

**Early determinants of cardiovascular risk in the young:
Two Dutch cohorts**

Lydia Esther Vos

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Early determinants of cardiovascular risk in the young: Two Dutch cohorts.

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University Medical Center Utrecht, The Netherlands.

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**Early determinants of cardiovascular risk in the young:
Two Dutch cohorts**

**Vroege determinanten van cardiovasculair risico
bij jongeren:
Twee Nederlandse cohorten**
(met een samenvatting in het Nederlands)

Proefschrift

Ter verkrijging van de graad van doctor aan de Universiteit te Utrecht
op gezag van de Rector Magnificus, Prof. Dr. W.H. Gispen
ingevolge het besluit van het College voor promoties
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Alan Lox

List of abbreviations

ARYA	Atherosclerosis risk in young adults
AUC	Area under the curve
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Common carotid intima-media thickness
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
HDL	High-density-lipoprotein
IGF-I	Insulin-like growth factor-I
LDL	Low-density-lipoprotein
MAP	Mean arterial pressure
MHS	Municipal health service
OR	Odds ratio
PP	Pulse pressure
PWA	Pulse wave analysis
PWV	Pulse wave velocity
ROC	Receiver operation characteristic curve
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error

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CHAPTER 1

Introduction

1.1

Background and rationale

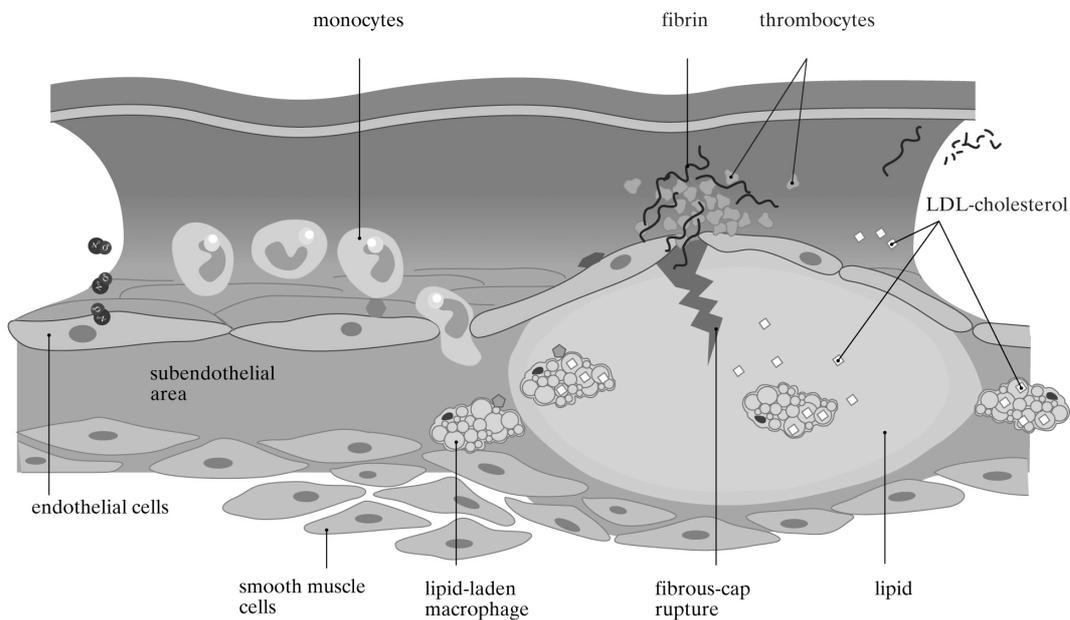
In most western societies, cardiovascular disease such as coronary artery disease, aortic aneurysm, and arterial disease of the lower extremities, are still a major cause of high mortality, of many quality adjusted years of life lost, and claim large parts of health budgets.^{1,2} In 2000 in The Netherlands, approximately 50,000 persons died of cardiovascular disease, accounting for 36% of the total mortality.³ From the nineteen-seventies onwards, there is a decrease in the age adjusted cardiovascular mortality rate, partly due to the improved technical and medical abilities to diagnose and treat disease. About 50% of the marked decline in mortality from coronary heart disease between 1980 and 1990 in the United States of America, could be attributed to treatment, 25% to secondary prevention and 25% to primary prevention.² However, these improvements have also led to an increase of the absolute numbers of patients with chronic heart failure.⁴ A thus continuing large impact on health commits us to next generations to search for new possibilities to reduce the burden of cardiovascular disease.

The complex and chronic process that eventually causes clinically manifest cardiovascular disease, is called atherosclerosis.⁵ It is a generic term for the thickening and hardening of the walls of the arteries. In 1911, Klotz and Manning⁶ introduced their study of the aorta of 90 cases between 1 and 73 years of age with this statement: "It is quite useless to argue the questions concerning the development of intimal sclerosis if we study the late stages of the disease alone..... If we wish to gain a true insight into the complex question of arterio-sclerosis we must attempt to follow the lesion from its early beginning." In those days, fatty streaks were considered as the earliest lesion of atherosclerosis. Klotz and Manning found a high prevalence of fatty streaks already in individuals below 30 years of age.

Initiation of potentially reversible fatty streaks occurs in childhood and adolescence (figure 1.1).^{5,7} Monocytes start to migrate from the blood into the arterial wall, become lipid-laden macrophages. Fatty streaks are formed by an increase of intracellular lipids, multiplying macrophages and smooth muscle cells. Around the third decade of life, the lipids locate extracellularly and the fatty streaks progress to intermediate and advanced lesions, which increase the arterial wall thickness. Fibrous plaques covering macrophages, lipid and debris, are formed around the fourth decade of life, and the amount of smooth muscle cells and collagen is increased. Rupture of the fibrous cap easily occurs, causing thrombotic deposits, hematoma's, fissures and the known clinically manifest cardiovascular diseases. From around the fifth decade in life, the process of arterial lumen blocking is enhanced.

The initiation and early process of atherosclerosis is accelerated by several risk factors.^{7,8} Table 1.1 shows a number of classic and putative new cardiovascular risk factors at young age.

Figure 1.1 Development of atherosclerosis⁹



Birth weight and intra-uterine environment as cardiovascular risk factors

It is postulated that low birth weight is associated with increased risk of chronic disease and mortality, also known as the “fetal origins hypothesis”. Although there have been a number of studies to support this hypothesis, these reports have also been criticized as being biased and for statistical error.^{10,11}

Furthermore, the mechanisms underlying the fetal origins hypothesis findings is debated. Initially, it was postulated that an unfavorable nutritional intra-uterine environment programs the fetus to adapt itself resulting in decreased fetal growth and chronic disease in later life.¹² Alternatively, fetal and parental genes may influence both size at birth and adverse future outcome.¹³⁻¹⁶

Blood pressure as risk factor in the young

Primary preventive medication benefits have been proven worthwhile in case of hypertension.¹⁷ However, preventive actions in the young and particularly in children have not been attempted in The Netherlands. To start preventive strategies in the young, first, individuals with increased risk of cardiovascular disease need to be identified. Studies showed that elevated blood pressure at late adolescence and young adulthood predicts high risk of future cardiovascular events.¹⁸⁻²⁰ Also, epidemiological investigations, such as the Bogalusa Heart study and the Muscatine study, suggest that childhood blood pressure predicts adult blood pressure.²¹⁻²⁴ However, it is unknown whether adolescent blood pressure as routinely measured by school health care, predicts hypertension or total cardiovascular risk in adulthood sufficiently accurate, to justify large-scale blood pressure screening.

Table 1.1 Classic and putative new pathophysiological factors associated with accelerated atherosclerosis at young age⁸

Genetic heredity	
Low birth weight:	<ul style="list-style-type: none"> - caused by genetic programming (polymorphism in promoter region of the insulin-like growth factor-I gene, mutations in glucokinase gene and D allele of the angioconverting enzyme) - caused by an unfavorable intra-uterine environment (undernutrition, toxins, maternal disease during pregnancy and maternal diet during gestation)
Insulin resistance and hyperinsulinemia	
Glucose intolerance	
Relative high blood pressure/hypertension	
Obesity/high waist-hip ratio	
Dyslipidemia:	<ul style="list-style-type: none"> - high levels of LDL-cholesterol, lipoprotein (a), apolipoprotein B-100 - atherogenic dyslipidemia (hypertriglyceridemia, low level of HDL-cholesterol, and small dense LDL-cholesterol) - low level of apolipoprotein A
Life style-related factors:	<ul style="list-style-type: none"> - smoking and chewing tobacco - low physical activity - unfavorable dietary profile (high dietary fat and energy intake, low intake of antioxidants) - (parental) socio-economic status
Microalbuminuria	
Elevated plasma levels of homocysteine	
Prothrombotic state:	<ul style="list-style-type: none"> - elevated plasminogen activator inhibitor-1 activity - high serum viscosity - high levels of plasma fibrinogen, tissue plasminogen activator antigen, and factors V, VII, and VIII - increased platelet aggregation
Leptin	
Bilirubin	
Inflammatory markers:	<ul style="list-style-type: none"> - elevated level of C-reactive protein and other markers
Endothelial dysfunction:	<ul style="list-style-type: none"> - indicated by elevated levels of Von Willebrand factor antigen and vascular and cellular adhesion molecules

LDL: low-density-lipoprotein; HDL: high-density-lipoprotein.

Two Dutch cohorts of the Atherosclerosis Risk in Young Adults (ARYA) study presented in this thesis, obtained data at birth, childhood and adolescence from the Dutch health care system. Around the age of 30 years the subjects were recently invited for re-examination of the cardiovascular risk. These data provided a unique opportunity to investigate some of the still unanswered etiologic and prognostic questions in the field of early determinants of cardiovascular disease.

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1.2

Outline

In this thesis, the presented studies are based on two Dutch population based cohorts of the Atherosclerosis Risk in Young Adults (ARYA) study (chapter 2). Characteristics recorded at birth and cardiovascular risk factors noted at adolescence, were obtained. Recently, they were screened for cardiovascular risk factors and vascular damage at young adulthood. The data of these two cohorts were studied on early determinants of cardiovascular risk in adolescents and young adults.

In chapter 3.1 we investigated whether a functional polymorphism in the promoter region of the insulin-like growth factor-I (IGF-I) gene, was observed as a genetic cause of the relation between birth size and cardiovascular risk at adolescence and young adulthood. The risk was determined by risk factors, arterial wall thickening and stiffness. Furthermore, in chapter 3.2 we comment on the relation between the genotype of the parents and the birth size of their first newborns.

The role of body mass index in the relation between birth weight and blood pressure at young adulthood, was assessed as an intermediate variable, a confounding variable and an effect modifier in chapter 4.1. In chapter 4.2 we related birth weight, -height and ponderal index to the absolute 10-year risk of coronary heart disease, using the Framingham risk score.

In chapter 5.1 the adolescent blood pressure was independently associated with the intima-media thickness of the carotid artery, a marker for life long exposure to atherosclerotic risk factor levels. Also we examined whether tracking and detracking of blood pressure levels from adolescence into young adulthood was associated with arterial wall thickening. Moreover, in chapter 5.2 we studied whether adolescent blood pressure as routinely measured by youth health care practice, predicts hypertension or total cardiovascular risk in adulthood with sufficient accuracy to warrant blood pressure screening.

The main results of the studies described in this thesis are reviewed in chapter 6. Also, the practical relevance is discussed and suggestions for future research are provided.

CHAPTER 2

The Atherosclerosis Risk in Young Adults (ARYA) study: rationale and design

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2.1 Abstract

Background: Despite recent advances in treatment, cardiovascular disease (CVD) is still health problem number one in western societies. Aiming at specific prevention strategies for high-risk individuals and shifting the available prevention programs towards younger age groups might increase the success of primary prevention. However, before addressing age-specific prevention programs, more insight in the determinants of early vascular damage and increased cardiovascular risk is warranted as well as insight in determinants of increased cardiovascular risk, including vascular damage, at an early age. The Atherosclerosis Risk in Young Adults (ARYA) study was specifically designed to address this issue. The ARYA study started off with studies evaluating 1) whether it is possible to predict cardiovascular risk at young adulthood by routinely measured adolescent data, and 2) evaluating the role of birth characteristics and adolescent characteristics to the development of vascular damage at young adulthood.

Methods: The ARYA study comprises of two cohorts of young adults. The cohort of Utrecht includes 750 young adults, aged 27-30 years. The cohort of The Hague includes 262 young adults born between 1963-1968. Data on birth characteristics, growth in early infancy as well as adolescent anthropometry, blood pressure, lipids, body mass index were obtained from the original medical records of the youth health care practice. In 1999/2001, the extent of subclinical vascular damage was measured using carotid wall thickness and aortic stiffness. Also, data on adult cardiovascular risk profile, bone density and central blood pressure were assessed, fasting blood was drawn and timed overnight urine samples were collected.

Conclusion: The ARYA study is aimed to provide data on early determinants of cardiovascular risk, including vascular damage, at an early age. This knowledge enhances the understanding of atherosclerosis development and cardiovascular disease risk and is needed to improve the available primary prevention programs.

2.2 Introduction

Despite recent advances in treatment, leading to a considerable reduction in cardiovascular mortality, cardiovascular morbidity is still health problem number one in the industrialized countries and rapidly increasing in the rest of the world. Aging of the population and an improved survival after non-fatal cardiovascular disease (CVD)-events bear heavily on medical costs. An improvement in primary prevention of CVD is essential in the fight against the cardiovascular epidemic as it may delay the development of atherosclerosis and hence reduce the incidence of CVD. However, before addressing age-specific prevention programs, more insight in the determinants of early vascular damage and increased cardiovascular risk is warranted as well as insight in determinants of increased cardiovascular risk,

including vascular damage, at an early age. The Atherosclerosis Risk in Young Adults (ARYA) study was specifically designed to address this issue. The ARYA study started off with studies evaluating 1) whether it is possible to predict cardiovascular risk at young adulthood by routinely measured adolescent data, and 2) evaluating the role of birth characteristics and adolescent characteristics to the development of vascular damage at young adulthood. In addition, the ARYA study enables studies on genetic traits as determinants of cardiovascular risk, assessed by measurement of risk factors and vascular damage. The rationale for these studies came from a number of observations.

Elevated blood pressure at late adolescence and young adulthood has been associated with an increased risk of cardiovascular morbidity and mortality.¹⁻³ In addition, several studies showed that a single standardized adolescent blood pressure relates to a relatively high blood pressure in young adulthood, and that combining several bi-annually blood pressure measurements improved the prediction.⁴⁻⁶ Also, other adolescent determinants appear to be related to future hypertension.⁷ Yet, whether routinely collected blood pressure measurements at adolescence predict elevated blood pressure at young adulthood is less clear, but relevant for routine clinical practice.

In 1989, Barker suggested that maternal undernutrition during sensitive periods of rapid growth results in a permanent change in infants' physiology and metabolism that lead to intrauterine growth retardation and an increased risk to develop chronic diseases, among others cardiovascular disease, in adult life.⁸ Since then this association has been confirmed in cohort studies among middle-aged and elderly subjects, from a range of countries.⁹⁻¹² However, the Barker's hypothesis is subject of heated debate in literature. Critics suggest that the observed relation between low birth weight and increased risk of cardiovascular disease in later decades should not be interpreted as causal. Selection bias, confounding, inconsistencies within and across studies as well as the influence of both genetic predisposition and postnatal growth are the most frequently raised objections.¹³⁻¹⁷ As far as we know, studies evaluating the relation between birth characteristics and vascular damage in young adulthood are scarce.¹⁸⁻²² The use of various vascular measures to further explore this relation in the young would add important information as subclinical vascular damage reflects the impact of cumulative exposure to adverse cardiovascular risk factors in preceding years. Furthermore, it may help to bridge the gap between studies in the young looking at the relation between birth characteristics and risk factors and studies in middle-aged mainly looking at the association between birth characteristics and CVD-events.

Longitudinal studies of children followed into young adulthood suggest that adverse life style and cardiovascular risk profile tend to 'track' into adulthood.²³⁻³⁰ Tracking refers to the relative stable position of an individual parameter, like body

mass index or blood pressure, within the group distribution over time. Reports from the Bogalusa Heart study showed positive associations between obesity in childhood and/or adolescence and an adverse cardiovascular risk profile at young adulthood.^{27,31-33} In addition, recent reports showed that childhood obesity was positively related to cardiovascular morbidity and mortality years hereafter.³⁴⁻³⁷ However, data on the relation between adolescent level of cardiovascular risk factors and early manifestation of vascular damage are limited but highly needed to enlarge the understanding in the etiology of cardiovascular disease.

The ARYA study population allows studies into the role of genetic traits in which the role of genetic traits can be evaluated on cardiovascular risk development at adolescence and young adulthood, both ages at which subjects are free from advanced cardiovascular disease. For instance, genetic traits involved in insulin resistance have been related to a low birth size, type 2 diabetes and cardiovascular diseases in later life.³⁸⁻⁴⁰ Such findings may partly explain association found in ARYA.

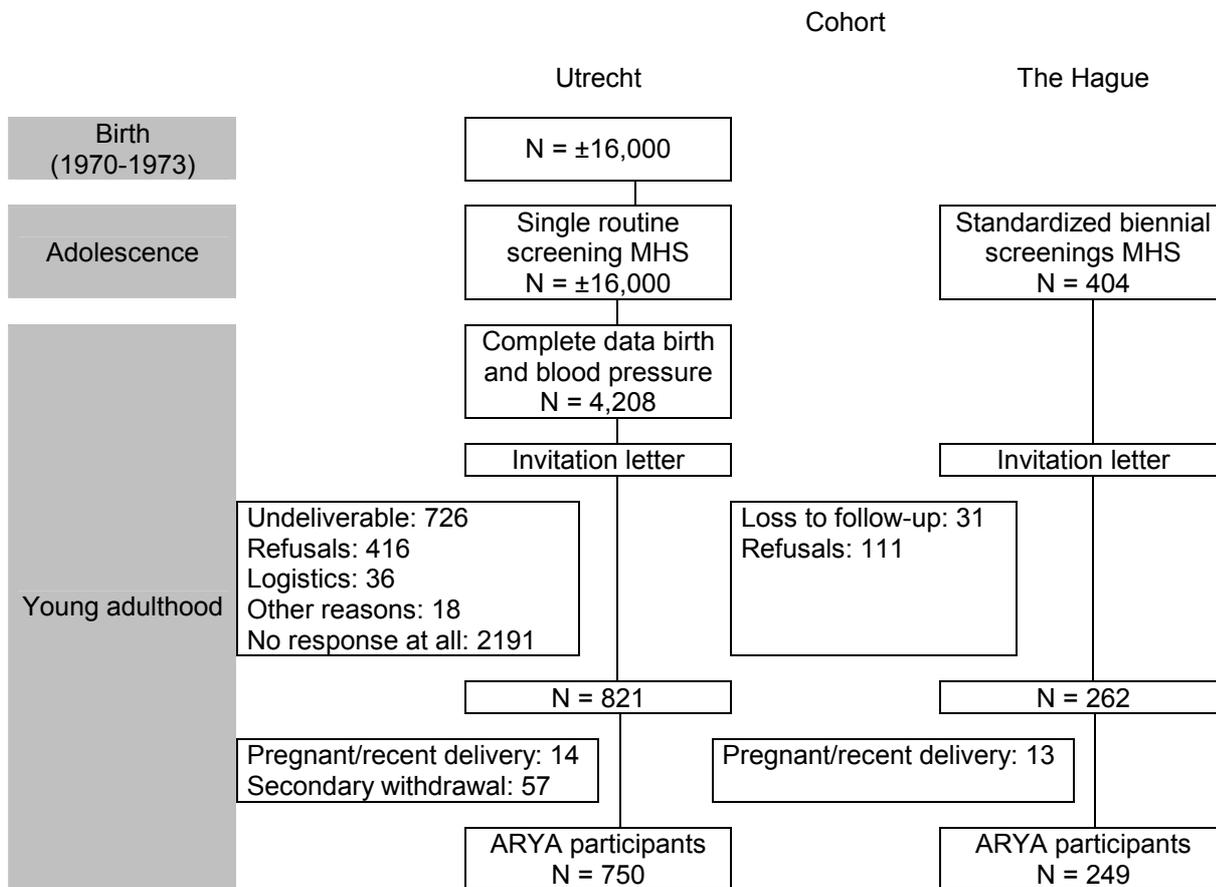
The ARYA study was designed to evaluate the early determinants, measured in these 3 predefined age-periods, of (subclinical) elevated blood pressure, vascular damage, and cardiovascular risk in healthy young adults.

2.3 Methods

Study population and selection procedure

The ARYA study comprises of two cohorts of young adults. The Utrecht-cohort includes 750 young adults born between 1970-1973, who attended secondary school in the city of Utrecht in The Netherlands and of whom original medical records were available from the youth health care practice. In The Netherlands virtually all children regularly visit the child health facilities of the Home Care Organizations and the Municipal Health Services, starting at four weeks until leaving secondary school at the age of 16 to 19 years. Information is routinely collected in health records by school doctors and nurses and kept at the Municipal Health Service. The 'The Hague-cohort' includes 262 young adults born between 1963-1968, who attended a particular secondary school in the city of the Hague, The Netherlands and of whom repeated adolescent lipid- and blood pressure measurements were available. The enrollment schemes of both cohorts are shown in figure 2.1. The ARYA study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, The Netherlands. All participants gave written informed consent. The general characteristics of the ARYA population are described in table 2.1 and 2.2.

The ARYA study participants are planned to be followed up by having repeated measurements over time (5 year intervals) of risk factors and vascular measures of functional and structural arterial characteristics and for the occurrence of cardiovascular events.

Figure 2.1 Selection procedures of the ARYA participants

MHS: Municipal Health Service.

The Utrecht cohort

To enroll the ARYA participants in Utrecht a stepwise procedure was used (figure 2.1): all available Municipal Health Service charts ($n=\pm 16,000$) were checked for the presence of adequately registered birth weight (birth weight notations with a \pm sign were excluded) and at least one blood pressure measurement during adolescence. All young adults with a complete chart ($n=4208$; 26.9%) were invited by mail (last-known parental address) to participate in the study. 2191/4208 (52.1%) individuals did not respond despite regular mailings, 726/4208 (17.3%) letters were returned due to an inadequate address, 470/4208 (11.2%) subjects declined to take part and 821/4208 (19.5%) were willing to participate. Of the eligible 821 young adults, 14 were excluded because of pregnancy and 57 declined to participate after they had given informed consent. Ultimately, 750 young adults completed participation in the Utrecht part of the ARYA study. From October 1999 to October 2000, the participants visited our research center twice, with a mean interval of 20.4 days (SD: 10.7), for evaluation of their cardiovascular risk profile and the extent of subclinical vascular damage.

To evaluate whether selection bias might be an issue we collected information about birth weight, systolic- and diastolic blood pressure as well as weight and height during adolescence from all charts matching our inclusion criteria in birth cohort 1970. None of these parameters was significantly different between responders and non-responders. Besides, the social economical status as well as the educational level of our participants was comparable to mean population levels as provided by the Central Office for Statistics in The Netherlands (www.cbs.nl).

The The Hague cohort

The Municipal Health Service of The Hague invited all first-year students starting in 1978 and 1979 at the Aloysius College, a secondary school. Of all invitees, 98% attended the study, one refused participation and five refused venipuncture. Information was biennially collected in a standardized way during seven years. At adolescence, an examination of the total cohort occurred at the end of September and March of each year between 9.00 and 15.00 hour within one week time. Blood pressure was measured three times within three minutes in sitting position at the right upper arm. A dynamap (Physiometrics SR-2) with a cuff of 13x23.5 cm was used. Body weight and height were measured without shoes. Blood was drawn 15 minutes after the blood pressure measurement. Total cholesterol was measured by the enzymatic CHOD-PAP method by Röschlau and the level of high-density-lipoprotein (HDL-) cholesterol was measured by the NA-P-wolframaat and $MgCl_2$ -precipitation by Prindler. A second WHO-recognized laboratory showed similar results for total cholesterol ($n=172$, $r=0.9733$) and HDL-cholesterol ($n=168$, $r=0.9450$). Recently, we invited all participants to measure cardiovascular risk factors at young adulthood. Of the 404 subjects initially attending the study, 262 (65%) subjects were willing to participate. 31 young adults were lost to follow-up, 111 subjects did not respond to the invitation, did not want to participate in the study or did not show up at the appointments (figure 2.1). Of these 262 subjects, 10 women were pregnant and 3 women gave birth less than five months ago. This resulted in a total of 249 (62% of the initially 404) participants in the cohort of The Hague. The participants were examined between November 2000 and February 2001 at their former secondary school in The Hague ($n=196$) or in the outpatient clinic of the Julius Center in Utrecht ($n=35$) by either one of the two first authors (LEV, AO). Of 16 subjects (of the 249), both visits took place elsewhere. Two young adults were not able to come over and sent results of two recent medical check ups. For the latter, the general practitioners and nurses were instructed by letter to examine the participants according to the overall protocol of the ARYA study. The blood pressure measurements, anthropometry and lipid values were recorded on standardized forms.

To evaluate possible selection, we compared adolescent data (visit 3) of the 249 participating and 155 non-participating and pregnant young adults. Both groups were

similar with respect to age, anthropometry, and single, double and triple blood pressure readings. Also, 49 of the 155 non-participating young adults completed a shortened questionnaire. Data at young adulthood was similar for gender, age, smoking habits, socio-economic status of the young adults and fathers, and cardiovascular diseases of the young adults and first-degree family members.

Birth characteristics and anthropometry in early infancy (*Utrecht cohort only*)

In The Netherlands, virtually all babies attend the Home Care Organization, a part of the youth health care practice, in their first 4 years of life. Growth and development are monitored, vaccinations are given and parental advice is given when needed. The first contact is scheduled when the newborn is about 4 weeks old and at that time data, provided by the mother, on pregnancy, delivery and birth characteristics are recorded.

For the purpose of the ARYA study, birth weight, birth length, gestational age, information about the delivery and data on weight and height in the first 4 years of life were obtained from the original charts of the Municipal Health Service. Since the presence of information on birth weight was one of the inclusion criteria for participation in the ARYA study, it was known in all participants but one, who was included by mistake. In contrast, length at birth and gestational age were known in 581/750 (77.5%) and 599/750 (79.9%) participants, respectively. Premature birth (n=32) was defined as birth before 37 weeks of gestation.

Adolescent parameters

Routine physical examinations of all Dutch children are performed by the Municipal Health Services during primary and secondary school period. Data at adolescence is shown in table 2.2.

For each Utrecht ARYA participant, information about adolescent blood pressure (a single measurement using a manual sphygmomanometer), -weight, -length and puberty (Tanner) stage was available from the original school health records. Data on smoking status, alcohol and the use of oral contraceptives at adolescence was present but had considerable number of missing values. Adolescent parameters were collected over minimally one and maximally four different points in time between the ages of 12-16 years. The number of individuals with multiple adolescent measurements declined gradually (n=750, n=291, n=38 and n=1 with at least 1, 2, 3 and 4 measurements, respectively).

For the The Hague cohort, blood pressure measurements, as described earlier, were available from baseline (1978-1979) until 1991-1992. During these visits also serum lipids and body mass index were assessed.

Table 2.1 General characteristics of the ARYA participants at adolescence

Adolescent parameters	Utrecht cohort		The Hague cohort	
	Men (n=352)	Women (n=398)	Men (n=223)	Women (n=164)
Age (years)	13.5 (1.1)	13.4 (1.2)	13.2 (0.7)	13.1 (0.7)
Systolic blood pressure (mmHg)	111.7 (12.5)	109.0 (11.3)	119.9 (12.4)	118.1 (11.9)
Diastolic blood pressure (mmHg)	67.4 (9.6)	66.0 (10.1)	67.4 (8.4)	67.7 (7.3)
Weight (kg)	49.2 (10.5)	49.9 (9.9)	46.8 (8.3)	47.9 (8.1)
Height (cm)	163.0 (10.3)	161.2 (8.0)	159.3 (8.9)	159.3 (7.0)
Body mass index* (kg/m ²)	18.3 (2.4)	18.9 (2.9)	18.3 (2.0)	18.8 (2.4)
Genital development (breast)*		3.6 (1.1)		-
Genital development (penis)*	2.9 (1.2)		-	
Pubic hair development*	2.9 (1.3)	3.7 (1.4)	-	-
Menstruation (%)		60%		-
Time between adolescent & adult measurement (years)	14.9 (1.3)	14.9 (1.3)	-	-

Values are expressed as mean (standard deviation).

