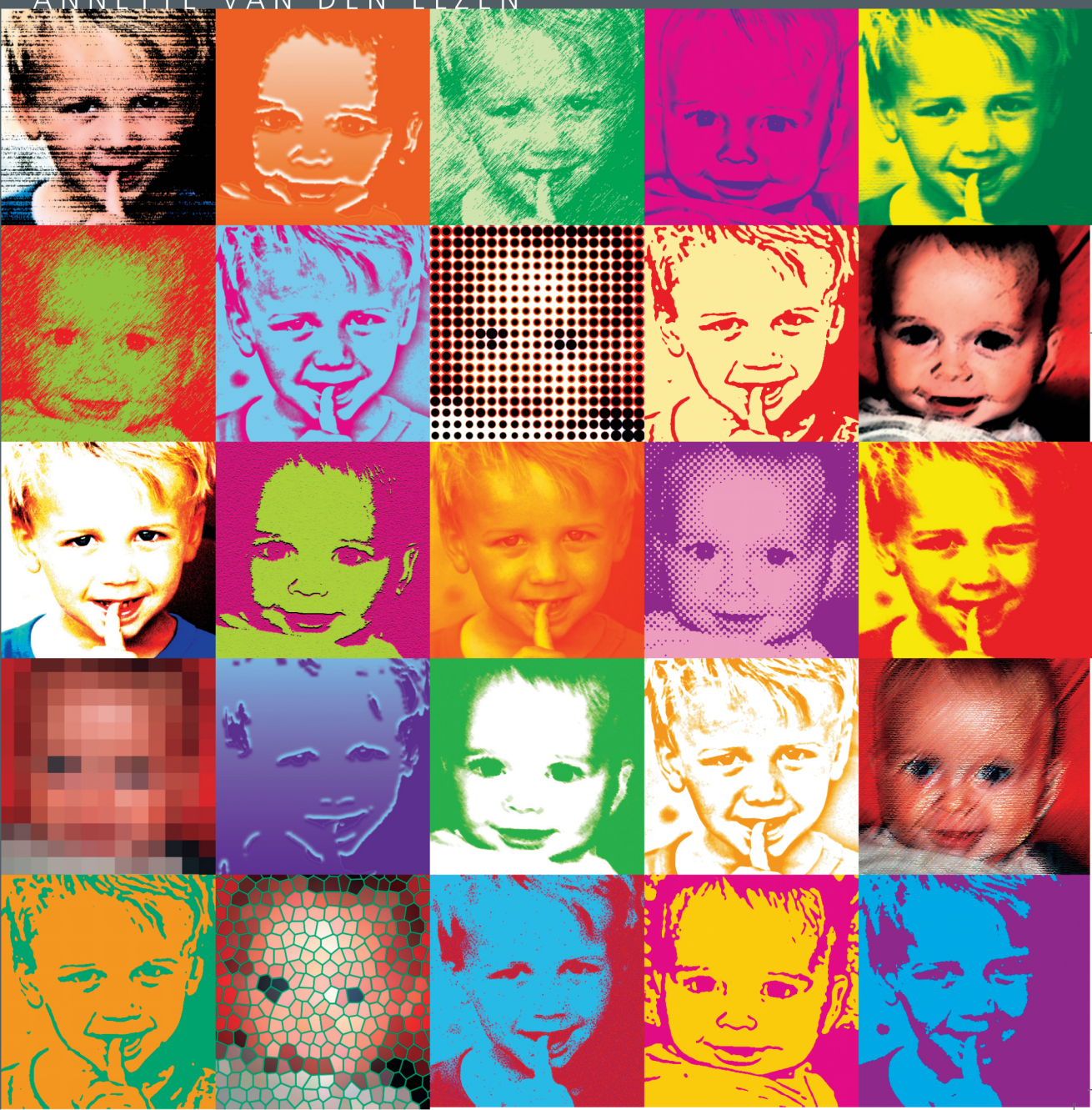
CHILDHOOD DETERMINANTS OF VASCULAR DAMAGE AND BODY MASS INDEX IN YOUNG ADULTHOOD

ANNETTE VAN DEN ELZEN



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CHILDHOOD DETERMINANTS OF VASCULAR DAMAGE AND BODY MASS INDEX IN YOUNG ADULTHOOD

Vroege determinanten van vasculaire schade en body mass index tijdens jong volwassenheid

PROEFSCHRIFT

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en volgens besluit van het College voor Promoties.

