of the most important findings of the present thesis

%FM: body fat percentage, FFA: free fatty acids, TG: triglycerides, ASP: acyl stimulating protein, LDL: low-density lipoprotein, apoB: apolipoprotein B, HDL: high-density lipoprotein, apoA1: apolipoprotein A1, IMT: carotid intima-media thickness.

* Male model, arrows indicate p-value<0.05, dashed arrows indicate p-value>0.10, female model is presented in Chapter 7. # Principal components resulting from PCA.

References

1. Barker DJ. The fetal and infant origins of adult disease. *BMJ (Clinical research ed.* 1990;301:1111.
2. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993;341:938-941.
3. de Bruin JP, Dorland M, Bruinse HW, Spliet W, Nikkels PG, Te Velde ER. Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev.* 1998;51:39-46.
4. Martyn CN, Gale CR, Jespersen S, Sherriff SB. Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. *Lancet.* 1998;352:173-178.
5. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ (Clinical research ed.* 2000;320:967-971.
6. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. *Hypertension.* 2000;36:790-794.
7. Law CM, Shiell AW, Newsome CA, Syddall HE, Shinebourne EA, Fayers PM, Martyn CN, de Swiet M. Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. *Circulation.* 2002;105:1088-1092.
8. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet.* 2003;361:1089-1097.
9. Gale CR, Martyn CN. Birth weight and later risk of depression in a national birth cohort. *Br J Psychiatry.* 2004;184:28-33.
10. Sayer AA, Cooper C. Fetal programming of body composition and musculoskeletal development. *Early Hum Dev.* 2005;81:735-744.
11. Leon DA, Koupil I, Mann V, Tuvemo T, Lindmark G, Mohsen R, Byberg L, Lithell H. Fetal, developmental, and parental influences on childhood systolic blood pressure in 600 sib pairs: the Uppsala Family study. *Circulation.* 2005;112:3478-3485.
12. Levy-Marchal C, Czernichow P. Small for gestational age and the metabolic syndrome: which mechanism is suggested by epidemiological and clinical studies? *Horm Res.* 2006;65 Suppl 3:123-130.
13. Mericq V. Low birth weight and endocrine dysfunction in postnatal life. *Pediatr Endocrinol Rev.* 2006;4:3-14.
14. Tideman E, Marsal K, Ley D. Cognitive function in young adults following intrauterine growth restriction with abnormal fetal aortic blood flow. *Ultrasound Obstet Gynecol.* 2007;29:614-618.
15. Barker DJ. The origins of the developmental origins theory. *J Intern Med.* 2007;261:412-417.
16. Singhal A. Does breastfeeding protect from growth acceleration and later obesity? *Nestle Nutr Workshop Ser Pediatr Program.* 2007;60:15-25; discussion 25-19.
17. Solomons NW. Developmental origins of health and disease: concepts, caveats, and consequences for public health nutrition. *Nutr Rev.* 2009;67 Suppl 1:S12-16.
18. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301:2234-2242.
19. Evensen KA, Steinshamn S, Tjonna AE, Stolen T, Hoydal MA, Wisloff U, Brubakk AM, Vik T. Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early Hum Dev.* 2009;85:239-245.
20. Wells JC. Historical cohort studies and the early origins of disease hypothesis: making sense of the evidence. *Proc Nutr Soc.* 2009;68:179-188.
21. Gluckman PD, Hanson MA, Buklijas T, Low FM, Beedle AS. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat Rev Endocrinol.* 2009;5:401-408.
22. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet.* 1999;353:1789-1792.