*According to Tanner stage.

Cardiovascular risk profile at young adulthood

Data at young adulthood is presented in table 2.2. At young adulthood (mean age: 28.4 years) cardiovascular risk factors were measured at our research center. All investigators were similarly instructed and blinded for data in the medical school health records. After a five minutes rest in sitting position, blood pressure was measured with a semi-automated device (Dynamap) without replacing the cuff between the two measurements at the left upper arm. After 5-15 minutes rest the measurement was repeated. The same procedure was followed at the second visit with a mean interval of 20.4 days (SD 10.7). Mean systolic- (SBP) and diastolic (DBP) blood pressure were calculated as the average of the 4 measurements. Pulse pressure was calculated as the difference between systolic and diastolic blood pressure. Peripheral (i.e. brachial) mean arterial pressure (MAP) was calculated as $(2 \cdot \text{DBP} + \text{SBP}) / 3$. In the ARYA study, the correlation between the duplicate adult blood pressure measurement at the first and second visit was 0.7 and 0.6 for SBP and DBP, respectively.

According to the JNC V definitions⁴¹, hypertension at young adulthood was defined as the average systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or use of anti-hypertensive medication (stages I-IV).

Body height was measured in standing position - without shoes - to the nearest 0.1 cm. Body weight was measured with indoor clothes - without shoes - to the nearest 0.5 kg. To compensate for clothes we subtracted 0.5 kg. Waist-to-hip ratio was measured with indoor clothes.

Table 2.2 General characteristics of the ARYA participants at young adulthood

Young adulthood	Cohort of Utrecht		Cohort of The Hague	
	Men (n=352)	Women (n=398)	Men (n=142)	Women (n=107)
Age (years)	28.4 (0.9)	28.4 (0.9)	34.3 (0.8)	34.3 (0.7)
Systolic blood pressure (mmHg)	131 (12)	121 (12)	134 (14)	121 (14)
Diastolic blood pressure (mmHg)	73 (8)	71 (8)	75 (9)	70 (9)
% of hypertensives*	24.7%	6.3%	29.6%	12.1%
Mean arterial pressure (mmHg)	92 (8)	87 (9)	95 (10)	87 (9)
Pulse pressure (mmHg)	58 (9)	50 (8)	59 (10)	51 (11)
Weight (kg)	83.5 (13.6)	71.3 (14.9)	85.4 (13.0)	69.5 (12.8)
Height (cm)	183.9 (6.7)	170.2 (6.4)	182.9 (7.0)	169.0 (6.4)
Body mass index (kg/m ²)	24.7 (3.7)	24.7 (5.0)	25.5 (3.5)	24.3 (4.0)
Waist-hip ratio	0.88 (0.06)	0.81 (0.06)	0.92 (0.06)	0.83 (0.07)
Total cholesterol (mmol/l)	4.8 (1.0)	4.8 (0.8)	5.3 (1.1)	4.9 (0.8)
HDL-cholesterol (mmol/l)	1.3 (0.3)	1.6 (0.4)	1.2 (0.3)	1.6 (0.3)
Triglycerides (mmol/l)	1.4 (0.9)	1.2 (0.6)	1.1 (0.7)	0.9 (0.4)
LDL-cholesterol (mmol/l)	2.9 (0.9)	2.7 (0.8)	3.6 (0.9)	3.0 (0.8)
Glucose (mmol/l)	5.2 (1.2)	4.8 (0.4)	5.3 (0.7)	5.0 (0.5)
Current smoker (%)	36%	27%	34%	25%
# Daily cigarettes in current smokers	12.9 (8.4)	14.5 (8.8)	13.7 (9.9)	11.8 (7.2)
# Years smoked in current smokers	9.6 (3.6)	10.3 (3.5)	13.8 (5.5)	14.3 (5.6)
Parental history myocardial infarction (%)	3.5%	3.9%	19.2%	17.9%
Parental history for diabetes (%)	5.6%	4.9%	7.4%	10.8%
Speed of sound (m/s)	1568 (30)	1566 (31)	1564 (26)	1564 (32)
Broadband ultrasound attenuation (dB/MHZ)	76.7 (16.1)	71.1 (14.7)	80.7 (14.5)	74.5 (14.2)
Quantitative ultrasound index	103.3 (18.1)	99.9 (17.8)	103.2 (15.9)	100.6 (18.1)
Urinary sodium excretion (mmol/24h)	131 (79)	127 (68)	125 (74)	111 (60)
Urinary potassium excretion (mmol/24h)	47 (22)	41 (17)	48 (19)	36 (14)
Urinary calcium excretion (mmol/24h)	3.8 (2.7)	3.2 (2.3)	3.9 (2.4)	3.5 (2.2)
Urinary total protein (mg/24h)	47 (37)	50 (60)	56 (27)	45 (22)
Urinary albumin excretion (mg/24h)	12 (19)	14 (54)	13 (8)	15 (18)
Urinary creatinine excretion (mmol/24h)	17 (5)	13 (9)	17 (4)	12 (3)
CIMT (mm)	0.49 (0.05)	0.48 (0.05)	-	-
Common carotid lumen diameter (mm)	6.19 (0.40)	5.66 (0.36)	-	-
Aortic PWV** (m/s)	6.5 (1.5)	5.5 (1.3)	-	-
10-year CHD risk {273}	3.1 (1.9)	0.3 (0.2)	4.9 (3.0)	0.7 (0.5)

Values are means with standard deviation.

LDL: low-density-lipoprotein, HDL: high-density-lipoprotein, CHD: coronary heart disease, PWV: pulse wave velocity.

*Hypertension is defined according to the JNC V definitions⁴¹: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg (hypertension stages I-IV).

**Adjusted for adult mean arterial pressure.

Fasting total cholesterol, HDL-cholesterol, triglycerides and glucose were determined using Vitros950 dry-chemistry analyzer (Johnson & Johnson, Rochester, New York, USA); LDL-cholesterol was calculated using the Friedewald formula. In timed-overnight urine samples sodium, potassium and calcium were determined using Beckman-Coulter Synchron LX20. The urinary excretions of creatinine and total protein were assessed by a Bio Rad Protein Assay, and microalbuminuria was assessed by ELISA (DAKO, Glostrup, Denmark). Blood-, urine- and DNA-samples were stored at -80°C for future research questions. In the cohort of The Hague, fasting blood was drawn of 240 young adults. Samples were kept at 4°C and the lipid profile and glucose level were determined within 4 hours. In The Hague, 187 samples were determined (LX-20 (Coulter-Beckman), in Utrecht 40 samples (Vitros950 dry-chemistry analyzer) and 13 samples were determined in laboratories elsewhere. Serum triglycerides values were logarithmically transformed to obtain a normal distribution in the analysis.

Information on smoking habits, alcohol intake, medical history, drug use and family history was obtained by a standardized written questionnaire. We obtained further information of the young adults by a written questionnaire on the highest education level (low/middle/high) as a measure of socio-economic status and cardiovascular family risk: hypertension of the first degree family members ($n=859$), myocardial infarction, stroke, diabetes and hypercholesterolemia.

In addition, all participants of the Utrecht cohort underwent a measurement of bone density at the right calcaneus assessed by the Sahara Clinical Bone Sonometer (Hologic, Waltham, USA).

Based on the information on the risk factors, the 10 years risk of the ARYA participants is estimated using the Framingham Heart Study risk score.⁴²

Common carotid intima-media thickness (CIMT) (Utrecht cohort only)

B-mode ultrasonography of both the left and right common carotid arteries was performed using a 7.5 MHz linear array transducer (Acuson Aspen, Mountain View, California, USA). When an optimal longitudinal image was obtained, it was frozen on the R-wave of the electrocardiogram and stored on S-VHS videotape.⁴³ This procedure was repeated at 4 predefined angles per side (180° , 150° , 120° , 90° for the right carotid artery and 180° , 210° , 240° , 270° for the left carotid artery) using the Meijer's Arc[®] (figure 2.2), allowing the collection of detailed information in a highly standardized and reproducible way. The ARYA study images were performed by 6 sonographers, which completed a uniform certification program. This program included a detailed training session in which the sonographer was familiarized with the ultrasound protocol and the ultrasound equipment, followed by the performance of 20 pre-certification scans in which a high level of proficiency had to be demonstrated. The actual measurements were performed off-line. The frozen images

Figure 2.2 Meijer's arc



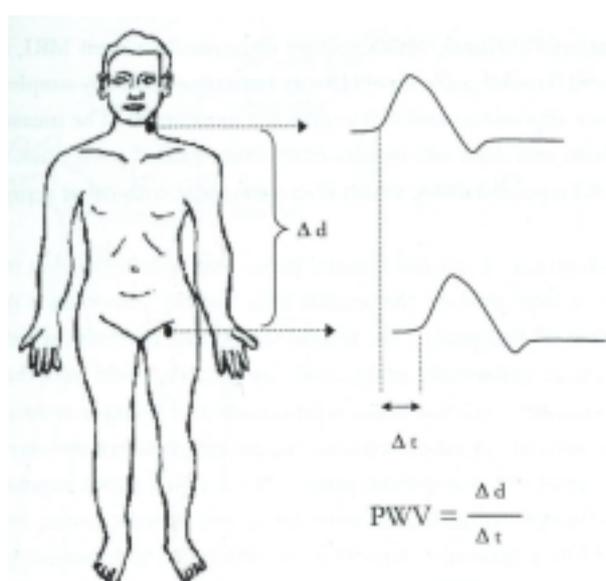
on the videotape were digitized and displayed on a screen using additional dedicated software as described in detail.⁴³ In short, the interfaces of the distal common carotid artery were marked over a length of 10-mm using an automated edge detection approach. The beginning of the dilatation of the distal carotid artery served as a reference point for the start of the measurement. The average of the intima-media thickness of the 8 predefined angles (each angles includes one image from the near wall and one image from the far wall) were used for each subject as a measure for current wall thickness of the common carotid artery. Two readers were engaged in the reading procedure of the echographic images. The reproducibility of the CIMT-measurement was assessed by scanning 21 subjects on a second occasion by another sonographer but read by the same reader. Absolute mean difference (SE) of the repeated measurements between visits was 0.012 mm (0.004) for mean intima-media thickness of both carotid arteries. The intraclass correlation coefficient was 0.84.

Aortic pulse wave velocity (PWV) (Utrecht cohort only)

In the ARYA study, PWV was measured non-invasively in the carotid-femoral segment using the SphygmoCor device (PWV Medical, Sydney, Australia) after extensive training. Due to equipment failure, usable PWV-data were available in only 524 participants of the Utrecht part of the ARYA cohort. As the subjects with PWV-measurement did not significantly differ from those without PWV-data in respect to

cardiovascular risk profile (except for age), we concluded that missing values were a random phenomenon. Participants were asked to lie down for 10 minutes before starting and to refrain from talking during the procedure. Two distances were measured in a straight line using a compass to reduce the influence of body contours: (a) from the sternal notch to the proximal sampling site on the carotid artery and (b) from the sternal notch to the distal sampling site on the femoral artery. The carotid to femoral path length was estimated by subtracting distance (a) from distance (b). PWV was determined by sequential acquisition of pressure waveforms from the carotid and the femoral arteries by applanation tonometry (Millar SPT 301 pressure transducer, Millar Instruments). The timing of these waveforms was compared with that of the R-wave on the simultaneously recorded ECG. PWV was determined by calculation of the difference in carotid to femoral path length divided by the difference in R-wave to waveform foot times (figure 2.3). The average of 10 successive measurements was used in the analyses to cover a complete respiratory cycle. The whole procedure was repeated 3 times per subject and the average PWV-value was used for the analyses. The reproducibility of the PWV-technique was assessed by repeating the procedure in a subset of 25 participants several weeks after the first visit. Absolute mean difference (+SE) in PWV of the repeated measurements between visits was 0.12 m/s (0.45). The intraclass correlation coefficient for repeated measurements was 0.67. Since the repeated measurements were performed on two different occasions and we failed to measure the blood pressure during the repeated visit, the moderate correlation could be explained in part by the variability in blood pressure over time (as described earlier).

Figure 2.3 Measurement of aortic pulse wave velocity⁴⁴



Δd = distance between the carotid artery and the femoral artery

Δt = time delay between arrival of the pulse wave in the carotid artery and the femoral artery

Determinants of central blood pressure indices from peripheral waveforms

The arterial pressure waveform varies throughout the arterial tree due to differences in vessel compliance and the phenomenon of wave reflection.⁴⁵ The pressure waveform at any point is a composite of the forward-going and reflected wave. O'Rourke et al. developed the technique of pulse wave analysis (PWA), making non-invasive derivation of central pressure waveforms possible.⁴⁶ This technique uses applanation tonometry to record pressure waves from peripheral arteries.

In the ARYA study, peripheral pressure waveforms were recorded from the radial artery at the wrist using micromanometer (Millar SPT 301, Millar Instruments). Ascending aortic pressure was derived from the central pressure waveform, using a transfer function⁴⁷ that is incorporated in the SphygmoCor device. Our results showed high correlation (Pearson's correlation coefficient: 0.94) between the brachial MAP, calculated using the formula $(2*DBP+SBP)/3$, and the central MAP, estimated by the transfer function. Theoretically, invalid estimation of the central blood pressure parameters by the transfer function would bias the relations between the cardiovascular risk factors and PWV if the error in the central MAP estimate is differential (i.e. the magnitude of the 'error' depends on the level of blood pressure). To our knowledge, however, a validation study in a young population with a broad blood pressure range, in which central MAP calculated with either the transfer function or manually (as the area under the generated central waveform) were compared according to levels of peripheral blood pressure, has not been performed.

2.4 Data analysis

The predictive capacity of adolescent blood pressure readings to discriminate between presence and absence of hypertension in young adulthood was quantified using univariate logistic regression analyses and estimated as logistic regression coefficients with their 95% confidence intervals. Reliability (goodness of fit) of the models was estimated using the Hosmer & Lemeshow test.⁴⁸ The prognostic ability to discriminate between young adults with and without hypertension was assessed using the Receiver Operation Characteristic curve (ROC-area).

The relationships between birth characteristics, adolescent parameters or adult cardiovascular risk factors and the two measures of subclinical vascular damage were evaluated by univariate and multivariate linear regression models. Subgroup analysis was performed only when the added interaction term was significant in the multivariate model and if a biological plausible mechanism was present. Since there was a statistically significant difference in CIMT between the two readers, all analyses using CIMT as dependent variable were adjusted for reader. In the analyses with PWV as the outcome variable and cardiovascular risk factors as independent variables, failure to correct for blood pressure could leave room for

residual confounding as PWV is related to wall elasticity and by that to distending pressure⁴⁹ while blood pressure is related to several cardiovascular risk factors such as body mass index, waist circumference, HDL-cholesterol and insulin. Mean arterial pressure is preferred to use for this correction because it remains constant between central and peripheral arteries while all other blood pressure parameters show pressure amplification along the arterial tree.⁵⁰

For analyses on genetic traits a multivariate linear regression model was used to study the relation between genotypes and cardiovascular risk factors, intermediate measures of vascular damage and birth characteristics as continuous variables.

2.5 Statistical power

Power calculations were performed for the main research questions of the ARYA study, i.e. the relation between birth weight and subclinical vascular damage at young adulthood, based on duplicate measurements of the vascular entities. In a study sample of 500 young adults, a CIMT-difference of at least 0.03 mm could be detected between subjects born at the lowest compared to subjects born at the highest quartile of the birth weight distribution, with a type-I error of 5% and a type-II error of 20%. Analogously, in a sample size of 500 subjects, a PWV-difference of at least 0.7 m/s was expected to be detected under identical limiting conditions. The sample size was increased from 500 up to 750 subjects after the decision to refrain, due to logistic reasons, from duplicate vascular measurements.

2.6 Discussion

The Atherosclerosis Risk in Young Adults (ARYA) study was specifically designed to evaluate 1) whether it is possible to predict cardiovascular risk at young adulthood by routinely measured adolescent data, and 2) evaluating the role of birth characteristics and adolescent characteristics to the development of vascular damage at young adulthood. In addition, the ARYA study enables studies on genetic traits as determinants of cardiovascular risk, assessed by measurement of risk factors and vascular damage.

In the ARYA study we are not able to use clinical events as outcome, since these subjects will not suffer from events for a long time. Therefore, we choose several types of outcomes, which are assumed to be markers of cardiovascular risk, i.e., surrogate outcomes. These include absolute risk factor levels, dichotomized risk factors using a cut off point at a certain percentile or clinically standardized cut off levels, a 10-years risk of coronary heart disease using the Framingham risk score, and the CIMT and PWV as indicators of vascular damage.

A surrogate endpoint is a biomarker that is intended to substitute for a clinical endpoint or end-organ damage and is positioned on the time axis in between the causal pathway.⁵¹⁻⁵³ There are two important reasons to use surrogate measures in stead of 'hard' endpoints in epidemiological studies. Firstly, the use of surrogate endpoints allows studies early in the process, i.e. before clinical manifestation of the disease of interest and thus at young age. Secondly, as surrogate endpoints can be measured in all participants, sufficient power can be attained in epidemiological studies with a smaller sample size. However, several criteria must be adhered before a measure can be used as a surrogate endpoint: 1) the measure must be valid and well reproducible 2) a biological plausible mechanism should underlie the pathway 3) the surrogate endpoint must be significantly related to both the risk factors and the disease of interest and 4) a change in risk factor level should induce change in the surrogate measure and 5) a change in the surrogate measure should be reflected in a change in risk of the disease of interest.

The surrogate marker, CIMT, assessed by high-resolution B-mode ultrasonography, is a non-invasive, reproducible method to quantify arterial wall thickening, atherosclerosis progression and carotid plaques⁵⁴. CIMT may be regarded as a valid marker of generalized atherosclerosis as it has been shown to be strongly associated with atherosclerosis in other parts of the arterial system⁵⁵⁻⁵⁹, with coronary calcification assessed by electron beam computer tomography⁶⁰ and to a lesser degree with coronary angiography.^{58,61,62} In middle-aged and elderly subjects, CIMT has been shown to be a strong predictor of subsequent cardiovascular morbidity and mortality.⁶³⁻⁶⁷ Furthermore, several population-based studies, predominantly in middle-aged subjects and the elderly, have shown significant associations between unfavorable cardiovascular risk profile and increased CIMT.⁶⁸⁻⁷² Stevens et al.⁷³ have shown that weight gain from early adulthood into middle adulthood was associated with increased CIMT. Similar results were observed for blood pressure and lipid levels.⁷⁴ In addition, results from the Tromso study⁷¹ showed positive associations between important cardiovascular risk factors and increased CIMT 15 years later. Besides, several studies have been conducted in which various interventions were shown to affect progression and regression of CIMT.⁷⁵⁻⁷⁸ However, data on the influence of change in CIMT on CVD-events as well as the usefulness of the CIMT-measurement in clinical care as a screening tool to improve risk assessment are scarce.⁷⁹ Nonetheless, the available evidence indicates that CIMT is a meaningful outcome measure in observational and intervention studies.

The surrogate marker PWV provides a non-invasive, reproducible and validated method to quantify arterial stiffness. Increased PWV has been shown to be a strong predictor of cardiovascular mortality in hypertensive subjects^{50,80}, in patients with end-stage renal disease⁸¹ and in healthy elderly.⁸² Moreover, cross-sectional studies in middle-aged and elderly subjects have shown that an unfavorable cardiovascular

risk profile is associated with increased arterial stiffness.⁸³⁻⁸⁷ In the young, most studies evaluating arterial stiffness are performed in high-risk children like children with diabetes mellitus⁸⁷, obesity⁸⁸ and hypercholesterolemia.⁸⁹ These studies had a cross-sectional design just like the two studies concerning healthy youngsters.^{91,92} However, an important drawback in all studies is insufficient adjustment for blood pressure, leaving room for residual confounding. Some evidence is present for stiffer arteries in children with a positive family history of myocardial infarction⁹² and familial hypercholesterolemia^{93,94} compared to age-matched healthy controls. Results about the impact of parental hypertension are less congruent.^{95,96} Several studies, particularly in the elderly, showed positive associations between aortic stiffness and atherosclerosis at different sites in the arterial tree.⁹⁷⁻¹⁰⁰ However, longitudinal studies evaluating the determinants of the progression of arterial stiffness as well as studies exploring the association between changes in arterial stiffness (more compliant) and improved survival are scarce and restricted to high-risk populations.^{100,101} Such evidence is needed to enhance the validity of PWV as a surrogate endpoint for CVD.

In summary, the ARYA study, together with two other cohort studies in children followed into adulthood, the Muscatine Study⁶⁰ and the Bogalusa Heart Study²⁷, is aimed to provide data on early determinants of cardiovascular risk, including vascular damage. This information will enhance our understanding of the underlying mechanism of atherosclerosis and CVD-development. Such knowledge is needed to improve the available primary prevention programs and to support discussion about new preventive strategies. Moreover, since the ARYA study disposes some other parameters, like urine samples, DNA and data on bone density, this cohort offers extensive possibilities for etiological research beyond those concerning atherosclerosis development. Finally, since the ARYA study includes young and healthy subjects, it provides an interesting cohort for further research on the development, differences and similarities of several manifestations of vascular damage characterized by different vascular measures, apart from CIMT and aortic PWV.

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CHAPTER 3

**A common functional polymorphism in
the promoter region of the insulin-like
growth factor-I gene**

3.1

A common functional polymorphism in the promoter region of the IGF-I gene is related to cardiovascular risk in adolescence and young adulthood but not with birth weight

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3.1.1 Abstract

Background: A common functional polymorphism in the promoter region of the insulin-like growth factor-I (IGF-I) gene may play an important role in the development of type 2 diabetes and myocardial infarction, and both pre- and postnatal body growth. However, the studies are limited and show contradicting results. Our aim was to study the relation between a functional polymorphism in the promoter region of the IGF-I gene and cardiovascular risk in adolescence and young adulthood, and birth size.

Methods: In a population based cohort study of 352 men and 398 women born in 1970-1973, birth characteristics, adolescent blood pressure and anthropometry were collected from the school health records. At young adulthood, we determined also fasting blood glucose, total cholesterol, triglycerides, HDL- and LDL-cholesterol and the IGF-I genotype. The common carotid intima-media thickness and pulse wave velocity as measures of subclinical cardiovascular damage were performed.

Results: Of the 729 subjects, 43% were homozygous carriers of the 19 CA repeat, 44% were heterozygous carriers and 13% were non-carriers. We observed a lower blood pressure in homozygous carriers, and a less atherogenic lipid profile at young adulthood (test for trend of LDL-cholesterol: $p=0.017$). Homozygous and heterozygous carriers were on average a 2.1 cm taller than non-carriers (test for trend at young adulthood: $p=0.030$). We could not observe an association between IGF-I genotype, and subclinical cardiovascular damage or birth size.

Conclusion: Homozygous carriers of a functional polymorphism in the promoter region of the IGF-I gene show a more favorable profile of cardiovascular risk factors in adolescence and young adulthood. A relation between the IGF-I gene and arterial wall thickness or wall stiffness could not yet be described at this young age.

3.1.2 Introduction

Cardiovascular diseases are still the major cause of morbidity and mortality in western countries. Already at young age, the development of the diseases is accelerated by cardiovascular risk factors such as hypertension, hypercholesterolemia and obesity.¹⁻³ The insulin-like growth factor-I (IGF-I) plays an important role in the development of cardiovascular diseases⁴, and both pre- and postnatal body growth.⁵ Hattersley et al. suggested that genes involved in insulin resistance caused a low birth size, type 2 diabetes and cardiovascular diseases in later life.⁶ This hypothesis has recently been confirmed by a study in the elderly, showing that subjects without the wildtype allele in the promoter region in the IGF-I gene had an increased risk of type 2 diabetes and myocardial infarction, and also low circulating IGF-I levels, reduced body height and low birth weight.^{7,8} However, a

second study with young adults showed contradicting results; no relations were described except for an increase of the IGF-I levels in non-carriers.⁹

To expand upon these conflicting results, we used data from the Atherosclerosis Risk in Young Adults (ARYA) study. We examined the relation between the functional polymorphism in the promoter region of the IGF-I gene and cardiovascular risk at adolescence and young adulthood, and birth characteristics.

3.1.3 Methods

The study population of the ARYA study comprises two cohorts: a cohort from the Dutch city of Utrecht consisting of 750 participants and a cohort from The Hague consisting of 262 participants. This report was restricted to the cohort of Utrecht because we determined the IGF-I genotype in this cohort only. The ARYA study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands. Written informed consent was obtained of all 750 participating young adults.

Registered risk factors at birth and adolescence

In The Netherlands virtually all children regularly visit the child health facilities of the Home Care Organizations and Municipal Health Service, starting at four weeks until leaving secondary school at the age of 16 to 19 years. Information was routinely collected and written down in health records by school doctors and nurses. Subjects of the ARYA study were born between 1970 and 1973 and attended secondary school in Utrecht. Records were selected on the presence of birth weight data and at least one adolescent blood pressure measurement, as previously described.¹⁰ A total of 750 subjects participated in the Utrecht cohort. Between the responders and non-responders born in 1970 (n=967), there were no differences in mean birth weight, and adolescent blood pressure and anthropometry.

We obtained information on birth weight (n=749), birth length (n=581), gestational age (n=599), delivery (n=688) and parity (n=736) noted one month after birth. Data from secondary school consisted of blood pressure measured by a sphygmomanometer (n=750), body height (n=750), weight (n=750) and sexual maturity determined by Tanner scale (n=656).

Risk factors at young adulthood

From October 1999 to December 2000 all participants underwent a physical examination during two visits at the outpatient clinic of the Julius Center. All investigators were similarly instructed and blinded for data in the medical school health records. After a five minutes rest in sitting position, blood pressure was measured by Dynamap at the left upper arm. After 5-15 minutes rest the

measurement was repeated. The same procedure was followed at the second visit with a mean interval of 20.4 days (SD 10.7). Body height was measured in standing position - without shoes - to the nearest 0.1 cm. Body weight was measured with indoor clothes - without shoes - to the nearest 0.5 kg. To compensate for clothes we subtracted 0.5 kg.

Of the 750 participants, fasting blood of 730 participants and non-fasting blood of 8 participants was drawn and stored at -20°C . When all the participants finally visited our outpatient clinic, levels of glucose, total cholesterol, triglycerides, high-density-lipoprotein (HDL-) cholesterol and low-density-lipoprotein (LDL-) cholesterol¹¹ were determined by Vitros950 dry-chemistry analyzer (Johnson & Johnson, Rochester, New York, USA).

We obtained further information on ethnicity, smoking (yes/no, packyears), alcohol consumption (yes/no), use of oral contraception (yes/no), cardiovascular family risk, and highest education level (low/middle/high) as a measure of socio-economic status, by a written questionnaire.

Arterial wall thickness and stiffness at young adulthood

The common carotid intima-media thickness of the right and left carotid artery was measured by six trained sonographers using a 7.5 MHz linear array transducer (Acuson Aspen). The common carotid intima-media thickness was measured at eight different angles (right carotid artery: 180, 150, 120 and 90 degrees, left carotid artery: 180, 210, 240 and 270 degrees) using the Meijer's Arc. A two dimensional image of the distal part of the common carotid artery was performed. An optimal image was frozen on the top of the R-wave of the electrocardiogram and recorded on tape. Two readers measured the common carotid intima-media thickness off line with a computerized analyzing system, over a length of 10 mm starting at the beginning of the dilatation of the bifurcation.¹² To assess reproducibility, the common carotid intima-media thickness of 21 participants was repeated at the same visit by another sonographer, and showed an absolute mean difference of 0.012 mm (standard error 0.004). The intraclass correlation coefficient for repeated measurements was 0.84.

The pulse wave velocity was measured between the carotid artery and the femoral artery using the SphygmoCor device. (Pulse wave velocity Medical, Sydney, Australia, Millar SPT 301 pressure reducer, Millar Instruments). The participants lied supine for 10 minutes before starting the measurement and were asked to refrain from talking. The carotid-femoral distance was calculated by subtracting: a) the distance from the suprasternal notch to the proximal sampling site on the carotid artery from: b) the distance from the suprasternal notch to the distal sampling site on the femoral artery. A compass was used to avoid the impact of body fat at the waist. The timing of the pressure waveform was compared with the timing of the R-wave at the electrocardiogram. Pulse wave velocity was calculated, dividing the carotid-

femoral distance by the difference in timing of the pressure waveform and R-wave. The average of 10 successive heartbeats was used to cover a complete respiratory cycle. To assess reproducibility, the pulse wave velocity of 25 participants was repeated within a mean time interval of three weeks. The absolute mean difference in pulse wave velocity was 0.12 m/s (standard error 0.45) and the intraclass correlation coefficient for repeated measurements was 0.67. Due to logistic reasons, the pulse wave velocity of only 524 subjects was measured and used in the analyses.

IGF-I genotype

Polymerase chain reaction was performed using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine (CA) repeat 1 kb upstream of the human IGF-I gene.¹³ The reaction was carried out in a final volume of 10 µl containing 50 ng of genomic DNA obtained from peripheral blood cells, 0.5 nmol/l forward primer (5'-ACCACTCTGGGAGAAGGGTA-3'), 0.5 nmol/l reverse primer (5'-GCTAGCCAGCTGGTGTATT-3'), 0.25 mmol/l 2'-dNTP, 2.2 mmol/l MgCl₂, 0.01% W1 (Gibco BRL), and 0.4 Taq DNA polymerase (Gibco BRL). Polymerase chain reaction was performed in 384 well plates (94°C 10 min; 35 polymerase chain reaction cycles of 30 s at 94°C, 30 s on 55°C, and 30 s on 72°C; 72°C 10 min; 4°C hold). Forward primers were labeled with FAM, HEX, or NED to determine the size of polymerase chain reaction products by autosequencer (ABI 3100, POP 4, filter set D, collecting time array 36 cm 7 s, peak-height between 100 and 2000, each lane containing three samples). The size of the polymerase chain reaction products was determined in comparison with internal ROX 500-size standard (Perkin Elmer).

3.1.4 Data analysis

The characteristics of men and women of the ARYA population were expressed as means with their standard deviation (SD). The Independent T-test was used to analyze gender differences. A multivariate linear regression model was used to study the relation between genotypes and cardiovascular risk factors, intermediate measures of vascular damage and birth characteristics as continuous variables. Because of the differences between men and women, gender was always included. We used a chi-square test to study the differences of IGF-I genotype between the babies in the lowest birth size stratum and the babies in the other strata. Therefore, all participants were categorized according to deciles of the birth size distribution.

At young adulthood, the mean of four blood pressure readings, sixteen carotid intima-media thickness measurements and three pulse wave velocity measurements were used for the analyses. Triglyceride values were logarithmically transformed to obtain a normal distribution. All analyses regarding the carotid intima-media thickness were adjusted for reader because of interobserver variation. Because pulse

wave velocity is related to wall elasticity and by that depend on blood pressure¹⁴, the analyses were adjusted for mean arterial pressure, calculated as: diastolic blood pressure + 1/3*(systolic blood pressure – diastolic blood pressure).

Analyses were carried out with the statistical analysis software SPSS 9.0. We used a cut off level of p=0.05 for statistical significance.

3.1.5 Results

Table 3.1.1 shows the general characteristics of the ARYA cohort according to gender. Men (46.9%) were born significantly heavier and taller and had a lower ponderal index. At adolescence, boys had a higher blood pressure and a lower body mass index. At young adulthood blood pressure, weight, body height and levels of fasting glucose, LDL-cholesterol and triglycerides were higher in men, and HDL-

Table 3.1.1 General characteristics of men and women of the ARYA cohort

	Men (n=352)	Women (n=348)
<i>Birth characteristics</i>		
Birth weight (kg)	3.48 (0.54)	3.37 (0.55)
Birth length (cm)	51.3 (2.5)	50.4 (2.5)
Ponderal index (kg/cm ³)	25.9 (3.1)	26.5 (3.1)
Gestational age (weeks)	39.8 (1.8)	39.8 (2.0)
<i>Adolescence</i>		
Age (years)	13.5 (1.1)	13.4 (1.1)
Systolic blood pressure (mmHg)	111.7 (12.5)	109.0 (11.3)
Diastolic blood pressure (mmHg)	67.4 (9.6)	66.0 (10.1)
Weight (kg)	49.2 (10.5)	49.9 (9.9)
Height (cm)	163.0 (10.3)	161.2 (8.0)
Body mass index (kg/m ²)	18.3 (2.4)	18.9 (2.9)
<i>Young adulthood</i>		
Age (years)	28.4 (0.9)	28.4 (0.9)
Systolic blood pressure (mmHg)	130.8 (12.0)	120.6 (12.3)
Diastolic blood pressure (mmHg)	73.1 (7.8)	70.9 (8.5)
Weight (kg)	83.5 (13.6)	71.3 (14.9)
Height (cm)	183.9 (6.7)	170.2 (6.4)
Body mass index (kg/m ²)	24.7 (3.7)	24.7 (5.0)
Total cholesterol (mmol/l)	4.8 (1.0)	4.8 (0.8)
LDL-cholesterol (mmol/l)	2.9 (0.9)	2.7 (0.8)
HDL-cholesterol (mmol/l)	1.3 (0.3)	1.6 (0.4)
Triglycerides (mmol/l)	1.4 (0.9)	1.2 (0.6)
Glucose (mmol/l)	5.2 (1.2)	4.8 (0.4)
Common carotid intima-media thickness (mm)	0.50 (0.05)	0.48 (0.05)
Pulse wave velocity (m/s)*	6.5 (1.5)	5.5 (1.3)

Values are means with standard deviation.

LDL: low-density-lipoprotein, HDL: high-density-lipoprotein.

*Pulse wave velocity is adjusted for mean arterial pressure.

Table 3.1.2 Allele distribution of the IGF-I promoter functional polymorphism

<i>Allele: number of base pairs</i>	<i>Genotype</i>	Men (n=340)	Women (n=389)
176 (X)		0 (0)	2 (0.3)
186 (X)		2 (2.9)	0 (0)
188 (X)		6 (0.9)	12 (15.7)
190 (X)		29 (4.3)	46 (5.9)
192 (Z)		439 (64.6)	493 (63.4)
194 (X)		142 (20.9)	146 (18.8)
196 (X)		49 (7.2)	58 (7.5)
198 (X)		13 (1.9)	21 (2.7)
Z/Z: 192/192	Homozygous	145 (42.7)	158 (40.6)
Z/X: 192/X	Heterozygous	149 (43.8)	177 (45.5)
X/X	Non-carrier	46 (13.5)	54 (13.9)

Data are n (%). The allele distribution is based on 2 alleles per participant.

IGF-I: insulin-like growth factor-I, Z: most frequent allele/wildtype, X: all other alleles.

cholesterol was lower than in women. The common carotid intima-media thickness and pulse wave velocity were higher in men. The mean time-interval between the measurements at adolescence and young adulthood was 14.9 years (SD=1.3).

IGF-I genotype

The IGF-I genotype was determined of 729 (97.2% of the 750) participants. Table 3.1.2 shows that eight alleles were identified. Genotype and allele distribution were in Hardy-Weinberg equilibrium ($p=0.44$). The most frequent allele in the promoter region of the IGF-I gene had a length of 192 base pairs, which is equivalent to a repeat of 19 cytosine-adenine (CA) base pairs. 86.5% of the population was either homozygous or heterozygous for the 192 base pair allele, suggesting that all other alleles originate from this so called wildtype allele (Z). All other seven alleles (X) were pooled and three possible genotypes were determined; of all subjects, 43% were homozygous by carrying two wildtype alleles, 44% were heterozygous carriers of the 19 CA repeat and 13% were non-carriers.

Blood pressure, lipid levels, body size, arterial wall thickness and stiffness

Table 3.1.3 describes that homozygous carriers had the lowest systolic and diastolic blood pressure, although statistical significance was only reached for adolescent diastolic blood pressure. The lipid profile was less atherogenic in homozygous carriers, showing a statistically significant test for trend in LDL-cholesterol.

Homozygous and heterozygous carriers were taller compared to non-carriers, a difference that was statistically significant at young adulthood. The mean body mass

Table 3.1.3 A functional polymorphism of the IGF-I gene and blood pressure and lipids

	Genotype				P (test for trend)	
	Homozygous	Heterozygous	P*	Non-carrier	P*	
<i>Adolescence</i>						
Systolic blood pressure (mmHg)	110 (0.68)	111 (0.95)	0.27	110 (1.37)	0.59	0.39
Diastolic blood pressure (mmHg)	66 (0.56)	68 (0.78)	0.006	67 (1.13)	0.33	0.07
Weight (kg)	49.3 (0.58)	49.6 (0.80)	0.67	49.5 (1.16)	0.80	0.72
Height (cm)	162.8 (0.53)	162.5 (0.73)	0.69	161.2 (1.05)	0.12	0.17
Body mass index (kg/m ²)	18.4 (0.15)	18.7 (0.21)	0.24	18.9 (0.30)	0.14	0.10
<i>Young adulthood</i>						
Systolic blood pressure (mmHg)	125 (0.69)	125 (0.96)	0.99	127 (1.39)	0.23	0.35
Diastolic blood pressure (mmHg)	72 (0.47)	72 (0.65)	0.46	73 (0.94)	0.15	0.39
Weight (kg)	76.2 (0.81)	78.0 (1.13)	0.11	75.6 (1.63)	0.71	0.75
Height (cm)	176.9 (0.37)	177.0 (0.52)	0.91	164.8 (0.75)	0.005	0.030
Body mass index (kg/m ²)	24.3 (0.25)	24.9 (0.35)	0.07	24.7 (0.50)	0.46	0.20
Total cholesterol (mmol/l)	4.77 (0.05)	4.90 (0.07)	0.06	4.87 (0.10)	0.32	0.13
LDL-cholesterol (mmol/l)	2.69 (0.05)	2.89 (0.07)	0.003	2.84 (0.10)	0.11	0.017
HDL-cholesterol (mmol/l)	1.47 (0.02)	1.42 (0.03)	0.10	1.47 (0.04)	0.96	0.55
Triglycerides (mmol/l)**	1.18 (1.12- 1.24)	1.17 (1.04- 1.33)	0.95	1.11 (0.95- 1.29)	0.23	0.33
Glucose (mmol/l)	4.98 (0.05)	5.04 (0.07)	0.41	4.95 (0.10)	0.79	0.91

Values are means (standard error of the mean) adjusted for gender.

IGF-I: insulin-like growth factor-I, LDL: low-density-lipoprotein, HDL: high-density-lipoprotein.

*P-values: genotype group versus homozygous carriers.

**Triglyceride values were logarithmically transformed and therefore 95% confidence interval is used.

index at both ages was the lowest in homozygous carriers, although not statistically significant. The common carotid intima-media thickness and pulse wave velocity did not materially differ between the genotypes (table 3.1.4). Adjusting for age did not alter the relations.

Birth size

We did not find a relation between the functional polymorphism in the promoter region of the IGF-I gene and birth weight, birth length, ponderal index, in the crude analyses or after additional adjustments for characteristics at birth, of the parents and participant. Homozygous carriers had a mean birth weight of 3.41 kg (standard error of the mean (SE)=0.03), which was not statistically significantly different from the birth weight of heterozygous carriers (3.43 kg (SE=0.04))(p=0.66) and of non-carriers (3.43 kg (SE=0.06))(p=0.66). The analyses with birth length and ponderal index as

Table 3.1.4 A functional polymorphism of the IGF-I gene and arterial wall thickness and stiffness

	Genotype			Non-carrier	P (test for trend)	
	Homozygous	Heterozygous	P*		P*	
Common carotid intima-media thickness (mm) adjusted for reader	0.487 (0.002)	0.489 (0.004)	0.57	0.481 (0.005)	0.22	0.45
Pulse wave velocity (m/s) adjusted for mean arterial pressure	6.01 (0.05)	5.92 (0.07)	0.18	6.07 (0.10)	0.51	0.98

Values are means (standard error of the mean) adjusted for gender.

IGF-I: insulin-like growth factor-I.

*P-values: genotype group versus homozygous carriers.

independent variables showed similar results. One exception is that the participants in the lowest decile of birth length, had a significantly lower percentage of homozygous carriers (27% of 49 individuals) than the other deciles (45% of 514 individuals)($p=0.03$).

3.1.6 Discussion

Our findings show an association between a functional polymorphism in the promoter region of the IGF-I gene and cardiovascular risk factors at adolescence and young adulthood. Homozygous carriers had a lower blood pressure, a less atherogenic lipid profile and were taller than non-carriers. The impact of these risk factors was not yet reflected in arterial wall thickness and stiffening.

To appreciate the findings of this report, four issues of the ARYA study need to be addressed. A strong feature of the ARYA study is that measurement error is minimized by using birth- and adolescent characteristics obtained from medical health records, instead of parental recall and self-report. Secondly, although only 750 of the 4208 invited subjects ultimately entered the study, and 70% of the young adults underwent the pulse wave velocity measurement, we have several reasons to assume that selection bias regarding the relations under study did not occur: the invited young adults were unaware of their IGF-I genotype, 87% of our participants had a 19 CA repeat in the promoter region of the IGF-I gene similar to other population based studies^{7,8,15-17}, data at birth and adolescence did not differ between the responders and non-responders born in 1970, and the obtained data between the participants with and without a pulse wave velocity measurement were similar. Third, in adults and elderly subjects, type 2 diabetes, myocardial infarction and mortality have repeatedly been related to the common carotid intima-media thickness¹⁸⁻²¹ and

pulse wave velocity.²²⁻²⁴ It is very likely to assume that these relations also exist in young adults. At young age, the common carotid intima-media thickness is associated with previous and current cardiovascular risk factors, such as a high blood pressure and a suboptimal lipid profile.^{1,10,25} Also, these risk factors are associated with cardiovascular diseases and mortality.^{3,26,27} Fourth, the polymorphism in the promoter region of the IGF-I gene appeared to be functional, as studies showed that it is related to the IGF-I level and body height.^{8,15,17}

The main finding of this report is that subjects with the wildtype of the functional polymorphism in the promoter region of the IGF-I gene show a more favorable profile of the cardiovascular risk factors at a young age. To our knowledge, one group from Rotterdam, The Netherlands^{7,8} has previously published on this specific functional polymorphism and cardiovascular risk factors. In contrast to our findings, they did not show a relation between genotypes, blood pressure and lipid profile, although homozygous carriers had a lower risk to develop type 2 diabetes and myocardial infarction. These contradicting results may be explained by the large differences between the two population based studies. In our study, participants had an age below the 30 years, where as the Rotterdam study was based on elderly subjects with a mean age of 66 years. When we use the same definition for hypertension (systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg or use of antihypertensive drugs), 2.3% of the 750 young adults in the ARYA study had hypertension, in contrast to 39% of the 900 participants of the Rotterdam study. Our participating young adults had a lower mean body mass index, waist-hip ratio, level of total cholesterol and were more often smokers (31%) than the participants from the Rotterdam (25%). Also, elderly are known to use a large variety of drugs, which may change the levels of the risk factors. Another explanation is that environmental or nutritional factors differ between our two study populations which also may interfere in the relation between the IGF-I genotypes and cardiovascular risk factors, for instance by the IGF-I level.²⁸ If so, environmental or nutritional factors act on the relation for decades longer in elderly subjects than in young adults.

Our findings show that the IGF-I gene was related to body height. Similar to us, the Rotterdam study⁸ and the U.K. study⁹ found that homozygous carriers were the tallest subjects. The fact that we found a statistically significant association at young adulthood but not at adolescence, may be explained by the different stages of maturity at time of measuring the body height. A fourth study did not mention the values of body height per genotype, but only described that the test for trend in women was not significant ($p=0.47$).¹⁷

We could not disclose an association between a functional polymorphism in the promoter region of the IGF-I gene and arterial wall thickness and stiffness at young adulthood as intermediate measures of cardiovascular damage. If a longer exposure to a high blood pressure and an unfavorable lipid profile is needed to develop

changes of the arterial wall, associations between the IGF-I genotype, carotid intima-media thickness and pulse wave velocity may emerge in the future.

Like Frayling et al.⁹ we could not confirm the results of Vaessen et al.⁷ who found that non-carriers had a low birth weight. Only when we stratified birth length in deciles, homozygous carriers had less often a low birth length. This result is based on a small subgroup with a low birth length and thus may be a chance finding. Nevertheless, Arends et al.²⁹ showed in a cohort of 124 short children with a mean age of 7 years and born small for gestational age, an association between birth characteristics and an intronic repeat in the promoter region of the IGF-I gene.

In conclusion, our findings show that homozygous carriers of a functional polymorphism in the promoter region of the IGF-I gene show a more favorable cardiovascular risk factor profile at adolescence and young adulthood. No impact on intermediate measures of vascular damage could be demonstrated at young adulthood.

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3.2

A polymorphism in the promoter region of the IGF-I gene of parents and a decreased birth size in newborns

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3.2.1 Introduction

Low birth weight and other measures of decreased fetal growth have been related to future cardiovascular morbidity and mortality.¹ It is postulated that an unfavorable nutritional intra-uterine environment programs the fetus to adapt itself resulting in decreased fetal growth and chronic disease in later life.² Alternatively, genes may influence both size at birth and adverse future outcome.³ Several studies have shown that a low birth weight of the newborn is related to increased maternal risk of ischaemic coronary disease, and increased risk of prenatal complications such as pre-eclampsia.⁴ Others described that birth weight in a newborn may be modified by a glucosekinase mutation initiating maternal hyperglycemia.⁵ These findings suggest that the maternal genotype may change the intra-uterine environment and indirectly affect birth weight.

Studies implied that non-carriers of a functional polymorphism in the promoter region of the insulin-like growth factor-I (IGF-I) had a low birth size and subsequently decreased adult height, type 2 diabetes and myocardial infarction.^{6,7} Findings of the Atherosclerosis Risk in Young Adults (ARYA) study previously suggested that the polymorphism of the young adults may be associated with cardiovascular risk, but not with their own birth weight. In this present study we used data of the ARYA study to examine associations between the polymorphism in the promoter region of the IGF-I gene in the ARYA participants and their first child's birth weight.

3.2.2 Methods and Results

The study population comprises 352 men and 398 women born between 1970 and 1973. Data on birth characteristics, blood pressure and anthropometry at adolescence were collected from the school health records. At age 27 to 30 years they were recently invited for re-examination and blood sampling. The IGF-I genotype was determined by polymerase chain reaction, using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine (CA) repeat 1 kb upstream of the human IGF-I gene.⁸ Birth weight of the newborns was collected by written questionnaire. A multivariate linear regression model was used for the analyses.

The ARYA participants had 129 first-born children. Of 42 participating men and 72 women, both the IGF-I genotype was determined and the birth weight of the first newborn had been documented. Of the 114 parents, 43% were homozygous carriers of the 19 CA repeat, 40% were heterozygous carriers of the 19 CA repeat and 18% were non-carriers of the 19 CA repeat. These proportions were similar to that of all 750 young adults participating the ARYA study, and other population based studies.^{7,9,10} Table 3.2.1 shows an association between infant's birth weight and the polymorphism in the promoter region of the IGF-I gene of one of its parents: the first-

Table 3.2.1 Associations between a polymorphism in the promoter region of the IGF-I gene of the parents, and birth weight of the first-born

<i>Genotype of the parents</i>	Birth weight of the first-born (g)		
	Of all parents (n=114)	Of the male parents (n=42)	Of the female parents (n=72)
Homozygous (n=49)	3496 (94)	3490 (145)	3500 (125)
Heterozygous (n=45)	3504 (136)	3341 (227)	3577 (174)
Non-carrier (n=20)	2912 (175)	3023 (272)	2839 (231)
	P=0.001*	P=0.09*	P=0.006*

Values are means (standard error).

*P-values: mean birth weight of the first-born of non-carrier parents versus homozygous parents.

born children of our non-carrier participants weighed on average 584 gram lower than of homozygous parents. This was more pronounced in the newborns of the female participants.

Adjustment for weight, height or body mass index of the parents had little effect on the difference in birth weight of newborns between carriers and non-carrier parents (data not shown). Also, other possible confounding variables of the young parents, such as weight, height, body mass index, level of education as a measure of socio-economic status, diabetes, hypertension, smoking and use of alcohol could not explain the observed association between parental genotype and birth weight in their offspring. Adjustment for gender of the first-born (n=103) did not change the results.

3.2.3 Comment

We show that the parents who were non-carriers of the 19 CA repeat had first-born children with a lower birth weight, than the children of homozygous and heterozygous parents. Birth weight was particularly lower in the first-born children of the non-carrier women. The relation between paternal genotype and birth weight of the newborn was in the same direction but not statistically significant.

This report provides evidence for the view that parental genotype may influence fetal growth. This may impact the risk of cardiovascular disease in later life. To understand the determinants and prognosis of a low birth weight, studies on both genetic and environmental causes of fetal growth and chronic disease programming, merit further investigation.

3.2.4 References

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CHAPTER 4

Birth size and cardiovascular risk in the young

4.1

Birth weight and future blood pressure: the role of attained body size

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4.1.1 Abstract

Background: Most reports in children and young adults only show inverse associations between birth size and blood pressure after adjustment for attained body mass index or other measures of body size. This may have led to spurious conclusions about the relation between birth weight and cardiovascular risk. We have attempted to clarify the role of attained body size along recently proposed analytical guidelines.

Methods: The ARYA study is a cohort that comprises 750 participants (46.9% men) born between 1970 and 1973. Birth characteristics and a single routine blood pressure measurement, weight and height in adolescence were available from the school health records of the Municipal Health Service. Recently, at young adulthood, blood pressure was measured twice at each of two visits.

Results: Inverse but non-significant relations between birth weight and systolic blood pressure were found at adolescence (linear regression coefficient: -1.2 mmHg/kg; 95% confidence interval -2.8 to 0.3) and at young adulthood (-0.7 mmHg/kg; -2.4 to 1.1). These relations became stronger after adjustment for attained body mass index (adolescence: -1.5 mmHg/kg; -3.0 to -0.01, young adulthood: -0.9 mmHg/kg; -2.5 to 0.8). There was a significant interaction between birth weight and body mass index in relation to young adult blood pressure levels. However, particularly in the highest tertile of body mass index at young adulthood was an inverse relation between birth weight and blood pressure observed (systolic blood pressure: -2.6 mmHg/kg; -5.3 to 0.2, diastolic blood pressure: -2.5 mmHg/kg; -4.3 to -0.6). In multivariate models, systolic blood pressure at young adulthood was more strongly related to a change of post-adolescent body mass within 15 years (1.1 mmHg/kg/m²; 0.8 to 1.3) than to birth weight (-0.7 mmHg/kg; -2.4 to 0.9). Similarly, systolic blood pressure was stronger related to young adult body mass index (0.8 mmHg/kg/m²; 0.6 to 1.0) than to birth weight (-0.9 mmHg/kg; -2.5 to 0.8).

Conclusion: Where the proposed analyses of our data seem to generally support the fetal origins hypothesis, results of additional analyses show a relation between low birth weight and high blood pressure particularly in young adults with high relative body weights. An unfavorable intra-uterine environment may be less important for future hypertension than excess change to high relative body weight in later life.

4.1.2 Introduction

In support of the fetal origins hypothesis, many studies in children and adults have reported inverse associations between birth size and cardiovascular risk factors and diseases.¹ The most frequent finding is a relation between low birth weight and high systolic blood pressure in later life.^{2,3} However, there are inconsistencies.⁴⁻⁷ One

frequently raised issue is about the role of attained body size in the relation between birth weight and blood pressure in later life.⁸⁻¹⁰ Relations between low birth size and high blood pressure often only became statistically significant after adjustment for measures of attained body size. Some longitudinal studies reported an intermediate role for attained body size, as a causal link in the chain of events leading from low birth weight to later high blood pressure, suggesting that postnatal body growth partially or totally explains the relation.⁷ This raises questions about whether or not attained body mass should be adjusted for as, theoretically, adjustment for intermediate variables leads to biased results.¹¹ In order to avoid false conclusions about the relation between birth size and later cardiovascular outcome and to facilitate comparability of results, it was recently proposed to use an analytical procedure for future studies on this subject.⁸ In a previous study which was analyzed along these lines it was found that predominantly birth weight, not later weight measures, was associated with blood pressure.¹² Using the same procedure, we have analyzed data from the Atherosclerosis Risk in Young Adults (ARYA) cohort to explore the role of attained body size in relation to birth weight and blood pressure.

4.1.3 Methods

Study design and population

The principal objective of the Atherosclerosis Risk in Young Adults (ARYA) study is to investigate early determinants of cardiovascular risk profiles in adolescence and young adulthood. The present study pertains to a cohort of subjects that was selected as follows. Dutch children are regularly screened at Home Care Organizations and Municipal Health Services for physical, mental, or behavioral disorders, starting at four weeks of age until leaving secondary school at the age of 16 to 19 years. Records of visits are kept in files stored in Municipal Health Service archives. From the archives in Utrecht, we screened all 16,000 files of subjects born between 1970 and 1973. Of these, 4208 files were eligible for ARYA as these contained both birth weight and adolescent blood pressure data. Using the latest available addresses, all 4208 now young adult subjects were invited in writing to be re-examined, and 2017 (47.9%) letters returned. Of these, 726 were undeliverable. Of the responders, 416 were not interested, 36 lived too far away, and 18 had other reasons. Out of 4208 invitees, 821 (19.5%) were willing to participate. Women who were pregnant at the time of the study or who gave birth less than 6 months preceding the study (n=14) were excluded. Despite initial agreement, 57 subjects did not enter the study. This left a total of 750 participants. Between the responders and non-responders born in 1970, there were no mean differences in birth weight, and adolescent blood pressure and anthropometry. The ARYA study was approved by

the Medical Ethical Committee of the University Medical Center Utrecht, The Netherlands. Written informed consent was obtained from all participants.

Measurements at birth and adolescence

From the records we retrieved data on birth weight (n=749), birth length (n=581), gestational age (n=599), delivery (n=688) and parity (n=736) noted one month after birth. Data extracted from routine secondary school screenings were included: blood pressure (measured by a sphygmomanometer, n=750), height, weight, sexual maturity determined by Tanner scale (n=656), occurrence of menstruation (n=350), and year of menarche (n=259).

Measurements at young adulthood

From October 1999 to December 2000 all participants had a physical examination at two visits in an outdoor clinic by two research nurses and two physicians. All were similarly instructed and blinded for all data in the medical files. After a five minutes rest in sitting position, blood pressure was automatically measured at the left upper arm by Dynamap (Dynamap Compact T critical Vital Answers and Vital Signs Monitor 8100) with a cuff size 51x15 cm or of 64x17 cm for participants weighing over 95 kilograms. After a 5 to 15 minutes rest the measurement was repeated. The same procedure was used at the second visit that followed after a mean 20.4 days (SD 10.7). The mean of the four blood pressure readings obtained at the two separate visits was used for the analyses.

Body weight and body height were measured with indoor clothes - without shoes - to the nearest 0.5 kg and 0.1 centimeter, respectively. We subtracted 0.5 kg from body weight to compensate for clothing.

Further information was obtained by written questionnaires. ARYA participants were asked to contact their parents and inquire for parental weight and height, weight gain and illnesses of the mother during pregnancy, smoking of the parents during pregnancy, and the delivery. Participants were asked about their own place of birth (The Netherlands, yes/no), current smoking (yes/no), alcohol use (yes/no), use of medication and oral contraception, and cardiovascular disease of the first-degree family members. Socio-economic status during adolescence and young adulthood was estimated by the highest education level reached, profession and annual income of the household of the parents and the participant.