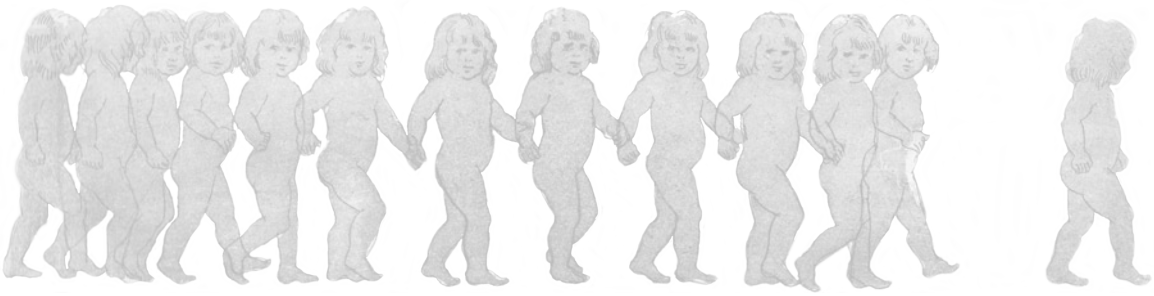
23. Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health? *Circulation.* 2004;109:1108-1113.
24. Singhal A. Early nutrition and long-term cardiovascular health. *Nutr Rev.* 2006;64:S44-49; discussion S72-91.
25. Ong KK. Size at birth, postnatal growth and risk of obesity. *Horm Res.* 2006;65 Suppl 3:65-69.
26. Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A. Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation.* 2007;115:213-220.
27. Ong KK. Catch-up growth in small for gestational age babies: good or bad? *Curr Opin Endocrinol Diabetes Obes.* 2007;14:30-34.
28. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab.* 2008;93:445-451.
29. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC. Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. *J Clin Endocrinol Metab.* 2008;93:4307-4314.
30. Leunissen RW, Stijnen T, Hokken-Koelega AC. Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol (Oxf).* 2009;70:245-251.
31. Leunissen RW, Gao Y, Cianflone K, Stijnen T, Hokken-Koelega AC. Growth patterns during childhood and the relationship with acylation-stimulating protein. *Clin Endocrinol (Oxf).* 2010;72:775-780.
32. Raitakari OT, Juonala M, Taittonen L, Jula A, Laitinen T, Kahonen M, Viikari JS. Pulse pressure in youth and carotid intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *Stroke.* 2009;40:1519-1521.
33. Johansson S, Norman M, Legnevall L, Dalmaz Y, Lagercrantz H, Vanpee M. Increased catecholamines and heart rate in children with low birth weight: perinatal contributions to sympathoadrenal overactivity. *J Intern Med.* 2007;261:480-487.
34. Bonamy AK, Martin H, Jorneskog G, Norman M. Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *J Intern Med.* 2007;262:635-642.
35. Bonamy AK, Andolf E, Martin H, Norman M. Preterm birth and carotid diameter and stiffness in childhood. *Acta Paediatr.* 2008;97:434-437.
36. Relton CL, Pearce MS, O'Sullivan JJ. The relationship between gestational age, systolic blood pressure and pulse pressure in children. *J Hum Hypertens.* 2008;22:352-357.
37. Norman M. Preterm birth--an emerging risk factor for adult hypertension? *Semin Perinatol.* 2010;34:183-187.
38. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* 2008;371:75-84.
39. Crump C, Sundquist K, Sundquist J, Winkleby MA. Gestational age at birth and mortality in young adulthood. *JAMA.* 2011;306:1233-1240.
40. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics.* 2003;111:986-990.
41. Euser AM, de Wit CC, Finken MJ, Rijken M, Wit JM. Growth of preterm born children. *Horm Res.* 2008;70:319-328.
42. Kytnarova J, Zlatohlavkova B, Kubena A, Markova D, Dokoupilova M, Plavka R, Zeman J. Post-natal growth of 157 children born as extremely premature neonates. *J Paediatr Child Health.* 2011;47:111-116.
43. Singhal A. Does early growth affect long-term risk factors for cardiovascular disease? *Nestle Nutr Workshop Ser Pediatr Program.* 2010;65:55-64; discussion 64-59.
44. Fanaro S. Which is the ideal target for preterm growth? *Minerva Pediatr.* 2010;62:77-82.

45. Yeung MY. Postnatal growth, neurodevelopment and altered adiposity after preterm birth--from a clinical nutrition perspective. *Acta Paediatr.* 2006;95:909-917.
46. Morsing E, Asard M, Ley D, Stjernqvist K, Marsal K. Cognitive function after intrauterine growth restriction and very preterm birth. *Pediatrics.* 2011;127:e874-882.
47. Lucas A, Fewtrell MS, Morley R, Singhal A, Abbott RA, Isaacs E, Stephenson T, MacFadyen UM, Clements H. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. *Pediatrics.* 2001;108:703-711.
48. Gillman MW, Rifas-Shiman SL, Camargo CA, Jr., Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA. Risk of overweight among adolescents who were breastfed as infants. *JAMA.* 2001;285:2461-2467.
49. Armstrong J, Reilly JJ, Child Health Information T. Breastfeeding and lowering the risk of childhood obesity. *Lancet.* 2002;359:2003-2004.
50. Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. Early nutrition and leptin concentrations in later life. *Am J Clin Nutr.* 2002;75:993-999.
51. Fewtrell MS, Morley R, Abbott RA, Singhal A, Stephenson T, MacFadyen UM, Clements H, Lucas A. Catch-up growth in small-for-gestational-age term infants: a randomized trial. *Am J Clin Nutr.* 2001;74:516-523.
52. Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet.* 2004;363:1571-1578.
53. Leunissen RW, Stijnen T, Boot AM, Hokken-Koelega AC. Influence of birth size and body composition on bone mineral density in early adulthood: the PROGRAM study. *Clin Endocrinol (Oxf).* 2008;69:386-392.
54. Willemsen RH, Leunissen RW, Stijnen T, Hokken-Koelega AC. Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab.* 2009;94:1695-1700.
55. Meigs JB. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am J Epidemiol.* 2000;152:908-911; discussion 912.
56. DiStefano C, Zhu M, Mîndila D. Understanding and Using Factor Scores: Considerations for the Applied Researcher. *Practical Assessment, Research & Evaluation.* 2009;14.
57. Hansson GK. Atherosclerosis--an immune disease: The Anitschkov Lecture 2007. *Atherosclerosis.* 2009;202:2-10.
58. Libby P. Inflammation in atherosclerosis. *Nature.* 2002;420:868-874.
59. Ballantyne CM, Hoogeveen RC. Role of lipid and lipoprotein profiles in risk assessment and therapy. *Am Heart J.* 2003;146:227-233.
60. de Ferranti SD, Rifai N. C-reactive protein: a nontraditional serum marker of cardiovascular risk. *Cardiovasc Pathol.* 2007;16:14-21.
61. Braunersreuther V, Mach F, Steffens S. The specific role of chemokines in atherosclerosis. *Thromb Haemost.* 2007;97:714-721.
62. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007;27:2292-2301.
63. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature.* 2006;444:875-880.
64. Pischon T. Use of obesity biomarkers in cardiovascular epidemiology. *Dis Markers.* 2009;26:247-263.
65. Silventoinen K, Kaprio J, Koskenvuo M, Lahelma E. The association between body height and coronary heart disease among Finnish twins and singletons. *Int J Epidemiol.* 2003;32:78-82.
66. Meigs JB, D'Agostino RB, Sr., Wilson PW, Cupples LA, Nathan DM, Singer DE. Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. *Diabetes.* 1997;46:1594-1600.
67. Chen W, Srinivasan SR, Elkasabany A, Berenson GS. Cardiovascular risk factors clustering features of insulin resistance syndrome (Syndrome X) in a biracial (Black-White) population of children, adolescents, and young adults: the Bogalusa Heart Study. *Am J Epidemiol.* 1999;150:667-674.
68. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA.* 2002;288:2709-2716.

69. Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? *J Pediatr.* 2008;152:160-164.
70. Kekalainen P, Sarlund H, Pyorala K, Laakso M. Hyperinsulinemia cluster predicts the development of type 2 diabetes independently of family history of diabetes. *Diabetes Care.* 1999;22:86-92.
71. Hanley AJ, Festa A, D'Agostino RB, Jr., Wagenknecht LE, Savage PJ, Tracy RP, Saad MF, Haffner SM. Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. *Diabetes.* 2004;53:1773-1781.
72. Power C, Thomas C. Changes in BMI, duration of overweight and obesity, and glucose metabolism: 45 years of follow-up of a birth cohort. *Diabetes Care.* 2011;34:1986-1991.
73. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *JAMA.* 2004;291:2847-2850.
74. Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *Br Med J (Clin Res Ed).* 1984;288:1401-1404.
75. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *JAMA.* 1999;282:1523-1529.
76. *Global Atlas on cardiovascular disease prevention and control*: World Health Organization; 2011.
77. Rabe-Hesketh S, Skrondal A. Classical latent variable models for medical research. *Stat Methods Med Res.* 2008;17:5-32.
78. Bots ML, Grobbee DE. Intima media thickness as a surrogate marker for generalised atherosclerosis. *Cardiovasc Drugs Ther.* 2002;16:341-351.
79. Pieterse K, van Dooren S, Seynaeve C, Bartels CC, Rijnsburger AJ, de Koning HJ, Klijn JG, van Elderen T, Tibben A, Duivenvoorden HJ. Passive coping and psychological distress in women adhering to regular breast cancer surveillance. *Psychooncology.* 2007;16:851-858.
80. Schur EA, Noonan C, Buchwald D, Goldberg J, Afari N. A twin study of depression and migraine: evidence for a shared genetic vulnerability. *Headache.* 2009;49:1493-1502.
81. Kline RB. *Structural models with observed variables and path analysis: I. Fundamentals, recursive models.* Vol 112. New York, NY: The Guilford Press; 1998.
82. Swamy GK, Ostbye T, Skjaerven R. Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. *JAMA.* 2008;299:1429-1436.
83. Ibanez L, Potau N, Enriquez G, de Zegher F. Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatr Res.* 2000;47:575-577.
84. de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW, te Velde ER. Morphometry of human ovaries in normal and growth-restricted fetuses. *Early human development.* 2001;60:179-192.
85. Ibanez L, Valls C, Cols M, Ferrer A, Marcos MV, De Zegher F. Hypersecretion of FSH in infant boys and girls born small for gestational age. *J Clin Endocrinol Metab.* 2002;87:1986-1988.
86. Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E. Low birth weight for gestational age and subsequent male gonadal function. *J Pediatr.* 2002;141:376-379.
87. Ibanez L, Potau N, Enriquez G, Marcos MV, de Zegher F. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. *Hum Reprod.* 2003;18:1565-1569.
88. Hernandez MI, Martinez A, Capurro T, Pena V, Trejo L, Avila A, Salazar T, Asenjo S, Iniguez G, Mericq V. Comparison of clinical, ultrasonographic, and biochemical differences at the beginning of puberty in healthy girls born either small for gestational age or appropriate for gestational age: preliminary results. *J Clin Endocrinol Metab.* 2006;91:3377-3381.
89. Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A. Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. *J Clin Endocrinol Metab.* 2007;92:1353-1357.
90. Boonstra VH, Weber RF, de Jong FH, Hokken-Koelega AC. Testis function in prepubertal boys and young men born small for gestational age. *Hormone research.* 2008;70:357-363.

91. Allvin K, Ankarberg-Lindgren C, Fors H, Dahlgren J. Elevated serum levels of estradiol, dihydrotestosterone, and inhibin B in adult males born small for gestational age. *J Clin Endocrinol Metab.* 2008;93:1464-1469.
92. Pearce MS, Groom A, Relton CL, Peaston RT, Pollard TM, Francis RM. Birth weight and early socio-economic disadvantage as predictors of sex hormones and sex hormone binding globulin in men at age 49-51 years. *Am J Hum Biol.* 2011;23:185-189.
93. Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H, Tachibana Y. Changes of serum gonadotropin levels and sex differences in premature and mature infant during neonatal life. *J Clin Endocrinol Metab.* 1983;56:1327-1331.
94. Massa G, de Zegher F, Vanderschueren-Lodeweyckx M. Serum levels of immunoreactive inhibin, FSH, and LH in human infants at preterm and term birth. *Biol Neonate.* 1992;61:150-155.
95. Meas T, Deghmoun S, Levy-Marchal C, Bouyer J. Fertility is not altered in young adults born small for gestational age. *Hum Reprod.* 2010;25:2354-2359.
96. Schoeters G, Den Hond E, Dhooge W, van Larebeke N, Leijs M. Endocrine disruptors and abnormalities of pubertal development. *Basic & clinical pharmacology & toxicology.* 2008;102:168-175.
97. Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. Environmental exposures and puberty in inner-city girls. *Environ Res.* 2008;107:393-400.
98. Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, Kushi LH, Erdmann C, Hiatt RA, Rybak ME, Calafat AM, Breast C, Environment Research C. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. *Environ Health Perspect.* 2010;118:1039-1046.
99. Sadrzadeh-Broer S, Kuijper EA, Van Weissenbruch MM, Lambalk CB. Ovarian reserve in young women with low birth weight and normal puberty: a pilot case-control study. *Gynecol Endocrinol.* 2011;27:641-644.
100. Lem AJ, Boonstra VH, Renes JS, Breukhoven PE, de Jong FH, Laven JS, Hokken-Koelega AC. Anti-Mullerian hormone in short girls born small for gestational age and the effect of growth hormone treatment. *Hum Reprod.* 2011;26:898-903.