4.1.4 Data analysis

General characteristics of the ARYA population are given as means and standard deviations. Along the proposed analytic procedure shown in figure 4.1.1⁸, we used linear regression models to examine the relation between blood pressure (dependent

variable) and birth weight (independent variable), separately for adolescence and for young adulthood. To study the effect of statistical adjustment for attained body size, body mass index (kg/m^2) was added as an independent variable. To assess effect modification, we added the body mass index and an interaction term (birth weight*body mass index) as independent variables.

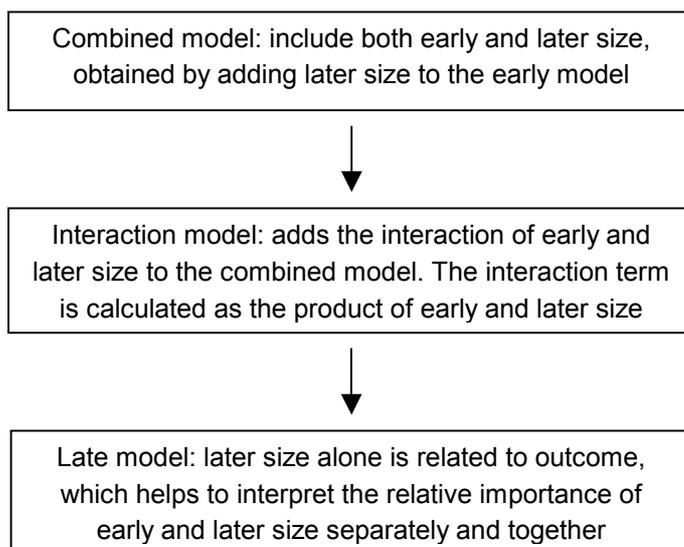
Some additional analyses were performed. To further examine the role of attained body size, we used linear regression to determine the relation between birth weight and blood pressure within each tertile of attained body mass index. In order to assess the role of previous body mass index in the relation between birth weight and blood pressure, we regressed young adult blood pressure on both birth weight and adolescent body mass index and separately on birth weight and post-adolescent body mass index change (young adult body mass index – adolescent body mass index).

All results are expressed as linear regression coefficients with corresponding 95% confidence intervals. All analyses were performed using the statistical analysis software SPSS 9.0.

4.1.5 Results

Table 4.1.1 shows the characteristics of the participants at birth, in adolescence and young adulthood. In the crude analysis (figure 4.1.1: early model) we found negative but non-significant relations between birth weight and blood pressure, both in

Figure 4.1.1 Recommended regression models



Analytical procedure adapted from Lucas et al.⁸ to deal with the role of current size in the relation between birth weight and outcome.

adolescence and in young adulthood (table 4.1.2). Adjustment for attained body mass index (figure 4.1.1: combined model) resulted in a more negative and now statistically significant inverse relation between birth weight and systolic blood pressure in adolescence, while it slightly increased the regression coefficient in young adulthood. Using attained weight or height as measures of body size showed similar results (data not shown). Table 4.1.3 shows that coefficients for the interaction terms birth weight*body mass index in relation to systolic blood pressure and diastolic blood pressure (figure 4.1.1: interaction model) were statistically significant in young adulthood. This result is graphically shown for systolic blood pressure in figure 4.1.2. The relation between young adult systolic blood pressure and body mass index (figure 4.1.1: late model) was 0.8 mmHg/kg/m², 95% CI 0.6 to 1.0, and for diastolic blood pressure 0.3 mmHg/kg/m², 95% CI 0.2 to 0.5.

We further examined the finding of interaction using linear regression analysis within each of the tertiles of attained body mass index. This indicated that within the highest young adult tertile of body mass index an inverse relation was present between birth weight and systolic blood pressure (-2.6 mmHg/kg: -5.3 to 0.2) and diastolic blood pressure (-2.5 mmHg/kg: -4.3 to -0.6). No such relations were found in the lowest and middle tertile of body mass index. To substantiate this somewhat

Table 4.1.1 General characteristics of the ARYA cohort

	Overall (n=750)	Men (n=352)	Women (n=398)
<i>Birth characteristics</i>			
Birth weight (kg)	3.418 (0.547)	3.479 (0.541)	3.365 (0.546)
Birth length (cm)	50.8 (2.5)	51.3 (2.5)	50.4 (2.5)
Ponderal index (kg/cm ³)	26.2 (3.1)	25.9 (3.1)	26.5 (3.1)
Gestational age (weeks)	39.8 (1.9)	39.8 (1.8)	39.8 (2.0)
<i>Adolescence</i>			
Age (years)	13.5 (1.1)	13.5 (1.1)	13.4 (1.1)
Systolic blood pressure (mmHg)	110.2 (11.9)	111.7 (12.5)	109.0 (11.3)
Diastolic blood pressure (mmHg)	66.6 (9.9)	67.4 (9.6)	66.0 (10.1)
Weight (kg)	49.6 (12.2)	49.2 (10.5)	49.9 (9.9)
Height (cm)	162.4 (9.1)	163.0 (10.3)	161.2 (8.0)
Body mass index (kg/m ²)	18.6 (2.7)	18.3 (2.4)	18.9 (2.9)
<i>Young adulthood</i>			
Age (years)	28.4 (0.9)	28.4 (0.9)	28.4 (0.9)
Systolic blood pressure (mmHg)	125.4 (13.2)	130.8 (12.0)	120.6 (12.3)
Diastolic blood pressure (mmHg)	71.9 (8.2)	73.1 (7.8)	70.9 (8.5)
Weight (kg)	77.0 (15.6)	83.5 (13.6)	71.3 (14.9)
Height (cm)	176.6 (9.4)	183.9 (6.7)	170.2 (6.4)
Body mass index (kg/m ²)	24.7 (4.4)	24.7 (3.7)	24.7 (5.0)

Values are means with their standard deviation.

Table 4.1.2 Body mass index and the association between birth weight and blood pressure in adolescence and young adulthood

<i>Birth weight</i>	Adolescence		Young adulthood	
	Systolic blood pressure	Diastolic blood pressure	Systolic blood pressure	Diastolic blood pressure
Crude	-1.2 (-2.8 to 0.3)	0.4 (-0.7 to 1.7)	-0.7 (-2.4 to 1.1)	-0.4 (-1.5 to 0.7)
Adjusted for attained body mass index	-1.5 (-3.0 to -0.01)	0.2 (-1.0 to 1.5)	-0.9 (-2.5 to 0.8)	-0.5 (-1.6 to 0.6)

Values are linear regression coefficients with 95% confidence intervals given in parenthesis.

Table 4.1.3 Interaction between birth weight and attained body mass index in relation to adolescent and young adult blood pressure

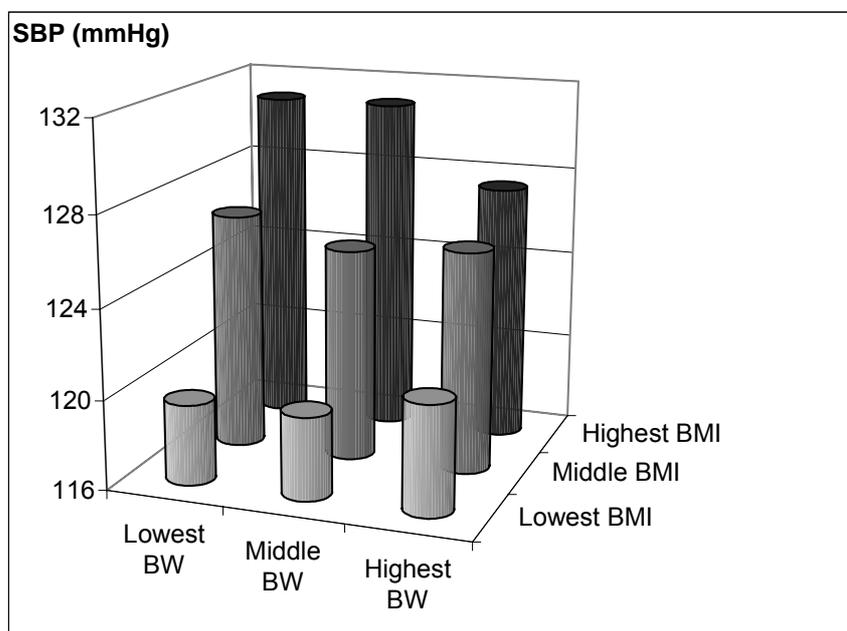
	Adolescence	Young adulthood
Model with systolic blood pressure		
Birth weight*body mass index (kg*kg/m ²)	-0.3 (-0.8 to 0.1)	-0.4 (-0.7 to -0.1)
Model with diastolic blood pressure		
Birth weight*body mass index (kg*kg/m ²)	0.02 (-0.4 to 0.4)	-0.3 (-0.5 to -0.1)

Values are linear regression coefficients (95% confidence intervals) of interaction terms birth weight*body mass index, in models with blood pressure as dependent variable and birth weight, body mass index and birth weight*body mass index as independent variables.

further, those who changed from the lowest quartile of birth weight (<3090 g) to the highest quartile of young adult body mass index (>27 kg/m²) had 7.2 mmHg (3.5 to 10.9) higher systolic blood pressure and 3.6 mmHg (1.3 to 5.9) higher diastolic blood pressure than the rest of the population. For comparison, those who were in the highest quartile of birth weight (>3750 g) and in the highest quartile of attained body mass index (>27 kg/m²) had higher systolic blood pressure levels but not statistically different from the rest of the population (2.9 mmHg: -1.0 to 6.8), while diastolic levels were virtually the same.

Regressing young adult systolic blood pressure on both birth weight and adolescent body mass index, showed that the coefficient of birth weight attenuated to -0.7 mmHg/kg, 95% CI -2.5 to 1.0, while the coefficient of adolescent body mass index was 0.4 mmHg/kg/m², 95% CI 0.05 to 0.8. For young adult diastolic blood pressure, the findings were comparable (birth weight: -0.5 mmHg/kg, 95% CI -1.5 to 0.6, adolescent body mass index: 0.2 mmHg/kg/m², 95% CI -0.008 to 0.4). Similar findings were obtained in models with birth weight and body mass index change from adolescence to young adulthood in relation to young adult systolic blood pressure

Figure 4.1.2 Mean systolic blood pressure according to tertiles of birth weight and attained body mass index



SBP: Systolic blood pressure at young adulthood, BW: Birth weight; BMI: Body mass index at young adulthood.

The linear regression coefficient for body mass index as effect modifier in the relation between birth weight and blood pressure is: -0.4 mmHg/kg (95% confidence interval -0.7 to -0.1).

(birth weight: -0.7 mmHg/kg, 95% CI -2.4 to 0.9 , post-adolescent body mass index change: 1.1 mmHg/kg/m²/15 years, 95% CI 0.8 to 1.3) and young adult diastolic blood pressure (birth weight: -0.5 mmHg/kg, 95% CI -1.5 to 0.6 , post-adolescent body mass index change: 0.4 mmHg/kg/m²/15 years, 95% CI 0.2 to 0.6).

Similar analyses with birth length instead of birth weight yielded similar but weaker relations. Although not the primary aim of this study, additional adjustment for characteristics at birth, of the parents and participant, like gestational age and socio-economic status of the parents and participant, did not materially change the findings.

4.1.6 Discussion

Our findings suggest that an association between low birth weight and high blood pressure is largely restricted to those with high-attained relative body weights.

To appreciate these findings some issues need to be addressed. The use of registered birth size data instead of parental recall and self-report data will have minimized misclassification. Data of adolescence were obtained from single routine health examinations, but measurement error may be presumed to be random. Our study population may be a selection of the general population as the response rate

was low. However, there were no differences in birth and adolescent data between responders and non-responders. Moreover, it seems unlikely that selection of subjects occurred on the basis of specific relations between birth characteristics and adolescent and adult blood pressure levels.

A large body of evidence seems to support the fetal origins hypothesis, in particular with respect to blood pressure. The initial hypothesis was that an adverse intrauterine environment, like a limited supply of nutrients, induces fetal and postnatal adaptation which itself leads to increased risk of chronic disease in later life.^{13,14} However, from the earliest reports onwards, the hypothesis has been subject to concerns about validity, causality, and publication bias.^{10,15-18}

Criticism has also involved analytical procedures that may have led to flawed interpretation in favor of the hypothesis. Many studies have reported associations that were adjusted for indexes of attained body mass, while these associations were not clearly present without such adjustment.^{7,19-22} Invariably, adjustments were intended to obtain the best estimate of the true underlying relation between birth weight, as a resultant of intra-uterine environment, and blood pressure. From that perspective, theoretically, adjustment is justified if it confounds the true relation between birth weight and blood pressure. Attained body size should then be a risk factor for high blood pressure, which it is²³, and be somehow related to birth weight, which it is also.^{24,25} It should not be an intermediate factor that causally links birth weight to blood pressure as adjustment for intermediate factors in simple analytic models will yield biased results.¹¹ The possibility of body mass index being an intermediate factor has been previously suggested^{7,21}, and later empirical evidence provided clues that (accelerated) postnatal growth and high-attained body mass are indeed important factors when relating birth size to cardiovascular risk factor profiles, coronary heart disease, or diabetes mellitus.^{7,26-29} However, in practice, effects of adjustment do not easily, if at all, distinguish between confounding or intermediate properties of a factor.

Following the recommended analytical procedure of Lucas et al.⁸, what do our findings mean? The authors state that there is support for the fetal origins hypothesis if results show the following: first, the 'early model' should have a statistically significant negative regression coefficient for birth weight, indicating that the lower the birth weight the higher the later blood pressure. In our study it was negative but not statistically significant. One reason might be lack of power as larger studies did show crude associations.³⁰ Second, adjustment for attained body mass index in the 'combined model' should further increase the negative coefficient of birth weight. This model is reported to actually study change of body weight as a determinant of blood pressure. Indeed, in our study it increased the negative coefficient. Third, the 'interaction model' should show a negative coefficient for the interaction term of birth weight and attained body mass, which was also the case in our study. This is

particularly suggested to indicate that shifts from low birth weight rankings in the distribution to high-attained body mass index rankings (de-tracking or centile crossing) would yield higher blood pressure levels. Finally, application of the 'late model' should show that attained body mass index is strongly positively associated with blood pressure, which again was the case in our study. Considering that our study was perhaps too small to detect crude associations between birth weight and blood pressure, the general interpretation might thus be that our findings support the fetal origins hypothesis.

However, further analysis indicates that both excess weight change and attained body mass level itself in later life is more important for blood pressure levels than intra-uterine causes of (too) low birth weight. Primary high blood pressure has various partly unknown origins, but high-attained body mass index is a well known strong determinant of high blood pressure levels, also in childhood.³¹ Thus, in a causative sense, youngsters with high blood pressure levels are a mix of those who grew to high-attained relative body weights from whatever their birth or later weights were, and of those with other reasons for high blood pressure. A specific group may be those who have excessively grown to high body mass coming from (too) low birth weights. The interaction model suggests that birth weight modifies the effect of body mass index on blood pressure, more in particular, that de-tracking of body weight to high levels would lead to high blood pressure. Indeed, those with the lowest birth weights in our study who de-tracked to the highest body mass index at young adulthood were part of those with the highest systolic and diastolic blood pressure levels. From our additional analyses, we infer that a relation between birth weight and blood pressure seems restricted to those with high-attained body mass. It was absent at lower body mass index levels. This would mean that there is no graded interaction between birth weight and body mass index, but that only babies who are light for their constitution or constitutionally small babies and become relatively heavy later are at risk of hypertension. Thus, it may be that (too) low birth weight babies, through high-attained body mass as an intermediate process, are particularly vulnerable to develop high blood pressure. A more likely explanation seems to be that such babies are particularly vulnerable to develop high body mass in young adulthood, with high blood pressure as a mere consequence of the latter.

The results of the recommended analytical procedure would generally support the fetal origins hypothesis. However, additional analyses show that a relation between birth weight and future blood pressure is largely restricted to those with high-attained relative body weights.

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4.2

Birth size is related to risk of coronary heart disease at young adulthood

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4.2.1 Abstract

Background: Several studies in the young have shown that low birth weight is associated with increased levels of individual cardiovascular risk factors. However, no studies have reported on relations between birth size and clustered cardiovascular risk profiles. The aim of this study was to investigate the association between birth size and the 10-year risk in young adulthood of coronary heart disease using the Framingham risk score.

Methods: The ARYA study is a population-based cohort that comprises 750 (46.9% men) subjects born between 1970 and 1973. Birth characteristics were obtained from school health records. At young adulthood, a duplicate blood pressure was measured in sitting position and repeated after approximately three weeks. Anthropometry was newly measured. Fasting levels of total-, HDL-, triglycerides and LDL-cholesterol were determined. Data about smoking and diabetes were obtained by questionnaire.

Results: In 723 subjects, the overall 10-year risk of coronary heart diseases in young adults was 1.6% (standard deviation=1.9), in men 3.0% (SD=1.9) and in women 0.3% (SD=0.2). Using a linear regression model, 1 SD lower birth weight (=0.54 kg) was associated with a 0.1% greater 10-year risk of coronary heart disease in the overall population (95% confidence interval: -0.19 to -0.004). A similar result was found with ponderal index at birth (β : -0.11% risk/SD ponderal index; 95% CI: -0.21 to -0.002). The inverse relations were stronger in men than in women. Adjustment for other characteristics at birth, of the participant and parents did not affect the relations.

Conclusion: Small birth size is associated with increased risk of coronary artery disease in young adulthood.

4.2.2 Introduction

One of the first reports supporting the “fetal origins hypothesis” was the observation that the highest rates of coronary heart disease were found in the area with the highest rate of neonatal mortality.¹ As neonatal mortality is correlated with small birth size, studies have tried to find relations between low birth weight and increased cardiovascular morbidity²⁻⁴ and mortality^{5,6} in advanced adulthood. In healthy young people however, studies were restricted to current cardiovascular risk factor levels, which were studied separately.⁷⁻¹⁰ Invariably, the primary interest of studies examining a single risk factor as dependent variable was the causality of associations, for example to find out whether low birth weight causes future high blood pressure levels in healthy youngsters.¹¹ However, if conclusions are to be expanded to the impact of birth size on an accurate estimate of future cardiovascular

disease risk in the young, clustering of risk factors is required. The use of risk scores has the advantage of combining the long-term contribution of several risk factors including their mutual relations and interactions, to an absolute risk of coronary heart disease. To our knowledge, using a risk score has not been done previously in young adults.

We have analyzed data of 750 participants of the Atherosclerosis Risk in Young Adults (ARYA) study, to examine the relation between birth size and the absolute 10-year risk of young adults for coronary heart disease. To that end, the Framingham risk score for risk factor categories was used, based on age, blood pressure, total- and high-density-lipoprotein cholesterol, diabetes and smoking.¹²

4.2.3 Methods

Data on birth weight was available in all 750 participants of the cohort of Utrecht. The ARYA study was approved by the Medical Ethical Committee of the University Medical Center, Utrecht, The Netherlands. Written informed consent was obtained of all 750 participating young adults.

Registered risk factors at birth

In The Netherlands, virtually all children regularly visit the child health facilities of the Home Care Organizations and Municipal Health Services, starting at the age of four weeks postpartum until leaving secondary school at the age of 16 to 19 years. Information is routinely collected by school doctors and nurses and written down in health records. Subjects of our study population were born between 1970 and 1973 and attended secondary school in Utrecht. The records were selected on the presence of birth weight data and at least one adolescent blood pressure measurement, the latter for research questions outside the realm of this report. If birth weight was approximated in the record (sign \pm), the subject was excluded. Out of the approximately 16,000 medical school records, 4208 fulfilled these criteria. All 4208 subjects were invited by letter at the last known address at adolescence by the Municipal Health Service of Utrecht, of which 2017 (47.9%) returned. 726 letters were undeliverable, and 416 responders had no interest in participation, 36 lived too far from our outpatient clinic, 18 had other reasons and 821 subjects (19.5% of the 4208) were willing to participate. Of the 821 subjects, pregnant women and women who gave birth less than 6 months before entering the study ($n=14$), were excluded. Despite initial agreement, 57 subjects did not enter the study, mostly due to lack of time and distance to the outpatient clinic. This resulted in a total of 750 participants in the Utrecht cohort. To evaluate selection, we compared data of responders and non-responders of the 4208 invitations who were born in 1970 ($n=967$). The mean birth weight, adolescent blood pressure and anthropometry were similar.

We obtained information on birth weight (n=749), birth length (n=581), gestational age (n=599), delivery (n=688) and parity (n=736) noted one month after birth.

Risk factors at young adulthood

From October 1999 to December 2000 the participants underwent a physical examination during two visits at the outpatient clinic of the Julius Center. All investigators were similarly instructed and blinded for the birth characteristics. After a five minutes rest in sitting position, blood pressure was measured by Dynamap at the left upper arm, and repeated after 5-15 minutes rest. The same procedure was followed at the second visit with a mean interval of 20.4 days (SD 10.7). Body height was measured in standing position - without shoes - to the nearest 0.1 cm. Body weight was measured with indoor clothes - without shoes - to the nearest 0.5 kg. To compensate for clothes we subtracted 0.5 kg.

Of the 750 participants, fasting blood of 730 participants and non-fasting blood of 8 participants was drawn and stored at -20°C . When all the participants finally visited our outpatient clinic, fasting serum levels of total cholesterol, high-density-lipoprotein (HDL-) cholesterol, triglycerides, low-density-lipoprotein (LDL-) cholesterol¹³ and glucose were determined by Vitros950 dry-chemistry analyzer (Johnson & Johnson, Rochester, New York, USA). In the non-fasting samples, total- and HDL-cholesterol were only assessed.

We obtained further information of the participant and parents on ethnicity, smoking (yes/no, packyears), alcohol consumption (yes/no), use of oral contraception (yes/no), highest education level (low/middle/high) as a measure of socio-economic status, and known cardiovascular diseases of the participant and of the first degree family members (myocardial infarction, cerebrovascular event, type 2 diabetes, hypertension, hypercholesterolemia (yes/no)), by a written questionnaire.

In our young and healthy population, we used the Framingham risk score as a proxy for future coronary heart disease. The Framingham risk score using categorized cardiovascular risk factors¹² was developed in a population based sample of 2489 men and 2856 women aged 30 to 74 years without overt coronary heart disease at the baseline examination. Study subjects were followed over a 12-year period for the development of coronary heart diseases: angina pectoris, recognized and unrecognized myocardial infarction, coronary insufficiency, and coronary heart disease death, according to previously published criteria.¹⁴ The individual 10-year probability of coronary heart disease was calculated using the β -coefficients from the Cox-proportional hazard models, using the continuous variables age and age-squared, the categorized variables total- and HDL-cholesterol and blood pressure, and the dichotomous variables diabetes and smoking. Cut-off values for total cholesterol (<200, 200 to 239, 240 to 279, and ≥ 280 mg/dl) and HDL-cholesterol (<35, 35 to 59, and ≥ 60 mg/dl) are similar to those used for the NCEP ATP II

guidelines.^{15,16} The mean of four blood pressure readings at young adulthood was calculated and blood pressure was categorized according to the JNC V definitions¹⁷: optimal blood pressure (systolic <120 mmHg and diastolic <80 mmHg), normal blood pressure (systolic 120 to 129 mmHg or diastolic 80 to 84 mmHg), high normal blood pressure (systolic 130 to 139 mmHg or diastolic 85 to 89 mmHg), hypertension stage I (systolic 140 to 159 mmHg or diastolic 90 to 99 mmHg), and hypertension stage II-IV (systolic \geq 160 or diastolic \geq 100 mmHg). When systolic and diastolic blood pressures fell into different categories, the higher category was selected. Blood pressure categorization was made without regard to the use of antihypertensive medication.

4.2.4 Data analysis

Univariate linear regression models were used to study the relations between birth size and the absolute 10-year risks of coronary heart disease in these young adults. As the Framingham risk scores were only provided for men and women separately¹², the relations with birth size measures in ARYA are given sex-specific. As the latter had reduced statistical power we added a multivariate linear regression analysis in the total ARYA population in which we adjusted for gender. To compare the results of the independent variables birth weight, -height and ponderal index, we used birth size divided by its standard deviation as independent variables.

Analyses were carried out with the statistical analysis software SPSS 9.0. Statistical significance was concluded if $p < 0.05$.

4.2.5 Results

Table 4.2.1 shows the characteristics at birth and young adulthood for the whole study population, and for men and women separately. At birth, male newborns had a statistically significantly higher birth weight and height and a lower ponderal index than female newborns. At young adulthood, blood pressure, body weight, height and waist-hip ratio, levels of LDL-cholesterol, triglycerides and glucose were higher in men, and HDL-cholesterol was lower. Due to missing values the Framingham risk score was calculated in 723 subjects of the 750 subjects. Men had a higher risk score.

In young men, studying the relation between birth size and the 10-year risk of coronary heart disease, a decrease of 1 standard deviation in birth weight and ponderal index was statistically significantly associated with an increased risk of 0.2% (table 4.2.2). In young women, the 10-year risk of coronary heart disease showed a borderline statistically significant inverse relation with birth length. The analyses

Table 4.2.1. General characteristics ARYA cohort

	Overall (n=750)	Men (n=352)	Women (n=398)
<i>Birth characteristics</i>			
Birth weight (kg)	3.42 (0.55)	3.48 (0.54)	3.37 (0.55)
Birth length (cm)	50.8 (2.5)	51.3 (2.5)	50.4 (2.5)
Ponderal index (kg/m ³)	26.2 (3.1)	25.9 (3.1)	26.5 (3.1)
Gestational age (weeks)	39.8 (1.9)	39.8 (1.8)	39.8 (2.0)
<i>Young adulthood</i>			
Age (years)	28.4 (0.9)	28.4 (0.9)	28.4 (0.9)
Systolic blood pressure (mmHg)	125.4 (13.2)	130.8 (12.0)	120.6 (12.3)
Diastolic blood pressure (mmHg)	71.9 (8.2)	73.1 (7.8)	70.9 (8.5)
Heart rate (beats/min)	65.3 (10.4)	63.9 (10.7)	66.6 (9.9)
Weight (kg)	77.0 (15.6)	83.5 (13.6)	71.3 (14.9)
Height (cm)	176.6 (9.4)	183.9 (6.7)	170.2 (6.4)
Body mass index (kg/m ²)	24.7 (4.4)	24.7 (3.7)	24.7 (5.0)
Waist-hip ratio	0.85 (0.07)	0.88 (0.06)	0.81 (0.06)
Total cholesterol (mg/dl)	187.2 (34.8)	187.3 (37.6)	187.1 (32.2)
HDL-cholesterol (mg/dl)	55.8 (14.0)	50.1 (11.5)	60.9 (14.0)
Triglycerides (mg/dl)	114.0 (63.7)	120.6 (76.0)	108.2 (49.6)
LDL-cholesterol (mg/dl)	108.4 (33.1)	112.9 (34.8)	104.5 (31.0)
Glucose (mg/dl)	89.6 (9.3)	92.2 (10.7)	87.2 (7.0)
Smoking (% yes)	31.0	35.9	26.6
Diabetes mellitus (% yes)	0.8	0.9	0.8
Framingham risk score*	1.56 (1.88)	3.02 (1.86)	0.27 (0.17)

Values are means (standard deviation) unless mentioned otherwise.

LDL: low-density-lipoprotein, HDL: high-density-lipoprotein.

*Framingham risk score¹²: 10-year risk of coronary heart disease using risk factor categories.

concerning birth length and ponderal-index were performed in a subgroup of 581 ARYA participants with available birth length measurements. These analyses have therefore less statistical power than those with birth weight. In order to gain statistical power we performed an additional analysis in the total ARYA population on the relation between birth characteristics and 10-year cardiovascular risk with adjustment for gender. This showed statistically significant inverse relations between and birth weight and risk (-0.10%/kg/SD; 95% CI -0.19 to -0.004), and with ponderal-index (-0.11%/(kg/m³)/SD; 95% CI -0.21 to -0.002), while there was a non-significant relation with birth length (0.03%/cm/SD; 95% CI -0.08 to 0.14).