Chapter 11



Summary

Chapter 1

This chapter provides a general introduction to the different aspects involved in developmental origins of adult health and disease. We also describe our study population, provide the aims of the studies performed, and present the outline of this thesis.

Chapter 2

Both preterm birth and small birth size for gestational age have been associated with increased risk for cardiovascular disease (CVD), but controversies still existed. In the study described in chapter 2, we aimed to investigate the effect of preterm birth on risk factors for CVD, independent of birth size. In the study, using data of 406 young adults, we showed that preterm birth is associated with higher systolic blood pressure, pulse pressure, blood pressure variability, and heart rate, and with lower diastolic blood pressure, also after adjustment for confounders including size at birth. We, therefore, concluded that young adults born preterm might have a higher risk for CVD than those born term, independent of birth size. There was no effect of gestational age on pulse wave velocity and carotid intima-media thickness, which is a marker of early stage atherosclerosis, but our study population is still relatively young, and it might well be that this effect will arise at an older age.

Chapter 3

Early postnatal weight gain has been associated with determinants of Cardiovascular Disease (CVD) and type 2 diabetes in adults born term. However, this association remained to be elucidated in adults born preterm. We, therefore, investigated the association of weight gain during different periods, and different weight trajectories in early life after preterm birth, with determinants of CVD and type 2 diabetes in early adulthood. We showed that accelerated gain in weight relative to length during the period from birth to term age, as well as during the first three months after term age, has adverse effects on body composition in early adulthood. Thus, the time window for effects of accelerated weight gain on body composition is wider in subjects born preterm than term. We also demonstrated that rapid weight gain after term age in subjects born preterm is an important determinant of body composition in later life, which is in line with results in subjects born term.

Chapter 4

The relationship between low birth weight and increased risk for metabolic syndrome (MetS) in later life has been frequently described, but mechanisms underlying this association remain unknown. In Chapter 4, we investigated associations of birth weight, gain in weight for length during early life, and adult IGF-I SDS, with number of MetS components, prevalence of MetS components and MetS, and other metabolic parameters. Our results indicate that the most important determinant of MetS is gain in weight relative to length SDS in the first three months of life, as this was associated with a higher number of MetS components at the age of 21 years,

in contrast with birth weight SDS, weight gain during the other three months periods in the first year of life, and adult IGF-I levels. Furthermore, gain in weight relative to length SDS in the first three months of life was associated with increased prevalence of MetS, higher prevalence of low HDLc, increased levels of CRP, and lower insulin sensitivity in early adulthood.

Chapter 5

In this chapter we present our study investigating associations between small birth size, prematurity, adult body size, and a broad range of biomarkers related to early stage atherosclerosis. Principal component analysis (PCA) was applied to identify combinations of biomarkers and their component-scores. This resulted in three principal components. Birth size and gestational age were not associated with any of the components, but adult fat mass was positively associated with the component Adverse Lipids and hsCRP (characterized by LDLc, ApoB, ApoA1, triglycerides, and CRP), and component Inflammatory markers (characterized by MCP-1, IL-8, sVCAM-1, and sICAM-1). We, therefore, concluded that small birth size is not associated with increased risk for atherosclerosis in early adulthood, in contrast to fat mass at age 21 years.

Chapter 6

In chapter 6, we present our study in which we identified combinations of known risk factors in early adulthood preceding type 2 diabetes, using PCA. Subsequently, we studied associations of early life growth trajectories with the identified principal components. We identified four combinations of type 2 diabetes risk factors. Gain in weight for length in the first three months of life was positively associated with the component characterized by insulin resistance, acute insulin response, and serum levels of CRP and triglycerides. Furthermore, subjects with catch-up in weight in the first year of life had higher adjusted scores for that component than those without catch-up growth, also after additional adjustment for birth weight SDS. We concluded that accelerated gain in weight compared to length in the first three months of life should be avoided to reduce the risk for type 2 diabetes in later life.

Chapter 7

Structural Equation Modeling (SEM) is an advanced statistical technique to unravel multidirectional associations and potential causal pathways in complex origins of diseases such as CVD. In chapter 7 we demonstrate our study using SEM to examine several pathways leading to vascular changes in early adulthood. We present a model of direct and indirect effects of fat mass leading to early stage atherosclerosis, measured by carotid intima-media thickness (cIMT). SEM showed that the pathways differed between males and females, with a larger effect of serum lipids on cIMT in males. We concluded that SEM is suitable in identifying models to unravel potential causal pathways in complex origins of diseases, and presented a model involving several pathways, showing that increased fat mass has an influence on risk for atherosclerosis, already at 21 years of age.