The relations were strengthened after adjusting for adult body mass index (data not shown). Other characteristics of pregnancy, at birth, for participants and parents such as parental smoking during pregnancy, gestational age, familiarity of disease, physical activity and socio-economic status, did not modify the relations.

Table 4.2.2 The percentage change in 10 year cardiovascular disease risk of young adults per standard deviation decrease in birth size

<i>Birth size</i>	Framingham risk score*		
	Overall (n=723) adjusted for gender	Men (n=338)	Women (n=385)
Birth weight (kg/SD)	-0.10 (-0.19 to -0.004)	-0.20 (-0.40 to 0.001)	-0.01 (-0.03 to 0.004)
Birth length (cm/SD)	0.03 (-0.08 to 0.14)	0.08 (-0.15 to 0.32)	-0.02 (-0.04 to <0.001)
Ponderal index (kg/m ³ /SD)	-0.11 (-0.21 to -0.002)	-0.22 (-0.44 to 0.004)	-0.01 (-0.03 to 0.01)

Values are linear regression coefficients with 95% confidence intervals.

Birth sizes per standard deviation (SD) were used as independent variables.

Coefficients for the overall group were obtained from models adjusted for gender.

*Framingham risk score¹²: 10-year risk of coronary heart disease using risk factor categories.

4.2.6 Discussion

To our knowledge, this is the first study to show that a relative low birth weight is associated with an increase in the 10-year risk of coronary heart disease in young adults, based on all of the most important cardiovascular risk factors clustered in a risk score.

One of the main advantages of this study is that measurement error of the determinants was limited by obtaining birth characteristics from the medical health records and not by parental recall or self-report. Birth weight and the risk factors were measured once and performed by several investigators, but if measurement error occurred it may be presumed to be random. Moreover, although the response rate was modest, it is not likely that selection of the participants, if any, was biased for the relation between birth- and adult characteristics. Birth weight was similar between the responders and non-responders. Furthermore, in our analysis we had to cope with the fact that the Framingham risk scores are sex-specific, while a pooled analysis of ARYA with gender in the risk score would have had more statistical power. The Framingham risk score was derived from a cohort that did also contain young adults, but was on average much older than the ARYA cohort. By using this score we assume that variable weights obtained from modeling in the Framingham cohort are constant over time and may thus be extrapolated to a cohort with only young individuals. We cannot exclude the possibility that this is not the case, but risk scores specific to the age group of ARYA are to our knowledge not available.

Many of the known cardiovascular risk factors have been studied separately in the young. In the young, birth size was shown to be inversely related to blood pressure^{2,11}, lipids^{8,18} and diabetes mellitus^{3,4}, and positively with adult obesity.^{4,19} Some of these findings were inconsistent^{9,10} or even flawed according to some.^{20,21} Obviously, manifest cardiovascular disease cannot be studied in the young, while

single risk factors can only partially describe the cardiovascular risk. Therefore, the ARYA study expands the available evidence as it has determined the relation between birth size and an aggregation of current risk factors, and consequently the most accurate available measure of risk.

The Framingham risk score consists of risk factors which track over decades and which predict future coronary heart risk already at young adulthood; high blood pressure²²⁻²⁴, atherogenic lipid profile^{25,26}, smoking²⁷ and diabetes.^{28,29} It has repeatedly been demonstrated that the Framingham risk score is effective in predicting coronary heart disease in several western populations³⁰⁻³², including a follow-up from the Framingham study.³³ The Framingham risk score was developed for a population-based sample of men and women without overt coronary heart disease at the baseline examination, similar to our study population. We used the Framingham risk score with risk factor categories, which was shown to be as accurate as a score based on continuous values of risk factors.¹² All the required risk factors for using this categorized score were measured in ARYA. Some cardiovascular disease risk factors however, are not included in the Framingham score although they do to some extent predict cardiovascular morbidity and mortality. One of these risk factors is obesity³⁴⁻³⁶, but it is excluded from the risk score because it is strongly associated with higher total cholesterol, lower HDL-cholesterol, higher blood pressure and diabetes³⁷, implying a small residual impact. The familiarity of disease was not included in the Framingham risk score because it was not uniformly available in the Framingham study. Also, information of physical activity was not available at the baseline levels. Socio-economic status was not mentioned. We adjusted for these risk factors in the associations between birth size and coronary risk, and showed no material effect.

Our main finding is that young adults born with low birth weights and low ponderal indexes had an increased 10-year risk of coronary heart disease. This relation with risk appeared - to some extent - to depend on gender. In men, both relations with birth weight and ponderal-index were particularly outspoken, indicating that those who were born light and thin, with low for height birth weights, were at the highest risk as young adults in developing cardiovascular disease. In women, it appeared to be those with low birth lengths, the small female newborns, who had the higher risk. It is of interest to note that not only young adult men had a higher risk than women, as is similarly shown in the literature^{38,39}, but also that birth size in men appeared to be more strongly associated with cardiovascular risk. This may suggest that, particularly in men, a higher risk originates in prenatal life through a determinant that affects both birth size and cardiovascular risk. However, given that the 10-year risk in young adult women was much lower than in men, it is possible that associations between birth size and risk are more difficult to detect in women. Noteworthy is that none of the relations was materially affected by adjustment for gestational age,

indicating that it was not being small for date at birth that can be held accountable. Neither had socio-economic status and familiarity of disease any impact on the relations.

Our findings in the overall study population indicate that with every standard deviation decrease in birth weight (0.54 kg), there was 1 excess coronary heart disease event out of 1000 young adults. In men, this decrease in birth weight was associated with 2 excess events. Although these numbers of events may not seem very high, it is emphasized that it concerns risk factor measurements in a young and healthy population. Cardiovascular disease generally becomes manifest after the age of forty, and therefore the number of diseased men will increase quickly with increasing age. Moreover, the event does not include only patients with angina pectoris, but also myocardial infarction, coronary insufficiency, and coronary heart disease death.

In summary, our findings provide support for the view that a relative low birth size is associated with an increased risk of coronary heart disease in young adults.

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CHAPTER 5

Adolescent blood pressure is related to cardiovascular risk at young adulthood

5.1

Adolescent blood pressure and blood pressure tracking into young adulthood are related to subclinical atherosclerosis

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5.1.1 Abstract

Background: Increased blood pressure at young adulthood is associated with cardiovascular morbidity and mortality. Longitudinal studies at young age are, however, limited. Our aim was to study the relations of adolescent blood pressure and tracking of blood pressure into young adulthood with subclinical atherosclerosis, as assessed by carotid intima-media thickness (CIMT), at the age of 28 years.

Methods: The ARYA study comprises of a community based sample of 750 subjects aged 27-30 years. Of 352 men and 398 women, at least one blood pressure measurement was recorded at a mean age of 13 years in the school health records. Recently, all participants completed a questionnaire on cardiovascular risk factors, had a fasting blood sample drawn and underwent an ultrasound examination of both common carotid arteries to assess CIMT.

Results: Linear regression showed that adolescent systolic blood pressure was associated with thickening of the intima-media (an increase of 7.5 μm in CIMT per standard deviation (SD) increase of systolic blood pressure; 95% CI 4.3 to 10.6). Similar relations were found for pulse pressure and mean arterial pressure. When gender, age and body mass index at adolescence and young adulthood and adult blood pressure were taken into account, the relations attenuated, but for pulse pressure remained statistically significant. Furthermore, subjects who tracked in the highest systolic blood pressure and pulse pressure levels from adolescence into young adulthood, showed the thickest CIMT.

Conclusion: Our findings strengthen the notion that elevated blood pressure at adolescence and a relative increase in blood pressure from adolescence to adulthood unfavorably affect cardiovascular risk, as indicated by increased CIMT.

5.1.2 Introduction

Cardiovascular diseases are the main causes of morbidity and mortality in western countries, and the chronic development of atherosclerosis is the responsible underlying mechanism. Although cardiovascular disease only becomes manifest at the age of 45 years or older, its subclinical roots are already found in childhood.¹ The progression of atherosclerosis is congruent with the level and number of cardiovascular risk factors, among which blood pressure is one of the main modifiable risk factors.²⁻⁴ It is known that blood pressure at childhood and adolescence is positively associated with blood pressure in early adulthood⁵⁻⁸ and that blood pressure at young adulthood is associated with cardiovascular morbidity and mortality after several decades.⁹⁻¹¹ However, longitudinal studies on adolescent blood pressure and adverse events are lacking. Only limited studies on adolescent blood pressure and vascular damage at a young adulthood are available. In a

postmortem study, increased systolic blood pressure and diastolic blood pressure in subjects aged between 2 and 39 years, were related to postmortem measured atherosclerosis.¹² Others found a positive relation between blood pressure and the CIMT in young adults, an intermediate measure of vascular damage, but these relations disappeared after adjustment for cardiovascular risk factors at adolescence.¹³

To further explore the role of adolescent blood pressure on early atherosclerosis, we used data of the Atherosclerosis Risk in Young Adults (ARYA) study. In particular, we examined the relation of indices of adolescent blood pressure with arterial wall thickening. In addition, we studied the importance of (de)tracking of blood pressure from adolescence to young adulthood on achieved CIMT.

5.1.3 Methods

The overall aims of the Atherosclerosis Risk in Young Adults (ARYA) study are evaluating 1) whether it is possible to predict cardiovascular risk at young adulthood by routinely measured adolescent data, and 2) evaluating the role of birth characteristics and adolescent characteristics to the development of vascular damage at young adulthood. The study population of the ARYA study comprises two cohorts: a cohort from the Dutch city of Utrecht with 750 participants and a cohort from The Hague with 262 participants. This paper is based on the Utrecht data only because CIMT was not measured in the The Hague participants. The ARYA study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands. Of all participants written informed consent was obtained.

Eligible participants were born between 1970 and 1973 and attended secondary school in Utrecht between the ages of 11 to ± 16 years. Of them, the school health records from the Municipal Health Service were screened. In The Netherlands virtually all children regularly visit the child health facilities of the Home Care Organizations and Municipal Health Service, starting at four weeks until leaving secondary school at the age of 16 to 19 years. Information on blood pressure (manually with a sphygmomanometer), height, weight, lifestyle, puberty stage and medical history is routinely collected and written down in health records by school doctors and nurses. Those health records were selected which contained information on the presence of birth weight and at least one blood pressure measurement when attending secondary school. Birth weight approximated with the sign \pm was excluded. Out of 15,592 medical school records, 4208 subjects were invited by letter at the last known address at adolescence by the Municipal Health Service, of which 2017 (47.9%) have returned. 726 (36.0%) returned due to unknown addresses, 416 (20.6%) subjects had no interest in participation, 36 (1.8%) lived too far from our

outpatient clinic, 18 (0.9%) had other reasons, and 821 of the 4208 subjects (19.5%) were willing to participate. Pregnant women and women who gave birth less than six months before entering the study (n=14) were excluded from participation. 57 subjects did not enter the study due to lack of time and distance to the outpatient clinic. This left a total of 750 participants in the Utrecht cohort. We compared birth data and adolescent data of responders and non-responders born in 1970. No differences were found.

From October 1999 to December 2000 all Utrecht participants had a physical examination during two visits at the outpatient clinic of the Julius Center by two trained research nurses and two medical doctors. All were similarly instructed and blinded for data in the medical school health records, including previous blood pressure. After five minutes of rest in sitting position, blood pressure was measured using a Dynamap at the left upper arm. The measurement was repeated after 5-15 minutes rest. The same procedure was followed at the second visit after a mean time-interval of 20.4 days (SD 10.7). The average of four readings was used in the analyses. Body weight was measured with indoor clothes - without shoes - to the nearest 0.5 kg. To compensate for clothes we subtracted 0.5 kg. Body height was measured in standing position - without shoes - to the nearest 0.1 cm.

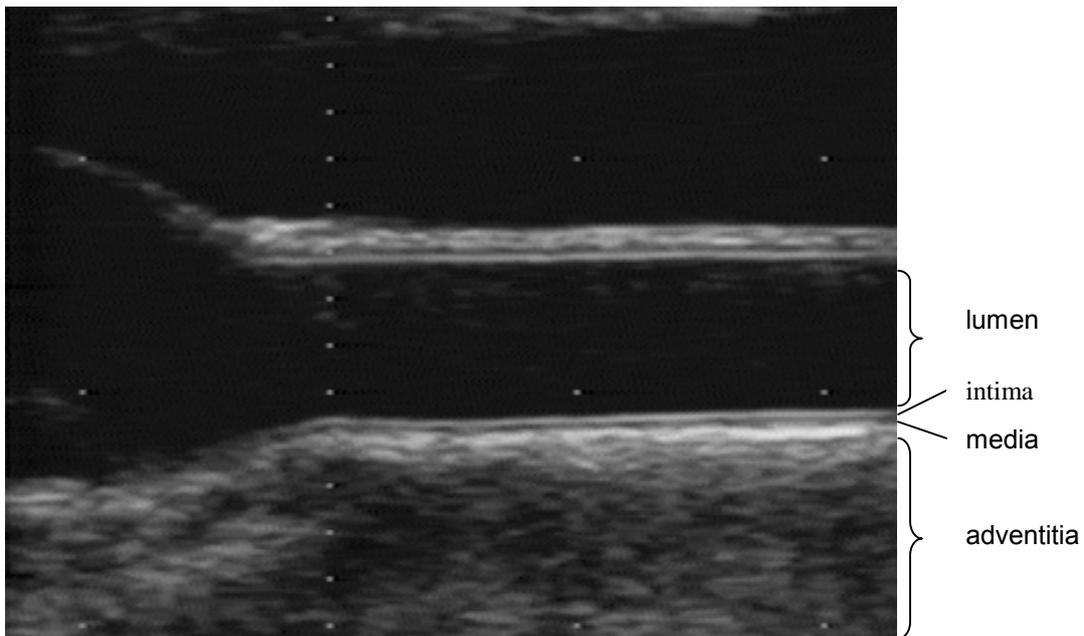
During the first visit at our outpatient clinic the CIMT of the right and left carotid artery was measured. This was performed by six trained sonographers using a 7.5 MHz linear array transducer (Acuson Aspen). The CIMT was measured at eight different angles (right carotid artery: 180, 150, 120 and 90 degrees, left carotid artery: 180, 210, 240 and 270 degrees) using the Meijer's Arc (figure 5.1.1). When an optimal two dimensional (2-D) image of the distal part of the common carotid artery was obtained (figure 5.1.2), it was frozen on the top of the R-wave of the electrocardiogram and recorded on tape. Two readers measured the CIMT off line with an automated edge detection program, over a length of 10 mm starting at the beginning of the dilatation of the bifurcation.¹⁴ Of the sixteen CIMT-measurements per participant (near and far wall and four angles and right and left carotid artery) the average intima-media thickness was calculated and used in all analyses. The reproducibility was assessed by scanning 21 participants for a second time at the next visit by another sonographer. The absolute mean difference was 0.012 mm (standard error ± 0.004). The intraclass correlation coefficient was 0.84. All sonographers and readers were blinded to the data in the medical health records and measurement results.

Further information on risk factors was obtained by a written questionnaire. Participants were asked to contact (one of) their parents. The questions were on ethnicity, smoking (yes/no), alcohol consumption (yes/no), use of oral contraception (yes/no), and cardiovascular risk factors and diseases in the family (did the first degree family members suffer from a myocardial infarction, stroke, or developed

Figure 5.1.1 Acquisition of a B-mode image at angle 270, using the Meijer's Arc



Figure 5.1.2 Typical 2-dimensional longitudinal B-mode image of the distal part of the common carotid artery. CIMT is measured over a distance 10 mm to the right of the vertical dotted line



The left side of the vertical line shows the carotid bifurcation. At the right site, the intima and media of the common CIMT are measured off line.

diabetes, hypertension and/or hypercholesterolemia (yes/no)). Socio-economic status at adolescence was estimated by the highest education level of father, and at young adulthood by the highest education level of the participant (low/middle/high).

5.1.4 Data analysis

We studied the role of blood pressure at adolescence and young adulthood and tracking of blood pressure on the development of atherosclerosis at young adulthood independent of gender, attained age and body mass index.

First, we estimated the relation of adolescent systolic blood pressure per standard deviation (independent variable) with CIMT (dependent variable) using linear regression models. Similar models were used for the independent variables diastolic blood pressure, pulse pressure (= systolic blood pressure – diastolic blood pressure) and mean arterial pressure (= diastolic blood pressure + 1/3 (pulse pressure) per standard deviation. As adolescent blood pressure was significantly related to gender, adolescent age and adolescent body mass index, the models were additionally adjusted for these factors.

Second, we estimated the association of adult blood pressure per standard deviation with CIMT, additionally adjusted for gender, adult age and adult body mass index.

Table 5.1.1 General characteristics ARYA cohort Utrecht in adolescence and young adulthood

	Men (n=352)		Women (n=398)	
	Adolescence	Young Adulthood	Adolescence	Young adulthood
Age (years)	13.4(1.1)	28.4(0.9)	13.4(1.1)	28.4(0.9)
Systolic blood pressure (mmHg)	111.7(12.5)	130.8(12.0)	109.0(11.3)	120.6(12.3)
Diastolic blood pressure (mmHg)	67.4(9.6)	73.1(7.8)	(10.1)66.0	70.9(8.5)
Pulse pressure (mmHg)	44.2(11.3)	57.7(8.9)	42.9(9.6)	49.6(8.1)
Mean arterial pressure (mmHg)	82.1(9.1)	92.3(8.5)	80.4(9.4)	87.5(9.2)
Weight (kg)	49.2(10.5)	83.5(13.6)	49.9(9.9)	71.3(14.9)
Height (cm)	163.0(10.3)	183.9(6.7)	161.2(8.0)	170.2(6.4)
Body mass index (kg/m ²)	18.3(2.4)	24.7(3.7)	18.9(2.9)	24.7(5.0)
Waist-hip ratio		0.88(0.06)		0.81(0.06)
Maturity	2.9(1.3)		3.7(1.4)	
Smoker (% yes)	4.0	35.8	5.8	26.6
Education level (% low (relative to middle and high))	39.7	15.5	32.6	10.9
CIMT (mm)		0.49(0.05)		0.48(0.05)

Tanner score (0-5) of pubic hair, Education level: of father during adolescence and of the participant at young adulthood.

Values are means (standard deviation) unless otherwise indicated.

Third, we studied the impact of adult blood pressure on the relation of adolescent blood pressure to CIMT, independent of gender, age and body mass index at both adolescence and young adulthood. This was done by first estimating the individual adolescent blood pressures independent of gender, adolescent age and adolescent body mass index: the individual blood pressure per standard deviation was adjusted for these factors in a linear regression model, and the individual outcome was called the adolescent blood pressure-residual. The adult blood pressure-residuals were similarly calculated by adjusting the adult blood pressure for gender, adult age and adult body mass index. Using linear regression analysis we studied the relation between the adolescent blood pressure-residuals and CIMT, and subsequently this relation was additionally adjusted for adult blood pressure-residuals.

Finally, we studied the relation between blood pressure tracking and CIMT. Tracking of blood pressure was defined as maintaining the same rank order in the blood pressure-distribution, and detracking of blood pressure as an increase or decrease of the rank order. We used the Spearman's rank correlation coefficients to obtain correlation- and tracking coefficients. To study both tracking and detracking from adolescence to young adulthood in one model, we defined four groups of subjects: a group with tracking of blood pressure-residuals below the median from adolescence to young adulthood (LL), a group with detracking of blood pressure-residuals from below the median to above the median (LH), a group of subjects with blood pressure-detracking from above the median to below the median (HL) and a group with blood pressure-tracking above the median (HH). A linear regression model was used to relate (de)tracking with CIMT, using dummy variables with LL as the reference group.

All analyses were carried out with the statistical analysis software SPSS 9.0. Statistical significance was concluded if $p < 0.05$.

5.1.5 Results

Table 5.1.1 shows the general characteristics of the ARYA cohort for men and women separately. The mean time-interval between the adolescent blood pressure measurement and CIMT measurement at young adulthood was 14.9 years (SD=1.3). At adolescence the blood pressure was higher in men, and body mass index and stage of sexual maturation were lower. The percentage of smokers and socio-economic status of the fathers during adolescence were higher among the participating women. At young adulthood, blood pressure, weight, height, waist-hip ratio, the percentage smokers and CIMT were higher in men.

Table 5.1.2 shows that systolic blood pressure, pulse pressure and mean arterial pressure at the age of 13 years are positively related to CIMT at young adulthood. The relations attenuated after adjustment for gender, adolescent age and adolescent

body mass index, but the associations with systolic blood pressure and pulse pressure remained statistically significant. Further adjustment for other factors did not affect the findings.

At the age of 28 years, systolic blood pressure and pulse pressure were related to CIMT after adjustment for gender, adult age and adult body mass index (4.3 $\mu\text{m}/\text{SD}$ systolic blood pressure mmHg (1.0 to 7.7) and 5.0 $\mu\text{m}/\text{SD}$ pulse pressure mmHg (1.7 to 8.4)). The relation with adult diastolic blood pressure and mean arterial pressure was smaller and not significant: 1.0 $\mu\text{m}/\text{SD}$ diastolic blood pressure mmHg (-2.0 to 4.0) and 2.5 $\mu\text{m}/\text{SD}$ mean arterial pressure mmHg (-0.6 to 5.7), respectively.

Our third question concerned the impact of adult blood pressure, measured by adjusting the relation between adolescent blood pressure and CIMT for adult blood pressure. Because blood pressure tracks from adolescence to young adulthood, an apparent association with adolescent blood pressure might appear, although the true relation exists between adult blood pressure and common carotid intima-media thickness. Therefore, we adjusted the relation between adolescent systolic blood pressure-residuals and CIMT, for adult systolic blood pressure-residuals. The relations between adolescent systolic blood pressure-residuals and CIMT attenuated slightly, and became statistically non significant. A similar finding was found for pulse pressure that, however, remained statistically significant (table 5.1.3).

Blood pressure showed a moderate degree of tracking from adolescence into young adulthood in our population. Overall, the *unadjusted* correlation (r) was 0.22 for systolic blood pressure ($p < 0.01$), $r = 0.09$ for diastolic blood pressure ($p < 0.05$), $r = 0.14$ for pulse pressure ($p < 0.01$) and $r = 0.14$ for mean arterial pressure ($p < 0.01$).

Table 5.1.2 Associations between adolescent blood pressure and CIMT at young adulthood

	Adolescence			
	Systolic blood pressure	Diastolic blood pressure	Pulse pressure	Mean arterial pressure
Common	7.5(4.3 to 10.6)	2.6(-0.6 to 5.9)	5.4(2.4 to 8.4)	5.2(1.9 to 5.8)
Adjusted for gender, adolescent age and adolescent body mass index	4.1(0.7 to 7.5)	-0.9(-4.3 to 2.4)	4.7(1.6 to 7.8)	1.1(-2.4 to 4.6)
Additionally adjusted for adolescent maturity, smoking and education level of father	4.5(0.7 to 8.2)	-0.9(-4.6 to 2.8)	4.8(1.5 to 8.1)	1.3(-2.6 to 5.1)

Values are linear regression coefficients: change in mean CIMT (μm) per standard deviation blood pressure (95% confidence interval).

Table 5.1.3 Relation of adolescent blood pressure with adult CIMT, adjusted for adolescent and adult risk factors levels

<i>CIMT at young adulthood</i>	Adolescence			
	Systolic blood pressure	Diastolic blood pressure	Pulse pressure	Mean arterial pressure
Adjusted for adolescent and adult risk factors*	4.1(0.6 to 7.7)	-0.9(-4.4 to 2.6)	4.7(1.4 to 7.9)	1.1(-2.5 to 4.7)
Adjusted for adolescent and adult risk factors* and adult blood pressure	3.4(-0.2 to 7.0)	-1.1(-4.6 to 2.5)	4.7(1.4 to 7.9)	0.6(-3.0 to 4.2)

Values are linear regression coefficients: change in mean CIMT (μm) per standard deviation blood pressure (95% confidence interval).

*Gender, adolescent age, adolescent body mass index, adult age and adult body mass index (see data analyses for details).

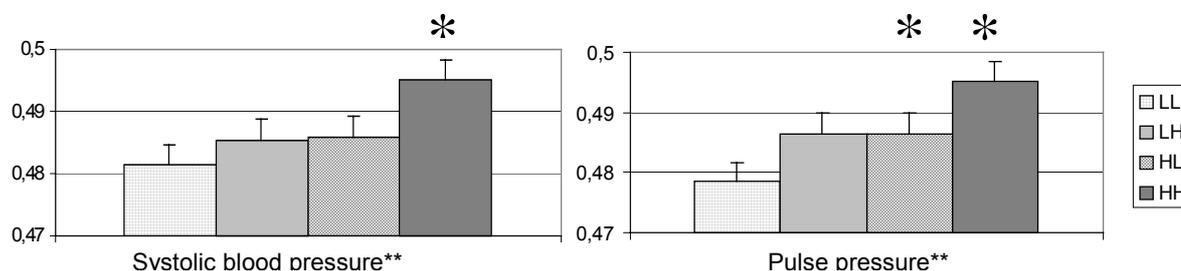
The correlation of the blood pressure-residuals was $r=0.19$ for systolic blood pressure ($p<0.01$), $r=0.08$ for diastolic blood pressure ($p<0.05$), $r=0.12$ for pulse pressure ($p<0.01$) and $r=0.13$ for mean arterial pressure ($p<0.01$). Of the 750 adolescents, 212 (28.3%) showed tracking of the systolic blood pressure-residuals above the median (HH). The tracking groups HL, LH and LL consisted of 162, 160 and 207 subjects, respectively. A similar number of subjects was observed in the tracking groups of diastolic blood pressure, pulse pressure and mean arterial pressure. Figure 5.1.3 shows that subjects tracking above the median of systolic blood pressure and pulse pressure (the HH group) had significantly larger CIMT than subjects tracking in the LL group. The subjects detracking between below and above the median (the LH and HL groups) had a comparable CIMT in between that of subjects tracking in either below or above the median. Pulse pressure showed similar results.

5.1.6 Discussion

We found that adolescent systolic blood pressure and pulse pressure levels are positively related to the thickening of the carotid arterial walls in healthy young adults. Furthermore, a high blood pressure at adolescence tracking into young adulthood was associated with the largest CIMT.

To appreciate the results of this study, some issues need to be addressed. A single and routine blood pressure examination by school doctors does not provide a standardized blood pressure level of an adolescent due to within individual and between observer variability. Therefore, we believe that our results reflect an underestimation of the true relations of adolescent blood pressure and blood pressure-tracking with CIMT. Secondly, although CIMT is regarded as a valid

Figure 5.1.3 CIMT (mm) and (de)tracking of systolic blood pressure and pulse pressure from adolescence to young adulthood



LL: tracking below the medians of adolescent systolic blood pressure- and pulse pressure-residuals into young adulthood, LH: detracking of blood pressure from below to above the median, HL: detracking from above to below the median, HH: tracking of blood pressure above the medians. Values represent the mean CIMT (mm) with standard errors.

*CIMT of this marked group of subjects is significantly thicker than that of the reference group LL.

**Systolic blood pressure- and pulse pressure-residuals: blood pressure per standard deviation adjusted for gender, age and body mass index at adolescence or young adulthood.

indicator of generalized atherosclerosis and cardiovascular risk in the middle aged and elderly subjects, it has been argued that at lower degrees of CIMT, and thus at younger age, the thickening may reflect a non atherosclerotic adaptive response to changes in shear and tensile stress.¹⁵ However, postmortem observational studies showed that raised fatty streaks are present in subjects as early as 15 through 34 years of age. These are regarded as the intermediate lesion in between the juvenile (flat) fatty streak and the raised lesion of atherosclerosis.² The raised lesions are related to cardiovascular risk factors.^{2,16} Also, cross-sectional studies showed that blood pressure and lipid levels in healthy young adults are associated with increased CIMT^{3,4,17,18} and other measurements of vascular damage such as coronary artery calcification¹⁹ These observations indicate that an increased CIMT at young adulthood most likely reflects exposure to cardiovascular risk factors, and as such confers risk of cardiovascular morbidity and mortality later in life.

Longitudinal data about the relation between cardiovascular risk factors and atherosclerosis in young adulthood are limited. In contrast to our findings, in the Muscatine study¹³ neither adolescent systolic or diastolic blood pressure were related to CIMT in healthy young adults after adjustment for adolescent total cholesterol in boys, and adolescent body mass index and total cholesterol in girls. Only when a time-weighted average of all (\pm six) diastolic blood pressure measurements were used in the multivariate analyses, a positive relation was found, in men only. The latter finding underlines the issue that variability in blood pressure measurements may attenuate the associations. In the Bogalusa Heart study¹², systolic blood pressure and diastolic blood pressure showed a positive relation with atherosclerosis measured postmortem in subjects till 39 years of age, but other risk factors were not

taken into account. Our findings are in agreement with data in middle aged²⁰⁻²³ and elderly subjects^{24,25}, showing that blood pressure is related to CIMT, measured after 19 months till 15 years of follow-up. In general, systolic blood pressure was more strongly related to CIMT than diastolic blood pressure, and this may result from the consistent finding that the diastolic blood pressure measurements are more variable than systolic blood pressure. In the ARYA study the correlation for systolic blood pressure was 0.7 and for diastolic blood pressure 0.6 between the first and second visit at young adulthood.

The ARYA findings expand the available evidence by indicating that adolescent pulse pressure is related to a thicker CIMT at the age of 28 years than adolescent systolic blood pressure. Studies on pulse pressure in adolescents and young adults are lacking, but longitudinal studies in middle aged and elderly subjects reported on pulse pressure^{21,25}, and similarly showed that the relation between CIMT and pulse pressure was stronger than systolic blood pressure. The rise in pulse pressure at older age is caused by a fall in diastolic blood pressure due to increased arterial stiffness of the large arterial vessels²⁶, and pulse pressure seems to be the best predictor of cardiovascular morbidity and mortality.^{11,27,28} In contrast, at young age both systolic blood pressure and diastolic blood pressure appear to be better predictors of cardiovascular morbidity and mortality than pulse pressure.⁹⁻¹¹ Yet, it is difficult to investigate whether pulse pressure is independently associated with cardiovascular risk, or does associate due to a strong correlation with systolic blood pressure. In our study, pulse pressure is strongly correlated with systolic blood pressure (0.8) and not with diastolic blood pressure (0.1). The difference in impact of these two blood pressure indices on the CIMT has to be elucidated by further research.

Previous studies demonstrated the presence of blood pressure-tracking in the young⁵⁻⁸. Yet, information on the relation between blood pressure-tracking and atherosclerosis is lacking at all ages. We showed that tracking in the highest systolic blood pressure and pulse pressure levels is related to the thickest CIMT and that subjects detracking have a mean CIMT in between that of subjects tracking in the lowest or highest levels. This might imply that in adolescents with a high blood pressure, whose adult blood pressure is below the median, exhibit a reduced rate of progression of the CIMT without abolishing the effect of the adolescent blood pressure on the arterial wall.

In conclusion, the results of the ARYA study provide evidence that routinely measured high adolescent systolic blood pressure and pulse pressure are related to an increased CIMT at the age of 28 years, independent of other variables at adolescence and young adulthood; age, body mass index, gender and adult blood pressure. Our results strengthen the notion that the process of rise of cardiovascular risk is already initiated at adolescence.

5.1.8 References

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5.2

Does a routinely measured blood pressure in young adolescence accurately predict hypertension and total cardiovascular risk in young adulthood?

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5.2.1 Abstract

Background: Studies with strongly controlled research settings suggest that childhood blood pressure predicts adult blood pressure. However, it is unknown if adolescent blood pressure as routinely measured by youth health care practices, predicts hypertension or total cardiovascular risk in adulthood sufficiently accurate to warrant blood pressure screening.

Methods: In the cohort of Utrecht, a single and routinely measured blood pressure and anthropometry at the age of 13 years were collected from school health records. In the cohort of The Hague, adolescents were examined biennially in a standardized manner with measuring blood pressure three times per visit and anthropometry. Recently, 998 young adults aged 30 years of both cohorts were newly examined. The mean of a duplicate blood pressure at two different occasions was used as outcome. Adolescent predictors of hypertension and of 10-year cardiovascular risk in young adulthood by adolescent characteristics were assessed by logistic regression and by Receiver Operation Characteristics (ROC) curve analysis.

Results: The Atherosclerosis Risk in Young Adults (ARYA) study comprised 167 (38 women, 129 men) young adults with and 831 without hypertension, defined as the average of all measurements at two visits of systolic blood pressure ≥ 140 mmHg and/or diastolic ≥ 90 mmHg. Both single adolescent systolic and diastolic blood pressure were associated with hypertension at young adulthood: odds ratio (OR): 1.04 per mmHg (95% confidence interval: 1.03 to 1.06), area under ROC-curve (AUC): 0.64 (0.59 to 0.69), and OR: 1.02 per mmHg (1.00 to 1.04), AUC: 0.54 (0.49 to 0.59), respectively. Gender, body mass index, and age at adolescence combined improved the prediction: AUC: 0.71 (0.67 to 0.75). There was a statistically significant gain in predictive value by adding systolic blood pressure: AUC: 0.74 (0.70 to 0.77) and the difference in AUC: 0.03 (0.002 to 0.06). However, this gain was limited in magnitude given the strong predictive value of gender. Within men and women, systolic blood pressure was the only predictor of hypertension 15 years later. In men, the OR was 1.03 per mmHg (1.01 to 1.04) and AUC: 0.59 (0.53 to 0.65) and in women 1.08 per mmHg (1.05 to 1.11) and AUC: 0.74 (0.67 to 0.82). However, an adolescent blood pressure of 120 mmHg or higher did not efficiently detect later hypertensive men, while it detected 57.9% of later hypertensive women. Predictors of the 10-year cardiovascular risk of young adults could only be studied in men, who had by far the highest risks. Again, only systolic blood pressure predicted risk. For systolic blood pressure predicting the 10-year risk score being above the 95th percentile the OR was 1.04 per mmHg (1.02 to 1.07), AUC: 0.67 (0.60 to 0.75).

Conclusion: A single routine blood pressure in young adolescent girls predicts hypertension in young adult women. In adolescent boys, blood pressure does not predict hypertension at young adulthood, but it predicts 10-year cardiovascular risk.

5.2.2 Introduction

To date, there are no studies with sufficiently long follow-up to directly show relations between childhood blood pressure levels and the actual occurrence of ischaemic cardiovascular disease many decades later. There are long-term follow-up studies indicating that elevated blood pressure in young adulthood is associated with cardiovascular morbidity and mortality.¹⁻⁶ Thus, elevated blood pressure in young adulthood is a marker for cardiovascular risk, which can be used as a meaningful surrogate endpoint in studies on childhood cardiovascular risk. Previous studies have shown that higher adolescent blood pressure levels are associated with higher blood pressure levels in young adulthood, and that combining several biennial blood pressure measurements improves prediction.⁷⁻⁹ However, these studies were performed under strongly controlled conditions both concerning research setting and measurements. Whether such results apply to common youth health care practice is unclear. It is specifically unknown if routinely measured blood pressure in children is sufficiently predictive for later hypertension to justify large-scale screening of blood pressure in childhood. Moreover, to our knowledge no studies on prediction with cardiovascular risk factors in children have taken total cardiovascular risk profiles in young adulthood as an endpoint.

In The Netherlands, school doctors and nurses of the youth health care practices regularly screen children's health states nationwide. Previously, routine blood pressure measurement was abandoned because its practical value in this screening was insufficiently known. The Atherosclerosis Risk in Young Adults (ARYA) study is a cohort of young adults of whom the medical records of the youth health care practices, including blood pressure, are still available. This provided a unique opportunity to study prediction of adult hypertension and total cardiovascular risk profiles by routinely measured blood pressure along with other simple and available screening characteristics at adolescence. Furthermore, ARYA includes a sub-cohort that enabled assessment of possible gain in predictive accuracy through using standardized and multiple blood pressure measurements at adolescence.

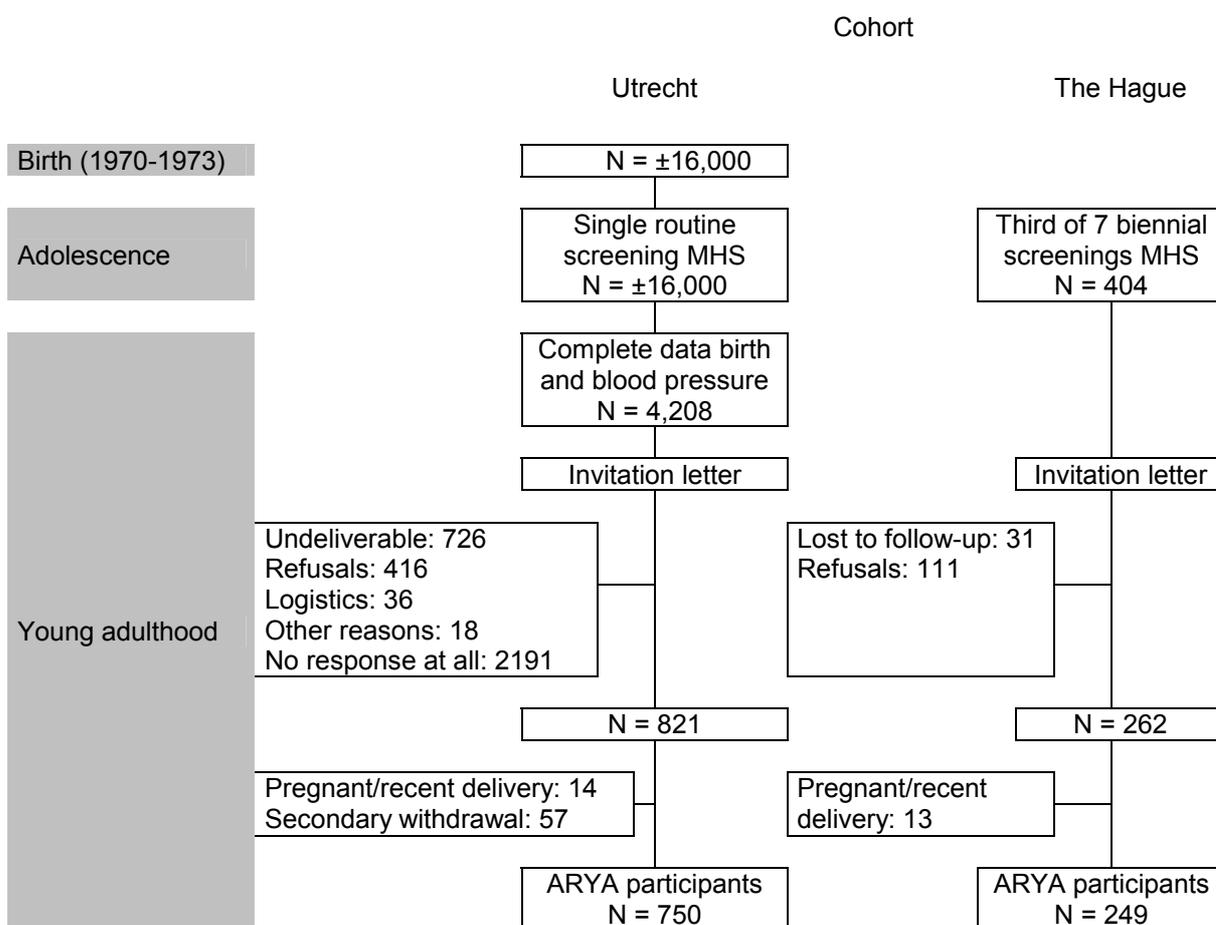
The aim of this study was to determine whether routinely measured blood pressure of young adolescents accurately predicts hypertension and 10-year cardiovascular risk in young adulthood. We particularly questioned whether such blood pressure measurement has additional value to already routinely collected indicators of later hypertension and cardiovascular risk. The results were meant to support a discussion about the relevance of blood pressure screening in Municipal Health Services.

5.2.3 Methods

The principal objective of the Atherosclerosis Risk in Young Adults (ARYA) study was to investigate early determinants of cardiovascular risk in the young. ARYA consists of a cohort from the Dutch city of Utrecht with 750 participants and a cohort from The Hague with 262 participants. The Medical Ethical Committee of the University Medical Center Utrecht, The Netherlands, approved the study. Written informed consent was obtained of all participants.

The enrolment schemes of both cohorts are shown in figure 5.2.1. In The Netherlands virtually all children regularly visit the child health facilities, starting at four weeks of age until leaving secondary school at the age of 16 to 19 years. Information is routinely collected in health records by school doctors and nurses. For the cohort of Utrecht, records of subjects born between 1970 and 1973 and attending secondary school in Utrecht were selected on the presence of birth weight data and approximated in the record (sign \pm), the subject was excluded. A single routine blood

Figure 5.2.1 Selection procedures of the ARYA participants



MHS: Municipal Health Service.

pressure was measured by a sphygmomanometer, as well as body height, weight and sexual maturity according to the Tanner scale. Between responders and non-responders born in 1970, there were no differences in mean birth weight, adolescent blood pressure and anthropometry.

The Municipal Health Service of The Hague invited all first-year students starting in 1978 and 1979 at the Aloysius College, a secondary school. Of all invitees, 98% participated, one refused participation and five refused venipuncture. Information was biennially collected by school doctors. Standard examinations of the total cohort took place at the end of September and March of each year between 9.00 and 15.00 hours within a time span of one week. This procedure was continued over seven years into later adolescence. Up to the age of 16 years, 11% of boys and 10% of girls were lost to follow-up, all due to changing school or moving to another city. Thereafter, loss to follow-up increased due to leaving school. At each examination, blood pressure was measured three times within three minutes in sitting position at the right upper arm. A dynamap (Physiometrics SR-2) with a cuff of 13x23.5 cm was used.¹⁰ Body weight and –height were measured without shoes. At visit 3, most of the adolescent pupils of two successive school years were examined (387 of 404).

Our first endpoint was hypertension in young adulthood. At young adulthood, all ARYA participants of both cohorts underwent a physical examination during two visits. The Utrecht cohort was examined at the outpatient clinic of the Julius Center and the cohort of The Hague was examined either at the Julius Center or in their former secondary school in The Hague, by either of the first two authors (LEV, AO). All examinations took place between October 1999 and February 2001. All examiners were similarly instructed and blinded for all childhood data. After a five minutes rest in sitting position, a first blood pressure was measured by Dynamap at the left upper arm. After another 5 to 15 minutes rest the measurement was repeated. The same procedure was followed at the second visit that took place after a mean interval of 20.4 days (SD 10.7). Hypertension at young adulthood was defined according to the JNC V definitions¹¹: systolic blood pressure ≥ 140 mmHg and/or diastolic ≥ 90 mmHg and/or use of anti-hypertensive medication (stages I-IV). We used the mean blood pressure of all measurements at two visits in the analyses.

Our second endpoint was the total risk profile in young adulthood. In the cohort of Utrecht, fasting blood of 730 and non-fasting blood of 8 young adults was drawn and stored at -20°C . At the last visit, serum levels of glucose, total cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol and low-density-lipoprotein (LDL-) cholesterol¹² were determined by Vitros950 dry-chemistry analyzer (Johnson & Johnson, Rochester, New York, USA). In the cohort of The Hague, fasting blood was drawn of 240 young adults. Samples were kept at 4°C and the lipid profiles and glucose levels were determined within 4 hours. Of these, 187 samples were

determined by using LX-20 (Coulter-Beckman, The Netherlands), 40 by Vitros950 dry-chemistry analyzer, and 13 in laboratories elsewhere. Serum triglyceride values were logarithmically transformed to obtain normal distributions in the analysis. Information on smoking, alcohol use, medication use, highest educational level (low/middle/high), family history of cardiovascular disease, and hypertension, myocardial infarction, stroke, diabetes or hypercholesterolemia of first degree family members (n=859) was obtained by a written questionnaire.

With the above mentioned measurements the total cardiovascular risk in young adulthood was calculated using a Framingham risk score.¹³ The score was developed in a population based sample of 2489 men and 2856 women aged 30 to 74 years old without overt coronary heart disease at baseline. Over a 12 year follow-up period the incidence of the following coronary heart diseases was recorded: angina pectoris, recognized and unrecognized myocardial infarction, coronary insufficiency, and coronary heart disease death, according to previously described criteria.¹⁴ The individual 10-year probabilities of coronary heart disease in ARYA participants was calculated using the β -coefficients from Cox-proportional hazard models fitted in the Framingham cohort, using continuous variables age and age-squared, categorized variables total- and HDL-cholesterol and blood pressure, and dichotomous variables diabetes and smoking. Cut-offs for total cholesterol (<200, 200 to 239, 240 to 279, \geq 280 mg/dl) and HDL-cholesterol (<35, 35 to 59, \geq 60 mg/dl) are similar to those used for the NCEP ATP II guidelines.^{15,16} The mean of blood pressure readings at young adulthood was calculated and blood pressure was categorized according to the JNC V definitions¹¹: optimal blood pressure (systolic <120 mmHg and diastolic <80 mmHg), normal blood pressure (systolic 120 to 129 mmHg or diastolic 80 to 84 mmHg), high normal blood pressure (systolic 130 to 139 mmHg or diastolic 85 to 89 mmHg), hypertension stage I (systolic 140 to 159 mmHg or diastolic 90 to 99 mmHg), and hypertension stage II-IV (systolic \geq 160 or diastolic \geq 100 mmHg). When systolic and diastolic blood pressures fell into different categories, the higher category was selected. Blood pressure categorization was made without regard to the use of anti-hypertensive medication.

5.2.4 Data analysis

Central estimators and variance measures were calculated for all relevant baseline characteristics for adolescence and young adulthood separately. The prediction of hypertension in young adulthood by adolescent characteristics was quantified using logistic regression analysis, and results expressed as odds ratios and areas under ROC curves with 95% confidence intervals.

A full model with blood pressure, gender, body mass index and age was first evaluated with a backward stepwise procedure, with removal of terms from the model

at a p-value exceeding 0.10.¹⁷ Areas under ROC curves were calculated to evaluate discriminative values of models. These procedures were first used to assess whether comparison of data from the Utrecht cohort (routine blood pressure) and data from the cohort from The Hague (standardized blood pressure) showed predictive benefit of measurement standardization. Similarly, comparisons of models were used to evaluate if various combinations of repeated blood pressure measurements in the cohort from The Hague would increase predictive accuracy. Differences in discriminating value between models were estimated by differences in area under ROC curves (AUC) with 95% confidence intervals, taking into account the correlation between models as they were based on the same cases.¹⁸ Reliability (goodness of fit) of the models was estimated using the Hosmer & Lemeshow test.¹⁹ Next, the absolute numbers of hypertension cases and non-hypertension cases were evaluated among categories of adolescent risk. Analyses were performed both for hypertension and 10-year cardiovascular risk in young adulthood. Analyses were carried out using SPSS 10.1.

5.2.5 Results

In table 5.2.1 the characteristics of participants as adolescents are given for both cohorts of ARYA. Boys had higher systolic blood pressures and lower weight and body mass index than women. In the Utrecht cohort girls had higher maturity scores than boys. Table 5.2.2 shows characteristics of participants as young adults. Men had higher systolic and diastolic blood pressure, weight, height and waist-hip ratio, while body mass index was similar in both sexes. One young adult with hypertension did not attend visit 3 at adolescence and therefore most of the following analyses pertain to 998 participants. The study population comprised 167 young adults (38 women and 129 men) with hypertension, 111 in the Utrecht cohort, 55 in the cohort of The Hague, and 831 without hypertension.

When analyzing data of the cohort of Utrecht and The Hague separately, similar results were found for blood pressure. The routine single adolescent blood pressure in the Utrecht cohort showed an odds ratio of 1.04 (95% confidence interval: 1.02 to 1.06) per mmHg increase of systolic and of 1.02 (95% CI: 1.00 to 1.05) per mmHg increase in diastolic blood pressure, when predicting adult hypertension. Within the cohort from The Hague, a single systolic blood pressure showed an odds ratio of 1.04 per mmHg (95% CI: 1.01 to 1.07) and for diastolic blood pressure of 1.01 (95% CI: 0.97 to 1.05). These associations did not change when the mean blood pressure was taken of either two or three adolescent readings per visit, nor when using the overall mean of all 6 measurements from visit 3 and the visit one year later (systolic blood pressure: OR 1.04; 95% CI: 1.01 to 1.07), or years later (data not shown). As

Table 5.2.1 General characteristics ARYA participants in adolescence

<i>Adolescence</i>	Cohort of Utrecht		Cohort of The Hague: Visit 3	
	Boys (n=352)	Girls (n=348)	Boys (n=223/232)	Girls (n=164/172)
Age (years)	13.5 (1.1)	13.4 (1.1)	13.2 (0.7)	13.1 (0.7)
Single/1 st SBP (mmHg)	111.7 (12.5)	109.0 (11.3)	119.9 (12.4)	118.1 (11.9)
Single/1 st DBP (mmHg)	67.4 (9.6)	66.0 (10.1)	67.4 (8.4)	67.7 (7.3)
Mean of 1 st + 2 nd SBP (mmHg)	-	-	118.1 (12.1)	116.5 (11.2)
Mean of 1 st + 2 nd DBP (mmHg)	-	-	66.6 (7.9)	66.7 (7.1)
Mean of 1 st + 2 nd + 3 rd SBP (mmHg)	-	-	117.1 (11.9)	115.2 (10.9)
Mean of 1 st + 2 nd + 3 rd DBP (mmHg)	-	-	66.0 (7.9)	66.1 (6.9)
Weight (kg)	49.2 (10.5)	49.9 (9.9)	46.8 (8.3)	47.9 (8.1)
Height (cm)	163.0 (10.3)	161.2 (8.0)	159.3 (8.9)	159.3 (7.0)
Body mass index (kg/m ²)	18.3 (2.4)	18.9 (2.9)	18.3 (2.0)	18.8 (2.4)
Maturity (Tanner score)	2.9 (1.3)	3.7 (1.4)	-	-

Values are means with standard deviation.

SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 5.2.2 General characteristics of ARYA participants in young adulthood

<i>Young adulthood</i>	Cohort of Utrecht		Cohort of The Hague	
	Men (n=352)	Women (n=398)	Men (n=142)	Women (n=107)
Age (years)	28.4 (0.9)	28.4 (0.9)	34.3 (0.8)	34.3 (0.7)
Systolic blood pressure (mmHg)	130.8 (12.0)	120.6 (12.3)	134.0 (13.9)	121.2 (14.2)
Diastolic blood pressure (mmHg)	73.1 (7.8)	70.9 (8.5)	74.8 (8.6)	70.2 (8.6)
Weight (kg)	83.5 (13.6)	71.3 (14.9)	85.4 (13.0)	69.5 (12.8)
Height (cm)	183.9 (6.7)	170.2 (6.4)	182.9 (7.0)	169.0 (6.4)
Body mass index (kg/m ²)	24.7 (3.7)	24.7 (5.0)	25.5 (3.5)	24.3 (4.0)
Waist-hip ratio	0.88 (0.06)	0.81 (0.06)	0.92 (0.06)	0.83 (0.07)
Total cholesterol (mmol/l)	4.8 (1.0)	4.8 (0.8)	5.3 (1.1)	4.9 (0.8)
HDL-cholesterol (mmol/l)	1.3 (0.3)	1.6 (0.4)	1.2 (0.3)	1.6 (0.3)
Triglycerides (mmol/l)	1.4 (0.9)	1.2 (0.6)	1.1 (0.7)	0.9 (0.4)
LDL-cholesterol (mmol/l)	2.9 (0.9)	2.7 (0.8)	3.6 (0.9)	3.0 (0.8)
Glucose (mmol/l)	5.2 (1.2)	4.8 (0.4)	5.3 (0.7)	5.0 (0.5)

Values are means with standard deviation.

LDL: low-density-lipoprotein, HDL: high-density-lipoprotein.

Table 5.2.3 Multivariate predictors in adolescence of hypertension at young adulthood after backward stepwise procedure

<i>Adolescence</i>	Odds ratio (95% CI) per mmHg		P-value
Single/first systolic blood pressure (mmHg)	1.04	(1.02 to 1.05)	<0.0001
Gender (male=1, female = 0)	4.38	(2.93 to 6.54)	<0.0001
Age (years)	0.81	(0.67 to 0.97)	0.02
BMI (kg/m ²)	1.09	(1.01 to 1.17)	0.03

In the full model diastolic blood pressure was included, but excluded in the backward stepwise procedure.

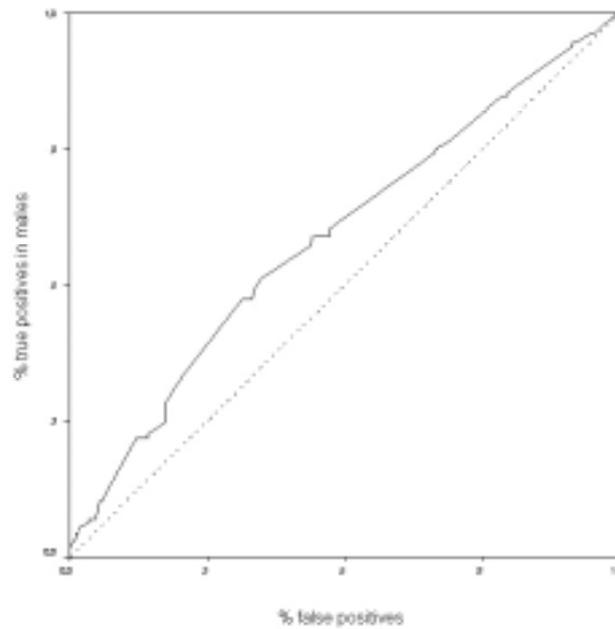
the predictive values of single blood pressure measurements did not differ between the two cohorts all data were pooled for further analyses on single early adolescent blood pressure data.

Table 5.2.3 shows results of multivariate logistic regression after a backward stepwise procedure. The full model included systolic and diastolic blood pressure, gender, age, and body mass index as possible predictors of young adult hypertension. The backward selection procedure removed diastolic blood pressure from the model. The area under the ROC curve (AUC) of gender, body mass index, and age at adolescence predicting hypertension at young adulthood was 0.71 (95% CI: 0.67 to 0.75), and with single adolescent systolic blood pressure added 0.74 (95% CI: 0.70 to 0.77). The difference in AUC of these two models was 0.03 (95% CI: 0.002 to 0.06), showing that systolic blood pressure had added predictive value. Clearly, gender was a strong independent predictor. Therefore, further analyses were performed for men and women separately.

Backward stepwise logistic regression was subsequently used in men and women separately starting with the same full model including systolic and diastolic blood pressure, age, and body mass index. Both in men and women, systolic blood pressure was the only remaining predictor of later hypertension. The odds ratio for male adolescent systolic blood pressure in mmHg was 1.03 (95% CI: 1.01 to 1.04) per mmHg. The corresponding ROC curve is shown in figure 5.2.2, with an AUC of 0.59 (95% CI: 0.53 to 0.65). The odds ratio for female adolescent systolic blood pressure in mmHg was 1.08 (95% CI: 1.05 to 1.11) per mmHg. The corresponding ROC curve is shown in figure 5.2.3, with an AUC of 0.74 (95% CI: 0.67 to 0.82).