Chapter 8

We hypothesized that alterations in gonadal function in young men are not due to preterm birth or being born SGA, but are due to other (environmental) factors. In the study described in chapter 8, we investigated the influence of preterm birth, birth length, and birth weight on serum levels of anti-Mullerian hormone, inhibin B, testosterone, SHBG, non-SHBG-bound testosterone, LH, and FSH in a group of 207 young men. We showed that preterm birth and SGA did not affect gonadal function. Preterm birth even showed a positive association with inhibin B after correction for several confounders. Higher socioeconomic status was associated with higher inhibin B levels, higher fat mass was associated with lower testosterone and SHBG levels, and maternal smoking during pregnancy was associated with increased LH and non-SHBG-bound testosterone levels. We concluded that preterm birth and SGA do not affect gonadal function in young men, whereas lower SES, higher fat mass, and maternal smoking during pregnancy do.

Chapter 9

In chapter 9, we investigated the association of preterm and SGA birth with ovarian function and the size of the follicle pool in young adult women. We, therefore, investigated the effects of gestational age at birth, birth weight SDS, birth length SDS, and adult size on serum anti-Müllerian hormone (AMH), LH, SHBG, testosterone, Non-SHBG-bound testosterone and androstenedione. We showed that there were no adverse effects of preterm and SGA birth on AMH levels and other gonadal function parameters. Older age at menarche and oral contraceptive use (OC-use) were related to lower AMH levels. Maternal smoking during pregnancy was associated with higher AMH levels, also after correction for SES, whereas lower SES was associated with lower AMH levels. Subgroup comparisons showed higher AMH levels in women born SGA with catch-up growth (SGA-CU) than in AGA controls, also after adjustment for several factors including gestational age. In conclusion, our results indicate that women born preterm and/or SGA do not have a smaller follicle pool size than women born at term and/or AGA.

Chapter 10

In this chapter, the main findings of the studies described in the present thesis are discussed, also in the context of current literature. We emphasized on clinical implications and gave directions for future research.

Samenvatting

Hoofdstuk 1

Dit hoofdstuk geeft een algemene inleiding over vroege determinanten van ziekten en aandoeningen die op volwassen leeftijd optreden. We beschrijven ook onze studiepopulatie, de doelstellingen van de uitgevoerde studies en de verdere opzet van het proefschrift.

Hoofdstuk 2

Premature geboorte en te klein zijn bij geboorte voor de zwangerschapsduur (small for gestational age (SGA)) veroorzaken beide een laag geboortegewicht. Verschillende studies hebben een relatie aangetoond tussen een laag geboortegewicht en een verhoogd risico op hart- en vaatziekten. Echter, tot op heden was de invloed van premature geboorte op deze relatie onbekend. In hoofdstuk 2 beschrijven we de studie waarin we de associatie hebben onderzocht tussen premature geboorte/zwangerschapsduur en verschillende risicofactoren voor hart- en vaatziekten, ongeacht het geboortegewicht. In deze studie hebben we laten zien dat premature geboorte geassocieerd is met een hogere systolische bloeddruk, polsdruk, bloeddrukvariabiliteit, hartslag en met een lagere diastolische bloeddruk. De resultaten bleven gelijk na correctie voor geboortegewicht en geboortelengte. Onze conclusie is dat prematuur geboren jongvolwassen mogelijk een hoger risico hebben op hart- en vaatziekten dan à term geboren jongvolwassenen en dat dit onafhankelijk is van grootte bij de geboorte. We vonden geen effect van zwangerschapsduur op Pulse Wave Velocity (meting van de arteriële stijfheid, PWV) en de intima media dikte van de carotis (cIMT) op jongvolwassen leeftijd, maar onze studiepopulatie is nog jong en het kan zijn dat deze associatie ontstaat op een latere leeftijd.

Hoofdstuk 3

In een eerdere studie hebben we laten zien dat teveel toename in gewicht ten opzichte van lengte in de eerste drie levensmaanden, gerelateerd is aan determinanten van hart- en vaatziekten en type 2 diabetes in à term geboren jongvolwassenen. Het was echter nog onzeker of deze associatie ook voorkomt bij prematuur geboren volwassenen. In hoofdstuk 3 beschrijven we onze studie waarin we gewichtstoename gedurende verschillende perioden in het eerste levensjaar na premature geboorte hebben bestudeerd, in relatie tot determinanten van hart- en vaatziekten en type 2 diabetes. We zagen dat meer gewichtstoename ten opzichte van lengtegroei gedurende de periode van geboorte tot à terme leeftijd, alsmede gedurende de eerste drie maanden na de à terme leeftijd, geassocieerd was met een slechtere lichaamssamenstelling op jongvolwassen leeftijd. We laten ook zien dat een te snelle gewichtstoename bij prematuur geboren kinderen na de à terme leeftijd een belangrijke determinant is van de lichaamssamenstelling op latere leeftijd, hetgeen vergelijkbaar is met de resultaten die we voorheen vonden bij à term geboren kinderen.