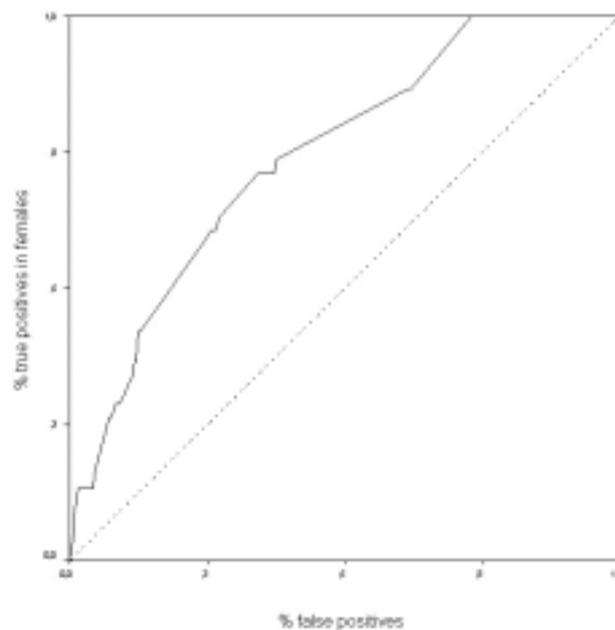
Thus, systolic blood pressure in early adolescent boys although statistically significantly associated with later hypertension but, according to the low AUC, did not discriminate between hypertensives and false positives. Within girls, however, systolic blood pressure predicted reasonably well. Table 5.2.4 shows numbers of hypertensives and non-hypertensives by categories of adolescent systolic blood

Figure 5.2.2 Receiver Operation Characteristic curve for adolescent systolic blood pressure in men predicting hypertension in young adulthood (129 hypertensives among a total of 365 men)



Area under the ROC curve is 0.59 (95% confidence interval: 0.53 to 0.65).

Figure 5.2.3 Receiver Operation Characteristic curve for adolescent systolic blood pressure in women predicting hypertension in young adulthood (38 hypertensives among a total of 504 women)



Area under the ROC curve is 0.74 (95% confidence interval: 0.67 to 0.82).

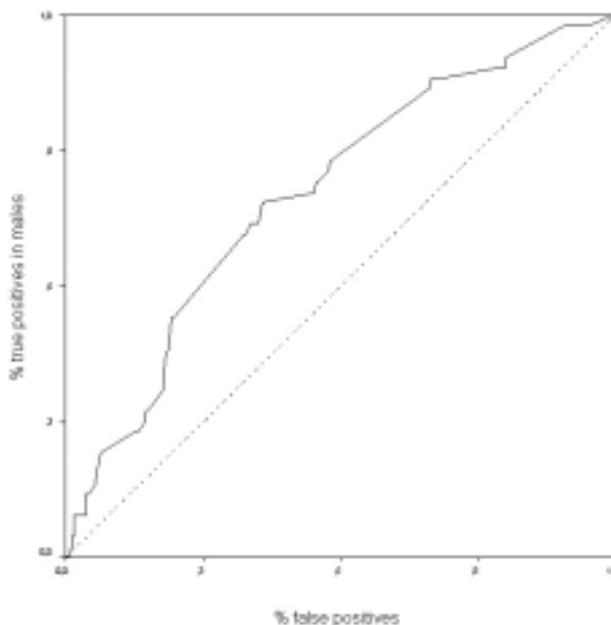
Table 5.2.4a Number of young adult men with and without hypertension across categories of adolescent systolic blood pressure

<i>Adolescent systolic blood pressure (mmHg)</i>	Total number of men (%)	Young adults with hypertension (%)		Young adults without hypertension (%)	
≤ 99	41 (8.3%)	8	(6.2%)	33	(9.0%)
100 – 119	278 (56.3%)	60	(46.5%)	218	(59.7%)
≥120	175 (35.4%)	61	(47.3%)	114	(31.2%)
Total	494 (100%)	129	(100%)	365	(100%)

Table 5.2.4b Number of young adult women with and without hypertension across categories of adolescent systolic blood pressure

<i>Adolescent systolic blood pressure (mmHg)</i>	Total number of women (%)	Young adults with hypertension (%)		Young adults without hypertension (%)	
≤ 99	63 (13.5%)	0	(0%)	63	(13.5%)
100 – 119	307 (60.9%)	16	(42.1%)	291	(62.4%)
≥120	134 (26.6%)	22	(57.9%)	112	(24.0%)
Total	504 (100%)	38	(100%)	466	(100%)

Figure 5.2.4 Receiver Operation Characteristic curve for adolescent systolic blood pressure in men predicting being above the 95% Framingham risk score in young adulthood



Area under the ROC curve is 0.67 (95% confidence interval: 0.60 to 0.75).

pressure for boys (table 5.2.4a) and girls (table 5.2.4b). For instance, an adolescent systolic blood pressure of 120 mmHg or higher in girls detected 57.9% of later hypertensive women, although there were only 38 women with hypertension in the total ARYA population (23% of all hypertensives). A low adolescent blood pressure in girls excluded young adult hypertension; i.e. 0% of the girls with a systolic blood pressure ≤ 99 mmHg developed hypertension at young adulthood. In boys, adolescent blood pressure did not discriminate later hypertensive and non-hypertensive men. However, the vast majority of young adult hypertensives were men, 129 of 167 (77%).

We proceeded with predicting the 10-year cardiovascular risk as calculated with the Framingham risk score. Expectedly, this could only be performed for men as the top 20% of the Framingham risk distribution consisted of men only (n=199), while only 3 women exceeded the 70th percentile of the Framingham risk score. In men, backward stepwise logistic regression showed that from a full model with systolic and diastolic blood pressure, body mass index and age, only systolic blood pressure was retained. The odds ratio for systolic blood pressure predicting the 10-year risk score being above the 95th percentile was 1.04 (95% CI: 1.02 to 1.07). The corresponding AUC of 0.67 (95% CI: 0.60 to 0.75) is shown in figure 5.2.4. Thus, for every mmHg higher systolic blood pressure there was a 4% increased risk of being in the 5% highest risk group as young adult men.

5.2.6 Discussion

Our findings show that a single routine systolic blood pressure measurement in young adolescent girls does predict later hypertension in young adulthood. In young adolescent boys, systolic blood pressure does statistically predict hypertension, but this has no practical relevance for screening at that age. However, in adolescent boys systolic blood pressure better predicted 10-year cardiovascular risk in young adulthood than hypertension.

A strong feature of ARYA is the use of data derived from clinical practice, which renders results that are directly relevant to that practice. We cannot exclude the possibility that there has been selection with regard to the prevalence of hypertension in our cohort. Non response of young adult hypertensives would probably imply an underestimation of predictive values. However, in the Utrecht cohort there was no difference between adolescent blood pressure and anthropometry of the responders and non-responders. Participation in the cohort of The Hague did not depend on adolescent data and several risk factors at young adulthood obtained by questionnaire.

Dutch youth health care services have in the past abandoned screening of blood pressure in adolescence based on insufficient knowledge of its practical value. There

are many longitudinal studies on the predictive properties of childhood blood pressure measurements, but these were invariably conducted under strongly controlled settings, while results are often not expressed in practically applicable ways.^{7-9,20,21} Many such studies do indicate that childhood blood pressure measurement, particularly when repeated, statistically predicts later blood pressure, but the predictive performance of measurements under practical conditions might be anticipated to be much less accurate. However, the decision of youth health care services to stop screening by blood pressure measurements was not evidence based. Rather, it was taken in the absence of evidence for practical usefulness. ARYA was designed to provide such evidence. Basically, our findings indicate that with systolic blood pressure measurement in young female adolescents, school doctors are able to detect a substantial number of future female hypertensives, particularly given nationwide screening activities. Obviously, adolescent screening would miss some 40% of future hypertensives and some 25% will be false positives. In adolescent boys at the age of around 13 years, screening of blood pressure, although statistically associated, does not seem useful to detect future hypertensives. Although, systolic blood pressure better predicted 10-year cardiovascular risk in young adult men, its discriminative value was not sufficiently high to warrant elaborate screening. Possibly, 13-year-old boys have less stabilized blood pressures than girls of that age. In the Utrecht cohort of ARYA, girls were substantially more sexually mature at this age than boys, which may partly underlie this gender discrepancy. This does not exclude the possibility that blood pressure screening in somewhat older boys may prove to be worthwhile. In the prediction model in the total ARYA population, particularly gender played a major role and to a lesser extent body mass index and age. Male gender and adolescent body mass index are indeed known risk indicators for hypertension and cardiovascular risk at large.²² Adding systolic blood pressure to these predictors yielded statistical but still limited gain in accuracy. Apparently, prediction models in young adolescence require gender specific derivation and practical application. Somewhat surprising was that using repeated blood pressure measurements for prediction did not increase predictive power as compared to prediction based on only one measurement. Other studies showed that combining repeated blood pressure measurements improved the prediction of later high blood pressure levels.^{7,23,24} The gain in precision of estimating the true underlying adolescent blood pressure was anticipated to be larger in our study using data from clinical practice, with larger measurement error and blood pressure variation.

It might be argued that further knowledge of hypertension risk factors in adolescence could further increase predictive possibilities. For instance, a family history of hypertension or cardiovascular disease has been shown by some to have predictive information concerning later hypertension,²⁵ although not supported by

others.²⁶ However, as these young adolescents are screened at school often without their parents being present, inquiring for a family history of hypertension or cardiovascular disease is probably too time consuming from a practical point of view and is likely to be subject to misclassification. In ARYA, the young adults were asked about a family history of hypertension, cardiovascular disease and diabetes mellitus. Data on family risk at the time of adolescence was not available and therefore could not be included in the predictive models. However, these data are likely subject to misclassification considering that it required recollection of data from more than 15 years ago. Moreover, it was considered questionable if young adolescents themselves will be able to accurately respond to such questions in practice. Predictive models were deliberately kept parsimonious with variables that can be objectively measured by physicians or nurses.

Cardiovascular disease is still the leading cause of morbidity and mortality in most Western countries and an increasing problem in many non-western societies.²⁷ There is growing consensus that primary hypertension has its origins in early life.²⁸ This had led many researchers to question whether or not prevention of hypertension and its sequelae, cardiovascular diseases, should be transferred from adult to early life. Except for population wide approaches, early prevention requires that high cardiovascular risk can be accurately estimated in early life. Moreover, it requires that health care systems have tools for effective and safe interventions in early life or for longer term monitoring of children at high risk. The most obvious intervention strategies are probably life style changes, as the efficacy and safety of long term aggressive interventions with medication are, for children, unknown. Sedentary lifestyles and obesity are increasingly threatening the health of youngsters.²⁹ Indicators of such lifestyles are shown to be predictors of obesity in later adolescence.³⁰ Adolescent overweight is shown to track into young adulthood and related to adult cardiovascular risk factors, including repeated blood pressure measurements.³¹ Also in adulthood, overweight contributes strongly to cardiovascular risk.³² In The Netherlands, the prevalence of obesity among children has strongly risen in between 1980 and 1997.³³ A recent systematic review shows that there is only limited data from intervention studies on obesity showing inconclusive results.³⁴ Small trials report effects of interventions on beneficial change in body mass index in adolescence, but these interventions do not seem suitable for large scale use by for instance youth health care workers in The Netherlands.³⁵ In The Netherlands, an observational evaluation of preventive action with regard to obesity, and unhealthy behavior in general, through youth health care intervention has not been very promising.³⁶ Still, the young adolescent body mass index was in our overall model a predictor of later hypertension. This, combined with our finding that of all hypertensives in ARYA more than 65% had a body mass index of 25 kg/m² or more

at young adulthood, underscores that future efficacious interventions to prevent adolescent obesity also has the potential to lower the adolescent blood pressure. Even if efficacious risk lowering interventions in adolescence cannot be easily achieved, there may still be merit in early screening of cardiovascular risk. Detection of high-risk adolescents enables longer term monitoring and possibly later targeted lifestyle or drug interventions.

One major distinction between our study and many other studies is that we have used routinely collected data in adolescence for prediction of carefully measured hypertension in young adulthood. Another important distinction with other studies is that a discussion on the relevance of early life screening cannot be solely based on for instance hypertension as a surrogate endpoint. The primary goal of cardiovascular screening at any age is to ultimately reduce the risk of disease, to which hypertension is only one contributor. The fact that in ARYA women the Framingham risk scores were so much lower than in men is not surprising. These were 10-year risk scores, while in women the actual occurrence of heart disease will on average be later in life than in men.⁵ In the year 2000 in The Netherlands, there were 151.570 hospital admissions for heart disease in men and 110.551 for women. Of these, 44% were younger than 65 years, but 49% of men versus 38% of women.³⁷ Notably, the Framingham risk score that we used was constructed for the prediction of coronary heart disease, while a higher percentage of Dutch women, compared to men, will get cerebrovascular disease.³⁷ A risk score providing specific baseline hazards for young adults with follow-up for longer than 10 years is to our knowledge not available. This does in our view not discard the discussion on early life screening of cardiovascular risk factors in girls. Our findings do directly apply to Dutch youth health care practice and support in our view a new discussion about the relevance of blood pressure screening, particularly in girls. Nowadays, blood pressure is very easily measured, not necessarily by highly trained physicians, using automated devices at relatively low cost. According to our findings, such activity would detect in an early stage more than half of all young adult hypertensive women who are nowadays generally not detected at this age. Early detection and possibly intervention would likely contribute to the prevention of long term exposure to high blood pressure. Given our plea for a renewed discussion on blood pressure screening, our data may also support a discussion on the age at which youngsters are to be screened. In The Netherlands, youth health care is well organized with a good infrastructure. However, also from within this organization there are critical evaluations as many of its screening activities are not evidence based, while some are shown to be ineffective.³⁸

Given our findings and the fact that cardiovascular disease has a continuous large impact on individuals and on public health, we believe that youth health care should resume a discussion about blood pressure screening in adolescents.

5.2.8 References

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CHAPTER 6

General discussion

6.1 Introduction

The technical and medical ability to diagnose and treat manifest cardiovascular diseases have markedly improved in the previous century. However, the cardiovascular diseases are still the major cause of mortality in most western societies and an increasing health threat in developing countries, claim large parts of health budgets and are responsible for many quality adjusted years of life lost.^{1,2} Whereas advances in myocardial infarction treatment have predominantly contributed to this decline in mortality, a trade-off is a concurrent rise in hospitalizations for heart failure, also in The Netherlands.³ A thus continuing large impact on health commits us to next generations to search for new possibilities to reduce the burden of cardiovascular disease.

Atherosclerosis is the primary process responsible for the clinically manifest cardiovascular diseases. Postmortem studies and some studies in healthy adolescents indicate that this process already starts in childhood and is accelerated by cardiovascular risk factors, like high blood pressure, obesity and dislipidemia. A relatively new and potentially fruitful area of further research on cardiovascular risk reduction is birth weight.

Preventive strategies aim to lower the incidence of cardiovascular disease. In general, there are two complementary primary preventive strategies. The whole population approach aims to influence risk in all individuals of the population for instance by general lifestyle measures. While the population at large would strongly benefit from such measures, the individual member of this population will only marginally benefit. In contrast, the high risk approach aims to lower risk in those with high disease risks only. High risk preventive approaches have been successful in adults, particularly by lowering blood pressure and elevated cholesterol levels.⁴ A marked decline in mortality from coronary heart disease between 1980 and 1990 in the United States of America, could be attributed to treatment for 50%, to secondary prevention for 25%, and to primary prevention for 25%.² Prevention here refers to measures taken in adults. However, as coronary heart disease originates in childhood, the concept of prevention in childhood may be considered.

In this chapter I will discuss issues regarding etiology and predictive capacity of cardiovascular risk factors at a young age. The role of low birth weight as a putative cardiovascular risk factor is discussed. Next, the predictive ability of adolescent blood pressure for hypertension and future coronary heart disease risk is studied.

Fetal origins hypothesis

One decennium ago, the “fetal origins hypothesis” was postulated⁵, based on the finding that low birthweight as a possible indicator of an unfavorable intra-uterine environment, was associated with chronic disease in later life, particularly

cardiovascular disease. The hypothesis was that fetal undernutrition in critical phases of pregnancy may initiate fetal adaptations. These adaptations were suggested to optimize the fetus' survival resulting in a small birth size with the adverse consequence of an increased risk of chronic diseases in the long term. Initially, a shortage of intra-uterine nutrients was considered to be responsible for the adaptation programming of the fetus by affecting the differentiation of the hypothalamic centers.⁶ Even before this hypothesis evolved, there were observations to support it. For example, a Dutch famine study on children who were fetuses at different phases of pregnancy during the great famine in the second world war in 1944, showed that nutritional deprivation in the first half of pregnancy resulted in relative adiposity in young men.⁷ Recently, it was found that small variations in the balance of macro-nutrients in the maternal diet during gestation may adversely affect blood pressure.⁸

6.2 Fetal origins hypothesis revisited

Although many reports have subsequently provided support for the fetal origins hypothesis, there was also increasing criticism as to whether the relation between birth size and cardiovascular risk truly exists. Critics were concerned about selection bias, confounding variables, publication bias and statistical errors in supportive reports.⁹⁻¹¹ For instance, the impact of body mass on the relation between birth weight and blood pressure in the young was discussed. Some longitudinal studies reported an intermediate role for attained body size, as a causal link in the chain of events leading from low birth weight to later high blood pressure.¹² Thus, adjusting for attained body size, if indeed an intermediate factor, would be statistically unjustified. Others found that babies born with a low birth weight and a relatively high body size at later age, also called catch-up growth or accelerated postnatal body growth, developed the highest blood pressure levels.^{13,14} These studies suggest that catch-up growth may partially or fully explain the relation between birth size and increased blood pressure, rather than decreased prenatal body growth reflected in low birth size itself.

Also, critics questioned the very nature of the fetal origins hypothesis.¹⁵ Research showed that an unfavorable intra-uterine environment other than caused by an imbalance of nutrients also could affect birth weight and disease in later life. In particular, maternal factors may induce an adverse intra-uterine environment, due to toxins of cigarettes, pregnancy complications and diseases like pre-eclampsia, or maternal genes. For instance, newborns of smokers are smaller and have a higher blood pressure in later life.¹⁶ Women with an increased risk of ischaemic heart disease more often had preterm deliveries, pre-eclampsia and small newborns.¹⁷ Moreover, maternal hyperglycemia may modify the birth weight of the newborn.¹⁸

Alternatively, the genes of the fetus itself could directly affect birth weight and future adverse outcomes. This concept has been confirmed by the finding that non-carriers of a polymorphism in the promoter region of the insulin-like growth factor-I (IGF-I) gene was related to low birth weight and increased risk of diabetes type 2 and myocardial infarction.^{19,20} Also, a glucosekinase mutation of the newborn which causes hyperglycemia in later life, has been associated with a decreased birth size.¹⁸

Moreover, an interaction between genetic factors of the fetus and an unfavorable intra-uterine environment caused by maternal factors, may underlie the nature of the fetal origins hypothesis.

Findings in the ARYA study

Our analyses described in chapters 4.1 and 4.2 appear to generally support the fetal origins hypothesis. They confirm previous findings that birth size may be related to future cardiovascular risk, but also show that birth weight may be less important for elevated blood pressure than excess change to a relative high body weight in later life. In chapter 4.1 we used the analytical recommendations of Lucas to assess the impact of body size.⁹ We observed a relation between low birth weight and increased blood pressure. Also, a change of body mass from adolescence to young adulthood was more strongly related to systolic blood pressure than birth weight. Similarly, body mass index levels at young adulthood were stronger related to adult blood pressure than birth weight.

Our additional analyses expand current knowledge, that a particular risk group may be those who have excessively grown to high body mass coming from (too) low birth weights. The interaction model from Lucas suggests that birth weight modifies the effect of body mass index on blood pressure, more in particular, that de-tracking of body weight to high levels would lead to high blood pressure. Indeed, those in our study with the lowest birth weights who de-tracked to the highest body mass index at young adulthood were part of those with the highest systolic and diastolic blood pressure levels. However, from additional analyses we infer that a relation between birth weight and blood pressure seems to be restricted to those with high attained body mass; it was absent at lower body mass index levels. This would mean that there is no graded interaction between birth weight and body mass index, but that only babies who are light for their constitution or constitutionally small babies and become relatively heavy later are at risk of hypertension. Thus, it appears that (too) low birth weight babies, through high attained body mass index as an intermediate process, are likely to develop high blood pressure. A more likely explanation seems to be that such babies are particularly vulnerable to develop high body mass in young adulthood, with high blood pressure as a mere consequence of the latter.

In chapter 4.2 we explored the existence of the fetal origins hypothesis, by using an aggregation of current risk factors as a comprehensive measure of cardiovascular

risk at young age. Obviously, clinically manifest cardiovascular disease cannot be studied in the young, while single and individual risk factors only partially describe the cardiovascular risk. In almost all previous reports on this subject only one cardiovascular risk factor in the young adult was studied. We feel that assessment of early determinants in relation to total cardiovascular risk is essential. In this chapter we describe a relation between low birth size and an increased Framingham risk score²¹ at young adulthood. The Framingham risk score uses several cardiovascular risk factors - such as age, blood pressure, lipids, diabetes and smoking – in combination to predict the 10-year risk of coronary heart disease accurately.

Furthermore, we examined the genetic explanations as an alternative for the fetal origins hypothesis. By way of example, we determined a polymorphism in the promoter region of the IGF-I gene which has recently been described as a possible modulator of birth size and risk of cardiovascular disease. In chapter 3.1, homozygous carriers of this polymorphism showed a more favorable profile of cardiovascular risk factors in adolescence and young adulthood, compared to heterozygous carriers and non-carriers. A relation between the polymorphism and arterial wall thickness or stiffness could not be found at this young age. Contrary to previous reports, no relation was observed between this polymorphism and birth size, suggesting that the polymorphism may not directly influence fetal growth.

However, when we further investigated the genotypes of the young adults in relation to the birth weight of their first newborns, we found that homozygous maternal and paternal carriers of the polymorphism in the promoter region of the IGF-I gene, had offspring with a decreased body weight (chapter 3.2). This latter report supports the view that parental genotype may influence fetal growth and subsequently the risk of cardiovascular disease in later life.

Comment

Data in this thesis provide some support to the fetal origins hypothesis, but also modifies the initial concept. In chapter 4.1 we show that a decreased birth size is associated with increased blood pressure levels at young adulthood, but is restricted to young adults with a high attained body mass index. This suggests that the hypothesis pertains to a select group of subjects. In chapter 4.2, we expand the available evidence by using a cluster of several individual cardiovascular risk factors as outcome variable, predicting 10-year risk of coronary heart disease. We observed that low birth size is associated with an increased risk score. However, we could not study the impact of body size in the relation between birth size and the Framingham risk score, as in chapter 4.1. The Framingham risk score is calculated by a formula which has included several risk factors. Although body mass is not one of them, the future cardiovascular risk modified by body mass index is taken over by these other

risk factors.²¹ Adjusting the relation between birth weight and the risk score for body mass index, and studying whether the association was restricted to particular subjects, is therefore difficult to interpret.

Next, our results suggest that the fetal IGF-1 gene is not directly involved in the fetal origins hypothesis, but parental IGF-1 genes may. These findings merit further research on genetic and environmental causes. For instance, we investigated only one single polymorphism on the promoter region of the IGF-I gene. It was previously shown that other mutations located near the IGF-I gene also affect birth weight and cardiovascular risk²², and other genes are described to be associated as well.¹⁵

In support of the current criticism, we consider the relative impact of birth weight on blood pressure in later life limited. We only found support for the hypothesis in particular subjects. In fact, an increased cardiovascular risk, i.e. high blood pressure, was limited to those who were born small and developed the highest body mass index. It is not known which mechanism is responsible for the latter finding. It may be that a child that is born (too) small is more vulnerable for excessive weight gain than babies born with a normal or heavy birth size. Perhaps overcompensatory mechanisms play a role in this vulnerability, for instance concerning the parental nourishment of (too) small babies. A second criticism is that the relation with blood pressure seems predominantly modified by postnatal body growth. Similarly, body mass index at later age is stronger related to blood pressure, than birth weight to blood pressure. These data indicate that postnatal risk factors, such as excessive change of body size and increased body mass index at later age, mainly explain the relation between birth weight and cardiovascular risk. Therefore, postnatal risk factors at young age seem to provide better tools for preventive strategies than birth weight.

6.3 Cardiovascular risk prediction in children

Several long-term follow-up studies have shown clear associations between elevated blood pressure level in late adolescence or young adulthood, and cardiovascular morbidity and mortality.²³⁻²⁸ This indicates that hypertension at young adulthood may be regarded as a surrogate outcome when studying cardiovascular risk in children for whom such long-term studies do not yet exist. Follow-up studies such as the Muscatine study and the Bogalusa Heart study, have shown that blood pressure levels measured in childhood and adolescence are positively associated with blood pressure in early adulthood.²⁹⁻³¹ Combining several biennial blood pressure measurements improved these associations.³²⁻³⁴ The finding that an elevated adolescent blood pressure is related to hypertension and thus increased cardiovascular risk at young adulthood, suggests that adolescents with a high risk of

future cardiovascular disease may be selected by measuring their blood pressure level.

However, the Muscatine and Bogalusa Heart study are epidemiological studies using similar research settings and measurements for each participant. Whether the results can be extrapolated to medical health care in general, remains to be determined, as practice itself generates variation. More in particular, it is unknown whether routinely measured blood pressure in children is sufficiently predictive for later hypertension and cardiovascular risk, to justify large-scale screening of blood pressure in adolescence. The ARYA study was designed to shed more light on these issues.

The Dutch health care system and ARYA study

In The Netherlands, virtually all children regularly visit the facilities of the Home Care Organizations and Municipal Health Service, starting at four weeks old until leaving secondary school at the age of 16 to 19 years. This system may provide a unique opportunity to screen subjects at young age and select those with an elevated risk of cardiovascular disease.

The ARYA study consists of two Dutch cohorts with predictor data from youth health care practices. The cohort of the Municipal Health Service of the city of The Hague was set up for specific research purposes and consists of standardized and biennial data on cardiovascular risk factors obtained at adolescence. The cohort of the city of Utrecht consists of routinely measured data from birth to late adolescence. At young adulthood, several cardiovascular risk factors were re-measured in participants of both cohorts. This provided the data to study the relative impact of routine versus standardized blood pressure measurement in the prediction of future risk of hypertension and the individual 10-year probability of coronary heart disease of young adults. The 10-year probability was assessed by calculating the Framingham risk score²¹ which clusters the impact of several cardiovascular risk factors.

Findings of the ARYA study

The findings presented in chapter 5.2 show that a single routine systolic blood pressure measurement in young adolescent girls predicts hypertension in young adulthood. In other words, measuring blood pressure once and non-standardized at adolescence distinguishes the female young adults with and without hypertension in the future. Adolescent screening of girls would select 60% of future hypertensives and some 25% will be false positives. This indicates that blood pressure screening in girls may be effective. In young adolescent boys, systolic blood pressure does predict hypertension, in statistical terms. However, the ability to adequately distinguish male young adults with and without hypertension is limited. This suggests that there is little

practical relevance of blood pressure screening in boys, at least at the age of 13 years.

We used as a second endpoint an assessment of the cardiovascular risk profile in young adulthood: the 10-year risk of coronary heart disease calculated on the basis of a Framingham risk score. Expectedly, the top 20% of this risk distribution consisted of males only, while only 3 women exceeded the 70th percentile. Therefore, we could only assess in men the relation between adolescent blood pressure and 10-year risk of coronary heart disease. A statistically significant association was found between an increased systolic blood pressure in adolescent boys and an increased risk score at young adulthood. Moreover, adolescent blood pressure in boys could distinguish young men with a 10-year risk score above and below the 95th percentile. This suggests that blood pressure screening to assess cardiovascular risk may not only be important in girls, but also in boys.

Comment

Currently, the only factors of cardiovascular relevance that are obtained by school doctors of the Dutch Municipal Health Services are gender and anthropometry as both were associated with cardiovascular risk in ARYA. In retrospect, both the introduction of blood pressure screening and its termination some years ago by Municipal Health Services were not based on accurate scientific evidence about cardiovascular risk screening in children. The results in chapter 5.2 indicate that the Municipal Health Service might reconsider the importance of screening for blood pressure as it predicts risk of hypertension in girls and coronary heart disease in boys. Obviously, an actual re-introduction of blood pressure screening requires more information for Municipal Health Services than health benefits alone (see Future research).