Hoofdstuk 4

Verschillende studies hebben een relatie aangetoond tussen een laag geboortegewicht en een verhoogd risico op Metabool Syndroom (MetS) op volwassen leeftijd, maar het onderliggende mechanisme hiervan bleef onbekend. In hoofdstuk 4 beschrijven we de studie waarin we onderzocht hebben wat het effect is van geboortegewicht, toename in gewicht ten opzichte van lengte gedurende verschillende periodes in het eerste levensjaar en het serum IGF-I gehalte op de leeftijd van 21 jaar, op componenten van MetS, de prevalentie van de componenten en MetS en verschillende andere metabole parameters op jongvolwassen leeftijd. We laten zien dat een te grote toename in gewicht ten opzichte van lengte in de eerste drie maanden na geboorte de belangrijkste determinant is van MetS op 21 jaar. Dit was geassocieerd met een groter aantal MetS componenten en een hogere prevalentie van MetS op jongvolwassen leeftijd, terwijl geboortegewicht en IGF-I hier niet mee geassocieerd waren. Een te grote toename in gewicht ten opzichte van lengte in de eerste drie maanden na de geboorte was ook geassocieerd met hogere CRP spiegels in het bloed en lagere insuline gevoeligheid op jongvolwassen leeftijd.

Hoofdstuk 5

In hoofdstuk 5 hebben we principale componentenanalyse (PCA) toegepast om combinaties van biomarkers, die gerelateerd zijn aan vroege stadia van atherosclerose, te identificeren en de componentenscores van deze combinaties te berekenen. De PCA analyse resulteerde in drie principale componenten. Vervolgens hebben we onderzocht of klein zijn bij de geboorte en premature geboorte van invloed zijn op de geïdentificeerde componenten. Ook onderzochten we de invloed van gewicht, lengte en lichaamssamenstelling op volwassen leeftijd op de componenten. Grootte bij de geboorte en zwangerschapsduur waren beide niet geassocieerd met de componenten, maar hogere scores voor de component bestaande uit ongunstige lipiden (LDLc, ApoB, lager ApoA, triglyceriden) en CRP en hogere scores voor de component bestaande uit ontstekingsparameters (MCP-1, IL-8, sVCAM-1 en sICAM-1) waren geassocieerd met meer vetmassa op volwassen leeftijd. Onze conclusie was dat klein zijn bij de geboorte niet geassocieerd is met een verhoogd risico op vroege stadia van atherosclerose op jongvolwassen leeftijd, maar dat een verhoogde vetmassa op 21 jarige leeftijd daar wel mee geassocieerd is.

Hoofdstuk 6

In hoofdstuk 6 beschrijven we de studie waarin we PCA hebben toegepast om combinaties te identificeren van risicofactoren om type 2 diabetes te ontwikkelen. PCA identificeerde vier componenten. Vervolgens hebben we associaties onderzocht tussen vroege groei, verschillende groeitrajecten en de geïdentificeerde componenten. Meer gewichtstoename dan lengtegroei in de eerste drie maanden na de geboorte was geassocieerd met de component bestaande uit insulineresistentie, acute insuline respons en CRP en triglyceride spiegels. Ook vonden we dat jongvolwassenen die inhaalgroei hadden vertoond in het eerste levensjaar, hogere scores hadden voor die specifieke component. Dit bleef significant na correctie voor geboortegewicht.

We concludeerden dat een hogere toename in gewicht ten opzichte van lengte in de periode na de geboorte vermeden moet worden om het risico op type 2 diabetes in het latere leven te verlagen

Hoofdstuk 7

Structural Equation Modeling (SEM) is een geavanceerde statistische methode die gebruikt kan worden om zonder voorgaande hypothese multidirectionele associaties en potentieel causale paden te ontrafelen van complexe ziekten zoals hart- en vaatziekten. In dit hoofdstuk laten we de studie zien waarin we, door gebruik te maken van SEM, verschillende paden hebben geïdentificeerd die leiden tot vasculaire veranderingen op jongvolwassene leeftijd. We presenteren een model met directe en indirecte effecten van vetmassa die uiteindelijk kunnen leiden tot verdikking van de intima-media van de carotis (cIMT), een vroeg stadium van atherosclerose. We toonden aan dat het padmodel verschilt voor mannen en vrouwen, met een groter effect van lipiden op cIMT bij mannen. Onze conclusie is dat SEM geschikt is voor het identificeren van potentieel causale paden van complexe ziekten. Tevens presenteren we een model waar uit blijkt dat een verhoogde vetmassa op de leeftijd van 21 jaar het risico op atherosclerose verhoogt

Hoofdstuk 8

Hoofdstuk 8 beschrijft de studie waarin we onderzochten of premature geboorte en te klein zijn bij de geboorte effect hebben op de gonadale functie van jongvolwassen mannen. Daartoe bepaalden we de invloed van premature geboorte, geboortelengte en geboortegewicht op bloedspiegels van anti-Müller hormoon (AMH), inhibine B, testosteron, SHBG, niet-SHBG-gebonden testosteron, LH, en FSH in een groep bestaande uit 207 jongvolwassen mannen. Uit onze studie bleek dat premature geboorte en SGA geboorte de gonadale functie van jongvolwassen mannen niet aantasten en dat premature geboorte zelfs geassocieerd was met hogere inhibine B spiegels. Verder vonden we dat de sociaal-economische status positief geassocieerd was met inhibine B spiegels, dat een hogere vetmassa geassocieerd was met lagere testosteron- en SHBG spiegels en dat LH en niet-SHBG-gebonden testosteron spiegels hoger waren als de moeder had gerookt tijdens de zwangerschap. We concludeerden dat premature- en SGA geboorte geen invloed hebben op de gonadale functie van jongvolwassen mannen, terwijl sociaal-economische status, een hogere vetmassa en roken van de moeder tijdens de zwangerschap de gonadale functie wel beinvoeden.