To appreciate the results of chapter 5.2, some issues need to be further addressed. Our findings show that blood pressure screening in boys aged 13 years had no relevance in predicting hypertension at young adulthood. It is possible that this relation was not observed due to less stabilized blood pressures of boys, compared to girls aged 13 years. In the Utrecht cohort, girls were more sexually mature at this age than boys, which may partly underlie this gender discrepancy. Therefore, blood pressure screening in somewhat older boys - at late adolescence - may prove to be worthwhile. The fact that only male adults had a high 10-year coronary heart disease risk was the reason to study its relation with adolescent blood pressure in boys only. This does not exclude the existence of a relation between adolescent blood pressure and risk of coronary heart disease in women. The cardiovascular risk in women increases after the fourth decennium in life, and this may result in an increased 20-year or 30-year Framingham risk score. If so, studying the relation between adolescent blood pressure and 20 or 30-year risk of coronary

heart disease in women would be indicated. However, to our knowledge, 20 or 30-year hazards are as yet not available in the literature.

6.4 Future research

The large impact of cardiovascular morbidity and mortality should be decreased worldwide, and improvement of primary prevention at young age could help to lower the burden. Studies on early determinants of cardiovascular risk presented in this thesis, show that the prediction capacity of cardiovascular risk by small birth size is low, as the relation is of limited magnitude and probably mainly explained by postnatal catch-up growth. Also, we show that blood pressure at adolescence predicts hypertension in female young adults and seems to predict coronary heart disease in men. Because of the latter, a renewed discussion on the introduction of blood pressure screening at adolescence may be worthwhile. However, several further questions concerning large-scale screening need to be answered.

First of all, in the ARYA studies the adolescent prediction of high risk of cardiovascular disease was solely based on blood pressure. Predictions based on more elaborate adolescent cardiovascular risk profiles might be investigated as well. Body mass index is of interest in that respect as it is a rational target for preventive interventions and is related to blood pressure and lipids. Another possibility to improve prediction, is to obtain additional data such as parental hypertension or a parental history of cardiovascular disease and the lipid and glucose levels at adolescence. However, most parents of the adolescents, are aged between 30 and 50 years and have no manifest cardiovascular disease or are in a subclinical stage. Nevertheless, children of the limited number of young parents with manifest cardiovascular disease are at increased risk themselves. Collecting further information about risk factors in school health care is hampered because invasive procedures are required to collect lipid and glucose levels at adolescence. This is in our view not applicable in large-scale screening programs, unless it could be shown that such measurements have marked additional predictive value. Nevertheless, such invasive procedures might be justified in the high-risk adolescents already selected by elevated blood pressure levels.

The prediction of cardiovascular risk may be further improved by more knowledge about prediction at different ages. We already mentioned that a blood pressure measurement in boys older than 13 years of age may improve the prediction of future hypertension.

Furthermore, we might optimize the knowledge about the best risk estimate at young adulthood to be predicted at adolescence. In the ARYA study we have used cardiovascular risk profiles and arterial wall properties in young adulthood as estimates of the risk of cardiovascular disease occurrence decades later. Such risk

estimates are commonly used in studies in the young in order to prevent to follow-up these young adults, for observing the actual disease to occur. However, although there are studies showing that high blood pressure levels at late adolescence and young adulthood are related to cardiovascular mortality, it is relevant to provide evidence about blood pressure in children and adolescents and future cardiovascular events. Like for young adult hypertension it is known that vascular damage measured by arterial wall thickening and stiffness in subjects above 35 years of age is related to cardiovascular mortality. Data suggest that such a relation likely exists at younger ages also, and ARYA participants were only slightly younger. However, studies might be performed to confirm the relation between vascular damage measured at younger ages with later cardiovascular events. It might also be attempted to find out whether a combination of classical cardiovascular risk factors and measurements of vascular damage provides a better predictor of cardiovascular morbidity.

From the point of view of mere disease prevention, our findings suggest that a renewed discussion on screening of blood pressure in adolescents may be worthwhile. However, this discussion cannot be solely supplied by data concerning health implications. These implications must be in balance with the economical costs. A particular question is whether nationwide screening of adolescents is more cost-effective than the current Dutch policy of case finding of for instance hypertensive adults. One strong argument for early nationwide adolescent screening is that it would probably detect a substantial number of future hypertensives that would otherwise be missed, as there are no national cardiovascular risk screening programs for adults in The Netherlands. Intervention on determinants of high adolescent blood pressure, particularly overweight and obesity, will lead to direct benefit of the child, as it will not only reduce cardiovascular risk directly but also risk of other diseases such as diabetes mellitus or diseases of the locomotor system. Information on cardiovascular risk in adolescence may be helpful in changing lifestyle behavior such as weight reduction which is shown to be currently difficult to achieve in the school health care setting.³⁵ Early effective intervention may be taken to reduce life-long exposure to high levels of risk factors. This in itself will expectedly contribute to a reduction of the occurrence of cardiovascular disease. A more practical argument for screening is that nowadays blood pressure is very easily measured using automated devices at relatively low cost, not necessarily by highly trained personnel. Thus, there are a number of positive arguments for screening adolescent risk. However, whether it will be more cost-effective than current case-finding practice in adults is a question that is still to be answered. A first step that we would consider is to find out at what economical costs young adolescent hypertensives would be detected, that would otherwise be missed by the current health care system.

Alongside economical costs, there are obviously other unintended effects of screening. Some 25% of girls in our study were wrongly predicted to be future hypertensives, while 40% of true hypertensives were not predicted as such in adolescence. One problem with implementation of cardiovascular screening in early life is that it concerns disease that will on average not occur before 4 to 5 decades later in life. However, the proposition that such disease can already be predicted in childhood should in our view lead to more awareness in school doctors and pediatricians that cardiovascular disease originates to a large extent in childhood. Therefore, it is important to show an etiological relation between adolescent blood pressure and actual damage of vessel walls in the young (chapter 5.1). Such a relation shows that early screening of blood pressure does not concern some remote disease, but concerns a process leading to disease that is already going on in youth. In our view, neither right nor wrong predictions should lead to stigmatization as high-risk children. Much more at issue here is that physicians should, by prediction, be helped to take evidence based decisions about intervention strategies, which may well include further monitoring of blood pressure. The latter implies that decisions about active interventions can be delayed until further insight in the development of blood pressure is obtained in an initially designated high-risk child. Early screening will obviously miss a substantial part of later true hypertensives. While an inherent problem of almost any type of screening activities, a solution will have to come from gain in predictive precision. Such gain may be derived from a reduction of measurement error, repeated measurements at much larger time interval for the purpose of monitoring high risk (early and late adolescence) and careful planning of the developmental stage of children at which measurements are performed. Another solution to missing high risk children is a population approach implying that general measures in childhood concerning healthy lifestyles will be taken. Given a recent worrisome rise in The Netherlands of the number of overweight and obese individuals, particularly including children, the necessity of such large scale measures can hardly be neglected.

Summarizing, even a simple routine measurement in childhood predicts the most important disease in our society. We feel that our findings support a renewed discussion with Dutch Municipal Health Services about the relevance of screening of blood pressure in adolescence. There are still several issues that have to be studied to further improve the predictive accuracy of cardiovascular risk and further information is required about cost-effectiveness of screening. ARYA findings justify such further endeavors towards early life prevention of cardiovascular disease.

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CHAPTER 7

Summary

Samenvatting

Dankwoord

List of publications

Curriculum vitae

Early determinants of cardiovascular risk in the young: Two Dutch cohorts.

Cardiovascular disease is the major cause of death in westernized societies, responsible for 35.5% of the total mortality in The Netherlands. Also, it claims large parts of health budgets and is responsible for many quality-adjusted years of life lost. Cardiovascular disease is based on thickening and hardening of the arterial walls – a process named atherosclerosis – and becomes clinically manifest after the fourth decade in life. The technical and medical abilities to diagnose and treat manifest cardiovascular diseases have markedly improved in the previous century. However, a trade-off is a concurrent rise in hospitalizations for heart failure, also in The Netherlands. The continuing marked impact on health commits us to next generations to search for new possibilities to reduce the burden of cardiovascular disease.

Preventive strategies aim at lowering incidence of cardiovascular disease. High-risk preventive approaches have been successful in adults, in particular for lowering blood pressure and cholesterol levels. The marked decline in mortality from coronary heart disease between 1980 and 1990 in the United States of America, has been attributed for 50% to treatment, for 25% to secondary prevention and for 25% to primary prevention. So far, prevention has primarily been targeted on adults. However, the roots of cardiovascular disease are in early life. Consequently, the possibilities for prevention in childhood may be considered.

There are several cardiovascular risk factors which may contribute to the early onset and early development of atherosclerosis. Low birth weight and an adverse intra-uterine environment are two possible new risk factors. However, the causality of the relation between birth weight and cardiovascular risk is heavily discussed and the presence of selection bias, confounding variables, publication bias and statistical errors have been proposed as alternative explanations. Furthermore, results from epidemiological studies suggest that childhood blood pressure may predict adult blood pressure. Yet, it is unknown whether adolescent blood pressure as routinely measured in school health care, predicts hypertension or cardiovascular risk in adulthood sufficiently accurate to warrant blood pressure screening.

To explore the early determinants of cardiovascular risk in adolescence and young adulthood, data of the Atherosclerosis Risk in Young Adults (ARYA) study was used (chapter 2). This observational study is based on data in young adults around 30 years of age selected from the general population, and comprises two Dutch cohorts. All children in The Netherlands, regularly visit the Home Care Organizations and Municipal Health Services at birth, childhood and adolescence for routine examinations. The findings are recorded in school health records. In the Utrecht

cohort 750 subjects participated. Members of this cohort were invited when birth weight and blood pressure were available in records from the youth health care practice. The second cohort – the cohort of The Hague - included 262 young adults. During secondary school, they participated in a longitudinal study on cardiovascular risk factors coordinated by the Municipal Health Service of The Hague. The adolescents were biannually measured in a standardized way. Recently, cardiovascular risk factors were remeasured in all young adults of both cohorts. Additionally, in the cohort of Utrecht the extent of early vascular damage was measured by two non-invasive techniques.

Since the nineties, there has been ample debate regarding small size at birth as a marker for an increased risk of cardiovascular disease. This concept is also known as the “fetal origins hypothesis”. It is thought that a fetus changes its physiology to adapt to an adverse nutritional environment, primarily to optimize the fetus’ survival, with unfavorable consequences occurring on the long term. However, critics suggested that, among others, the underlying cause of a relation between birth size and cardiovascular disease is genetic. Recently, a study showed that a polymorphism in the promoter region of the insulin-like growth factor-I (IGF-I) gene, is related to both low birth weight and increased risk of cardiovascular diseases. Therefore, we studied in chapter 3.1 the relation between a functional polymorphism in the promoter region of the IGF-I gene, birth weight and cardiovascular risk in adolescence and young adulthood. Homozygous carriers of the polymorphism show a more favorable profile of cardiovascular risk factors and an increased height in adolescence and young adulthood, compared to heterozygous carriers and non-carriers. No relation between the IGF-I gene and birth size nor with arterial wall thickness or stiffness could be found. This indicates that the IGF-I gene may be associated with increased cardiovascular risk factor levels at young age. Contrary to the ARYA study, in which birth weight was not an intermediate factor in the relation between the polymorphism and cardiovascular risk, it has been suggested in previous studies that parental genes do underlie the fetal origins hypothesis. In chapter 3.2 we examined the association between a polymorphism in the promoter region of the IGF-I gene in young parents and their first child’s birth weight. The first born children of non-carrier parents weighed on average 584 gram lower than of homozygous parents. This was especially found in the newborns of the female participants in the ARYA study. This present report provides evidence for the view that parental genotype may influence fetal growth of their newborns.

A second criticism on the “fetal origins hypothesis” is that an inverse relation between birth weight and blood pressure artificially results from adjustment for attained body size. When body size is an intermediate factor rather than a confounder, the

adjustment may induce error. In chapter 4.1 we further analyzed the impact of body mass index. The analysis of our data, as proposed by other investigators, seems to support the fetal origins hypothesis, as low birth weight appear to be associated with increased blood pressure. However, additional analysis showed that the relation is restricted to those with low birth weight and high attained relative body weights. In agreement with other studies, we observed that an unfavorable intra-uterine environment may be less important for future hypertension than an excess gain in body weight in later life.

Reports in the young have shown that low birth weight is associated with increased levels of particular cardiovascular risk factors. However, to our knowledge, no studies have reported on the relation between birth size and the overall cardiovascular risk profile, the most comprehensive measure of risk. In chapter 4.2 we describe that a lower birth size is continuously related with an adverse risk profile in young adulthood, supporting the fetal origins hypothesis. A standard deviation decrease in birth weight (540 g) was associated with one excess coronary heart disease event in 1000 subjects.

Postmortem studies showed that the subclinical process of atherosclerosis starts already in childhood, and was more pronounced in the young with increased risk factor levels, among which blood pressure is one of the main modifiable. However, these studies are limited in healthy young subjects. Therefore, we studied adolescent blood pressure levels and blood pressure tracking into young adulthood in relation to vascular damage (chapter 5.1). Vascular damage was measured by the common carotid intima-media thickness, a marker for generalized atherosclerosis. Systolic blood pressure and pulse pressure at adolescence and arterial thickening were positively associated, independent of blood pressure, gender, age and body mass index. Furthermore, adolescents of whom the systolic blood pressure and pulse pressure tracked above the medians into young adulthood had the thickest arterial walls. These findings strengthen the evidence that atherosclerosis develops at early age and that the development is enhanced by elevated blood pressure.

Epidemiological studies have suggested that childhood blood pressure predicts adult blood pressure. It is, however, unknown whether adolescent blood pressure as routinely measured in school health care, predicts hypertension or total cardiovascular risk in adulthood sufficiently accurate to warrant blood pressure screening. In chapter 5.2 we show that adolescent blood pressure in boys and girls predicts hypertension 15 years later. Measuring the blood pressure once, multiple times, routinely or standardized did not affect the data. A single routine blood pressure measurement in young adolescent girls could distinguish 60% of the hypertensive women at young adulthood. In young adolescent boys, blood pressure did not distinguish young adults with and without hypertension, but an elevated blood

pressure predicted 10-year cardiovascular risk in young adulthood as estimated by the cardiovascular risk profile.

In chapter 6 we conclude that there is evidence to support the fetal origins hypothesis. The direct effect of a small birth weight is, however, too limited to justify preventive strategies only targeted at the intrauterine environment. The impact of postnatal change of body size and attained body mass index at young adulthood seems to have a bigger impact on future cardiovascular risk.

Furthermore, we confirm relations between elevated blood pressure at adolescence and hypertension at young adulthood. In addition, in girls the adolescent blood pressure is able to distinguish those with and without future hypertension. Thus, blood pressure screening at adolescence could select those with an increased risk of cardiovascular disease. The Municipal Health Service may therefore reconsider the option to re-introduce the blood pressure measurement at adolescence, to assess future cardiovascular risk.

Vroege determinanten van cardiovasculair risico bij jongeren: Twee Nederlandse cohorten.

Cardiovasculaire ziekten zijn de belangrijkste oorzaak van sterfte in westerse landen. In Nederland zijn deze ziekten verantwoordelijk voor 35.5% van de totale sterfte. Ook eisen cardiovasculaire ziekten grote delen van het budget voor gezondheidszorg op, en zijn ze verantwoordelijk voor vele jaren met verminderde kwaliteit van leven. Cardiovasculaire ziekten worden primair veroorzaakt door verdikking en verharding van de arteriële vaatwanden, een proces dat atherosclerose wordt genoemd. De ziekten manifesteren zich klinisch meestal na het vierde decennium. De technische en medische mogelijkheden om cardiovasculaire ziekten in de kliniek te diagnosticeren en te behandelen zijn sterk verbeterd in de vorige eeuw. Echter, de keerzijde is een stijging van het aantal hospitalisaties door hartfalen, ook in Nederland. Deze voortschrijdende en grote invloed op de gezondheid is een belangrijk argument om te zoeken naar nieuwe mogelijkheden om ook het ontstaan van cardiovasculaire ziekten te verminderen.

Preventieve maatregelen kunnen bijdragen aan het verlagen van de incidentie van cardiovasculaire ziekten. In volwassenen is de hoog risico benadering succesvol gebleken, voornamelijk door een verlaging van het bloeddruk en cholesterol niveau. Een opvallende sterftedaling door coronaire hartziekten tussen 1980 en 1990 in de Verenigde Staten, is voor 50% toegeschreven aan behandeling, voor 25% aan secundaire preventie en voor 25% aan primaire preventie. Tot nu toe zijn preventieve maatregelen voornamelijk toegepast bij volwassenen. Echter, de oorsprong van cardiovasculaire ziekten begint al op jonge leeftijd. Preventie bij kinderen zou daarom overwogen kunnen worden.

Er zijn verschillende cardiovasculaire risicofactoren die bijdragen aan een vroege start en ontwikkeling van atherosclerose. Het geboortegewicht en daaraan gerelateerd de intra-uteriene omgeving is mogelijk een nieuwe risicofactor. Niettemin wordt er hevig gediscussieerd over wat de werkelijke oorzaak is van de relatie tussen geboortegewicht en cardiovasculair risico. Er is geopperd dat selectie bias, confounding, publicatie bias of statistische fouten alternatieve verklaringen kunnen zijn voor de relatie. Verder suggereren epidemiologische studies dat de bloeddruk, gemeten bij kinderen, de bloeddruk op volwassen leeftijd voorspelt. Het is echter onbekend of een routinematig gemeten bloeddruk door de Jeugdgezondheidszorg, hypertensie of cardiovasculair risico bij volwassenen voorspelt en voldoende accuraat is om op bloeddruk te screenen.

Om vroege determinanten van cardiovasculair risico bij adolescenten en jongvolwassenen te onderzoeken, werden de gegevens van de Atherosclerosis Risk in Young Adults (ARYA) studie geanalyseerd (hoofdstuk 2). Deze observationele

studie bestaat uit 2 Nederlandse populatiecohorten en is gebaseerd op jongvolwassenen in de leeftijd van rond de 30 jaar. Alle inwoners van Nederland worden vlak na de geboorte, als kind en als adolescent door de Thuiszorg en de Jeugdgezondheidszorg routinematig onderzocht. De medische gegevens worden in statussen genoteerd. In het cohort van Utrecht namen 750 jongvolwassenen deel. Ze werden uitgenodigd als het geboortegewicht en een bloeddruk tijdens de adolescentie genoteerd stond in de status van de Jeugdgezondheidszorg. Het tweede cohort, het cohort van Den Haag, bestond uit 262 jongvolwassenen. Tijdens de middelbare school namen zij deel aan een longitudinale studie over cardiovasculaire risicofactoren, gecoördineerd door de afdeling Jeugdgezondheidszorg van de GG&GD Den Haag. Als adolescent werden zij twee keer per jaar op een gestandaardiseerde manier onderzocht. Recent werden de cardiovasculaire risicofactoren bij de jongvolwassenen in beide cohorten weer gemeten. Tevens werd in het cohort van Utrecht de vroege vasculaire schade bepaald met behulp van twee niet-invasieve technieken.

Sinds de negentiger jaren zijn er veel discussies geweest over een laag geboortegewicht als marker voor een toegenomen risico op cardiovasculaire ziekten. Dit fenomeen is ook wel bekend als de “fetal origins hypothesis”. Er wordt verondersteld dat een foetus zijn fysiologie aanpast aan zijn slechte voedingsomgeving, in eerste instantie om zijn eigen overleving te optimaliseren, maar met ongunstige consequenties op de lange termijn. Echter, critici suggereren dat bijvoorbeeld ook de genen de relatie tussen een geboortegewicht en cardiovasculaire ziekten verklaren. Recent toonde een studie aan dat een polymorfisme in de promotor regio van het insulín-growth factor-I (IGF-I) gen, geassocieerd is met zowel een laag geboortegewicht als met een verhoogd risico op diabetes type 2 en hartinfarct. Daarom bestudeerden wij in hoofdstuk 3.1 de relatie tussen het polymorfisme in de promotor regio van het IGF-I gen, geboortegewicht en cardiovasculair risico bij adolescenten en jongvolwassenen. Homozygote dragers van het wildtype polymorfisme toonden een verbeterd profiel van cardiovasculaire risicofactoren en een toegenomen lichaamslengte tijdens adolescentie en jongvolwassenheid, vergeleken met de heterozygote dragers en degenen die het wildtype polymorfisme niet droegen. Een relatie tussen het polymorfisme, geboortegewicht en arteriële wanddikte en wandstijfheid werd niet aangetoond. Dit geeft aan dat het polymorfisme geassocieerd kan zijn met een verhoogd cardiovasculair risico op jonge leeftijd, maar dat geboortegewicht geen intermediaire factor lijkt te zijn in deze relatie. In tegenstelling tot de ARYA studie, waarbij het geboortegewicht geen intermediaire factor bleek te zijn in de relatie tussen het polymorfisme en cardiovasculair risico, is er gesuggereerd dat de genen van de ouders de fetal origins hypothesis kunnen verklaren. In hoofdstuk 3.2 onderzochten

wij de associatie tussen een polymorfisme in de promotor regio van het IGF-I gen bij jongvolwassen ouders en het geboortegewicht van hun eerstgeboren kind. De eerstgeborenen van de ouders die het wildtype polymorfisme niet droegen, wogen gemiddeld 584 gram minder dan van de homozygote ouders. Deze relatie werd vooral gevonden bij de eerstgeborenen van een vrouwelijke deelnemer van de ARYA studie. Deze bevinding levert aanwijzingen voor de visie dat het genotype van de ouders de foetale groei van hun kinderen kan beïnvloeden.

Een tweede punt van kritiek op de foetale origine hypothese is dat een inverse relatie tussen geboortegewicht en bloeddruk artificieel kan ontstaan door te corrigeren voor lichaamsomvang. Wanneer lichaamsomvang een intermediaire factor is in plaats van een confounder, kan het corrigeren een statistische fout induceren. In hoofdstuk 4.1 analyseren wij de invloed van body mass index nader. Wanneer wij de data analyseerden zoals voorgesteld door andere onderzoekers, steunden de resultaten in het algemeen de foetale origine hypothese, aangezien een laag geboortegewicht geassocieerd was met een verhoogde bloeddruk. Echter, de relatie tussen een laag geboortegewicht en een verhoogde bloeddruk bleek alleen aanwezig te zijn bij jongvolwassenen met een hoog lichaamsgewicht. In overeenstemming met andere studies toonden wij ook aan dat een ongunstige intra-uteriene omgeving minder belangrijk is voor de ontwikkeling van hypertensie, dan een buitensporige toename van de lichaamsomvang op latere leeftijd.

Artikelen over jongeren hebben aangetoond dat een laag geboortegewicht geassocieerd is met verhoogde niveaus van individuele cardiovasculaire risicofactoren. Er zijn echter aan ons geen studies bekend die een relatie tussen het geboortegewicht en het cardiovasculair risicoprofiel beschreven. In hoofdstuk 4.2 beschrijven wij dat een kleine geboorteomvang geassocieerd is met een verhoogd risico op coronaire hartziekten bij jongvolwassenen. Dit ondersteunt de foetale origine hypothese. In de onderzoeksgroep was een daling van één standaarddeviatie van het geboortegewicht (540 gram) geassocieerd met één geval van coronaire hartziekte op de 1000 jongvolwassenen.

Postmortale studies toonden aan dat het subklinische proces van atherosclerose al op jonge leeftijd begint, en vooral aanwezig was bij jongeren met meerdere risicofactoren. Echter, het aantal studies bij jonge en gezonde personen is beperkt. Daarom bestudeerden wij de bloeddrukniveaus bij adolescenten en tracking van bloeddruk van adolescentie naar de jongvolwassenheid, in relatie tot vasculaire schade (hoofdstuk 5.1). Vaatschade werd gemeten door de intima-media dikte van de grote halsslagader, een marker voor gegeneraliseerde atherosclerose. De systolische bloeddruk en polsdruk bij adolescenten waren positief geassocieerd met arteriële wandverdikking, onafhankelijk van de bloeddruk bij jongvolwassenen,

geslacht, leeftijd en body mass index. Ook blijken de adolescenten bij wie de systolische bloeddruk en polsdruk boven de mediaan bleef tijdens adolescentie én jongvolwassenheid, de dikste arteriële wanden te hebben. Deze bevindingen bevestigen dat atherosclerose zich al op jonge leeftijd ontwikkelt en dat de ontwikkeling versterkt wordt door een verhoogde bloeddruk.

Epidemiologische studies suggereren dat de bloeddruk gemeten bij kinderen, de bloeddruk op volwassen leeftijd voorspelt. Het is echter onbekend of een door de Jeugdgezondheidszorg routinematig uitgevoerde bloeddrukmeting tijdens de adolescentie, hypertensie of cardiovasculair risico voorspelt bij jongvolwassenen en voldoende accuraat is om te screenen op bloeddruk. In hoofdstuk 5.2 tonen wij aan dat een adolescente bloeddrukmeting bij meisjes en jongens, geassocieerd is met hypertensie 15 jaar later. Het eenmalig, meervoudig, gestandaardiseerd of routinematig meten van de bloeddruk veranderde de gegevens niet. Een eenmalige bloeddrukmeting bij meisjes maakte het mogelijk om 60% van de jongvolwassen vrouwen die hypertensie kregen te selecteren. Bij jongens maakte een bloeddrukmeting geen onderscheid tussen de jongvolwassen mannen met of zonder hypertensie, maar voorspelde wel het 10-jaars risico op coronaire hartziekten op jongvolwassen leeftijd.

In hoofdstuk 6 concluderen wij dat onze bevindingen de fetal origins hypothesis steunen. Het effect van een laag geboortegewicht is echter te beperkt om preventie alleen te richten op de intra-uteriene omgeving. De invloed van postnatale verandering van het lichaamsgewicht en het gewicht op jongvolwassen leeftijd, lijkt meer impact te hebben op het cardiovasculair risico in het latere leven.

Vervolgens tonen wij de relatie aan tussen een verhoogde bloeddruk tijdens de adolescentie en hypertensie bij jongvolwassenen. Bovendien kan de adolescente bloeddruk bij meisjes onderscheid maken tussen degene die wel of geen hypertensie ontwikkelen in de toekomst. Concluderend, screenen van bloeddruk tijdens de adolescentie maakt het mogelijk om jongeren te selecteren met een verhoogd risico op cardiovasculaire ziekten. De Jeugdgezondheidszorg kan met deze gegevens heroverwegen of zij de bloeddrukmeting tijdens de adolescentie zouden willen herinvoeren om toekomstig cardiovasculair risico te bepalen.

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Curriculum vitae

Lydia Esther Vos was born on July 21st, 1972, in Groningen, The Netherlands. She graduated from secondary school in 1990 at the Nijmeegse Scholengemeenschap Groenewoud in Nijmegen. In 1992 she obtained her propedeutics of Biomedical Sciences at the University of Leiden, and started Medical School at the University of Leiden. In 1996 she worked for 6 months in the laboratory of Immunogenetics and Transplantation at the Brigham and Women's Hospital, and Harvard Medical School, Boston, United States of America. She obtained her medical degree in February 1999. Thereafter, she worked for 5 months at the editorial office of the "Nederlands Tijdschrift voor Geneeskunde" (Dutch Journal of Medicine). In August of that same year she started the research project "Atherosclerosis Risk in Young Adults" described in this thesis, at the Division of Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht (supervised by Prof. dr. D.E. Grobbee and dr. C.S.P.M. Uiterwaal). She obtained her MSc degree in Clinical Epidemiology at The Netherlands Institute of Health Sciences, Erasmus University, Rotterdam, in August 2002. In November 2002 she started research at the Department of Dermatology, University Medical Center Leiden, on a multinational epidemiological project on human papillomaviruses, UV-light and non-melanoma skin cancers in transplant patients (supervised by dr. J.N. Bouwes Bavinck). In November 2003 she will start her training in Dermatology at the University Medical Center Leiden (supervised by Prof. dr. R. Willemze).