Hoofdstuk 9

In hoofdstuk 9 presenteren we de studie waarin we associaties onderzochten tussen premature- en SGA geboorte en ovariële functie en het aantal (antrale) follikels bij jongvolwassen vrouwen. Daartoe hebben we de invloed onderzocht van zwangerschapsduur, geboortegewicht en geboortelengte op bloedspiegels van AMH, LH, SHBG, testosteron, niet-SHBG-gebonden testosteron en androsteendion. We lieten zien dat er geen nadelige effecten zijn van premature-

en SGA geboorte op AMH spiegels en andere parameters van gonadale functie. Een oudere leeftijd bij de eerste menstruatie en gebruik van orale anticonceptie waren wel geassocieerd met lagere AMH spiegels. Verder was roken door de moeder tijdens de zwangerschap geassocieerd met hogere AMH spiegels op jongvolwassen leeftijd, ook na correctie voor SES, terwijl lagere SES geassocieerd was met lagere AMH spiegels. Bij de subgroepvergelijkingen vonden we hogere AMH spiegels bij SGA-CU vrouwen dan AGA controles, ook na correctie voor verschillende factoren waaronder zwangerschapsduur. Onze conclusie was dat premature- en SGA geboorte geen invloed hebben op ovariële functie en het aantal (antrale) follikels bij jongvolwassen vrouwen.

Hoofdstuk 10

In dit hoofdstuk worden de belangrijkste bevindingen besproken van de studies beschreven in dit proefschrift, ook in de context van de huidige literatuur. We leggen de nadruk op klinische implicaties en geven aanwijzingen voor toekomstig onderzoek.

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Curriculum Vitae

Gerthe F. Kerkhof was born in Groningen, the Netherlands, on June 11, 1986. She passed her secondary school exam at the "CSG Willem de Zwijger" in Schoonhoven in 2004. In the same year she started her medical training at the Medical Faculty of the Erasmus University of Rotterdam. She graduated on the theoretical part of her medical training in 2008. During her study, in 2006, she started a masters in Clinical Research at the Netherlands Institute for Health and Sciences, for which she attended four courses at the Johns Hopkins School of Public Health, Baltimore, USA. In 2009 she obtained her Master of Science degree in Clinical Research. During her study she performed a research project on the effects of preterm and small for gestational age birth on gonadal function in young adult men, at the department of Pediatric Endocrinology of the Erasmus MC – Sophia Children's Hospital (supervisor Prof. dr. A.C.S. Hokken-Koelega). This resulted in a research fellowship which started in February 2009 at the same department. The research performed during that period is presented in this thesis. In June 2012 she will continue her Medical training. Together with Ludo Guns she lives in Breda.

List of Publications

Leunissen RW, **Kerkhof GF**, Stijnen T, Hokken-Koelega AC. 2008 Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. J Clin Endocrinol Metab 93(11):4307-4314

Leunissen RW, **Kerkhof GF**, Stijnen T, Hokken-Koelega AC. 2009 Timing and tempo of First-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. JAMA 301(21):2234-2242

Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T, Hokken-Koelega AC. 2009 Influence of preterm birth and birth size on gonadal function in young men. J Clin Endocrinol Metab 94(11):4243-4250

Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Visser JA, Laven JS, Hokken-Koelega AC. 2010 Influence of preterm birth and small birth size on serum anti-Müllerian hormone levels in young adult women. Eur J Endocrinol 163(6):937-944

Kerkhof GF, Duivenvoorden HJ, Leunissen RW, Hokken-Koelega AC. 2011 Pathways leading to atherosclerosis: a structural equation modeling approach in young adults. Hypertension 5(2):255-260

Breukhoven PE, **Kerkhof GF**, van Dijk M, Hokken-Koelega AC. 2011 Long-term impact of GH treatment during childhood on body composition and fat distribution in young adults born SGA. J Clin Endocrinol Metab 96(12):3710-3716

Breukhoven PE, **Kerkhof GF**, Willemsen RH, Hokken-Koelega AC. 2012 Fat mass and lipid profile in young adults born preterm. J Clin Endocrinol Metab *in press*

Kerkhof GF, Breukhoven PE, Leunissen RW, Willemsen RH, Hokken-Koelega AC. 2012 Does Preterm Birth Influence Cardiovascular Risk in Early Adulthood? J Pediatrics *in press*

Leunissen RW, **Kerkhof GF**, Stijnen T, Hokken-Koelega AC. 2012 Effect of birth size and catch-up growth on adult blood pressure and carotid intima-media thickness. Horm Res Paediatr *in press*

Kerkhof GF, Leunissen RW, Hokken-Koelega AC. Early Origins of the Metabolic Syndrome: Role of Small Size at Birth, Early Postnatal Weight Gain and Adult IGF-I. J Clin Endocrinol Metabol *in press*

Kerkhof GF, Willemsen RH, Leunissen RWJ, Breukhoven PE, Hokken-Koelega AC. Health Profile of Young Adults Born Preterm: Negative Effects of Rapid Weight Gain in Early Life. *Submitted*

Kerkhof GF, Naas M, Breukhoven PE, Drexhage HA, de Maat MP, Hokken-Koelega AC. Biomarkers of Early Stage Atherosclerosis in Young Adults: Effects of Small Size at Birth, Prematurity, and Adult Body Size. *Submitted*

Breukhoven PE, de Ridder MA, **Kerkhof GF**, Hokken-Koelega AC. Alterations in Body Composition, Lipid Levels and Blood Pressure after Discontinuation of GH in subjects born SGA: Results of a 2 year Longitudinal Study. S*ubmitted*

Kerkhof GF, Leunissen RW, Hokken-Koelega AC. Early Origins of Type 2 Diabetes: Growth Trajectories in Early Childhood and Risk for Type 2 Diabetes in Young Adults. *Submitted*

PhD Portfolio

Erasmus MC Department of Pediatrics, Subdivision of Endocrinology
Research School: Molecular Medicine
PhD period: February 2009 – June 2012
Master of Science Clinical Research, Nihes: 2006 – 2009
Promotor: Prof. Dr. A.C.S. Hokken-Koelega

General academic courses	**Year**
English Biomedical Writing and Communication	2009
Good Clinical Practice	2009
Basic and translational Endocrinology	2011
Research skills	
Master of Science in Clinical Research	2006-2009
Weekly research meeting, department Pediatric Endocrinology	2008-2012
Seminars and workshops	
PhD Day Erasmus MC	2009-2011
Writing Successful Grant Proposals	2011
Photoshop and Illustrator CS5 for PhD-Students	2011
Indesign CS5	2012
(Inter)national conferences	
LWEPES/ESPE 8th joint meeting, New York, USA (poster presentation)	2009
8th Research Day Pediatrics, Rotterdam, Netherlands (oral presentation & poster presentation)	2009
Annual Molecular Medicine Day, Rotterdam, Netherlands (poster presentation)	2010
49th Annual Meeting of the ESPE, Prague, Czech Republic (poster presentation)	2010
9th Research Day Pediatrics, Rotterdam, Netherlands (poster presentation)	2010
The Power of Programming Conference, Munich, Germany	2010
Developmental Origins of Health & Disease 7th world congress, Portland, Oregon USA (oral presentation & poster presentation)	2011
50th Annual Meeting of the ESPE, Glasgow, Scotland (2 poster presentations)	2011

Teaching activities

Meeting of the Dutch Advisory board on Growth Hormone (oral presentation)	2009
Supervising student, keuzeonderzoek, Erasmus MC	2011
Masterclass Pediatric Endocrinology II – SGA (oral presentation)	2011

Symposia

Genetic and non-genetic causes of growth hormone deficiency and hypopituitarism	2008
Early Origins of diabetes type 2 and cardiovascular disease – relevance for clinical practice	2008
Meeting Diabetes Platform Erasmus MC	2009-2010
Annual Research Meeting Dept. Obstetrics and Gynaecology Erasmus MC	2009-2010
Early growth, infant feeding and long-term risk for metabolic and cardiovascular disease	2009
The Analysis of Growth curves	2009
Recent genetic findings in children born SGA	2009
Metabolomics of the obese	2009
Disorders of Sex Development	2012

Other

PedENDO-GYN meetings	2009
Research meeting Dept. of Obstetrics & Gynaecology (2 oral presentations)	2010
Peer review of manuscripts for scientific journals:	2009-2012

- American Journal of Human Biology
- Clinica Chimica Acta
- Hormone Research
- Journal of Clinical Endocrinology and Metabolism
- Circulation
- Human Reproduction

List of abbreviations

AGA:	Appropriate for Gestational Age
AIR:	Acute Insulin Response to glucose
AMH:	Anti-Müllerian Hormone
ApN:	Adiponectin
ApoA-1:	Apolipoprotein A-1
ApoB:	Apolipoprotein B
ASP:	Acylation Stimulating Protein
BMI:	Body Mass Index
CRP:	C-Reactive Protein
CV:	Coefficient of variation
CVD:	Cardiovascular Disease
DBP:	Diastolic Blood pressure
DI:	Disposition Index
DM2:	Type 2 Diabetes Mellitus
DXA:	Dual-Energy X-ray Absorptiometry
FFA:	Free Fatty Acids
FM:	Fat Mass
FSIGT:	Frequently Sampled Intravenous Glucose Tolerance Test
FSH:	Follicle-Stimulation Hormone
GA:	Gestational Age
HDLc:	High Density Lipoprotein cholesterol
HR:	Heart rate
IGF-I:	Insulin like Growth Factor-I
IGFBP-3:	Insulin like growth factor binding protein-3
IL-8:	Interleukin-8
IMT:	Intima Media Thickness
IR:	Insulin resistance
ISS:	Idiopathic Short Stature
IUGR:	Intra-uterine Growth Retardation
LBM:	Lean Body Mass
LDLc:	Low Density Lipoprotein cholesterol
LH:	Lute inizing Hormone
MCP-1:	Monocyte Chemotactic Protein-1
MetS:	Metabolic Syndrome
MR:	Multiple linear regression
NCEP:	National Cholesterol Educational Program

OC-use:	Oral Contraceptive pill use
PCA:	Principle Component Analysis
PWV:	Pulse Wave Velocity
SBP:	Systolic Blood pressure
SDS:	Standard Deviation Score
SEM:	Structural Equation Modeling
SES:	Socioeconomic Status
SGA:	Small for Gestational Age
SGA-CU:	born small for gestational age with catch-up growth
SGA-S:	born small for gestational age with short adult stature
SHBG:	Sex Hormone Binding Globulin
Si:	Insulin Sensitivity
sICAM-1 :	soluble Intracellular Adhesion Molecule-1
sVCAM-1:	soluble Vascular Adhesion Molecule-1
TC:	Total Cholesterol
TG:	Triglycerides