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CONTENTS

ABBREVIATIONS

BMI	body mass index
bp	base pairs
CCA	common carotid artery
CI	confidence interval
CVD	cardiovascular disease
DBP	diastolic blood pressure
EPOZ	Epidemiological Preventive Study Zoetermeer (Epidemiologisch Preventief Onderzoek Zoetermeer)
HDL	high-density lipoprotein
IGF-1	insulin-like growth factor-1
IMT	intima-media thickness
LDL	low-density lipoprotein
MAP	mean arterial pressure
PP	pulse pressure
PWV	pulse-wave velocity
SAS	Statistical Analysis System
SBP	systolic blood pressure
SD	standard deviation
SE	standard error

My heart leaps up when I behold
A rainbow in the sky
So was it when my life began
So is it now I am a man
So be it when I shall grow old,
Or let me die!
The Child is father of the Man

William Wordsworth (1770-1850)

LIST OF PUBLICATIONS

van den Elzen AP, Semmekrot BA, Bongers EM, Huygen PL, Marres HA.
Diagnosis and treatment of the Pierre Robin sequence: results of a retrospective clinical study and review of the literature.
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Families and the natural history of blood pressure. A 27-year follow-up study.
Am J Hypertens. 2004;17:936-40.

van den Elzen APM, Sierksma A, Oren A, Vos LE, Witteman JCM, Grobbee DE, Hendriks HFJ, Uiterwaal CSPM, Bots ML.
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Van den Elzen APM, Uiterwaal CSPM, de Ridder MAJ, Hofman A, Witteman JCM.
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Submitted.

voor papa

GENERAL INTRODUCTION

1

Throughout the past 50 years, cardiovascular disease (CVD) has been the leading cause of morbidity and mortality in most modern societies. In 2002, almost 50,000 persons died of CVD in the Netherlands, accounting for 33.7% of all deaths.[1] Aging of the population and improved survival after CVD events bear heavily on medical costs. In order to delay the development of atherosclerosis and hence decrease the incidence of CVD, it is essential to improve primary prevention of CVD. About four or five decades ago, the concept of risk factors was introduced, largely as a result of the work done in the Framingham Heart Study.[2, 3] Since then, extensive research has been done to examine the role of risk factors in the development of atherosclerosis and CVD. It has been established that one can successfully reduce the incidence of CVD by reducing or even eliminating risk factors for this disease. In addition, the decrease in the age-adjusted cardiovascular mortality rate, that is seen from the 1970s onwards, can be attributed to improved treatment, but also to secondary prevention and primary prevention, each accounting for a 25% decrease in CVD mortality.[4] Many successful preventive campaigns have aimed at creating more awareness among adults and drawing attention to people's own responsibility concerning their body and health. In the current view, the best way to reduce one's risk for CVD is to live as healthy as possible by exerting regular physical activity, refraining from smoking, consuming alcohol only moderately and by a healthy diet.[5] Furthermore, much research has been devoted to gaining insight into less traditional factors that contribute to the initiation and progression of CVD. For example, in recent years, inflammation is recognized as a putative cause of atherosclerosis.[6]

Yet, despite these important successes and new insights into the pathogenesis of CVD in the past years, unfavourable trends in some coronary risk factors, including increasing rates of smoking, obesity[7] and decreasing levels of physical activity,[8] have contributed to a slowing of the rate of decline in age-adjusted mortality from CVD. Recently, it has been suggested that a proper preventive strategy requires interventions targeting children and young adults.[9] Already from conception, genetic factors, environmental factors and cultural factors emerge that influence eventual heart health (see figure 1-1). For example, parents play an important role in the development of the risk factor profile of their children, not only genetically but also environmentally. Children share their parents' behaviour e.g. eating habits, exercising, smoking and drinking habits.

LIFE

(from the perspective of risk to heart health)

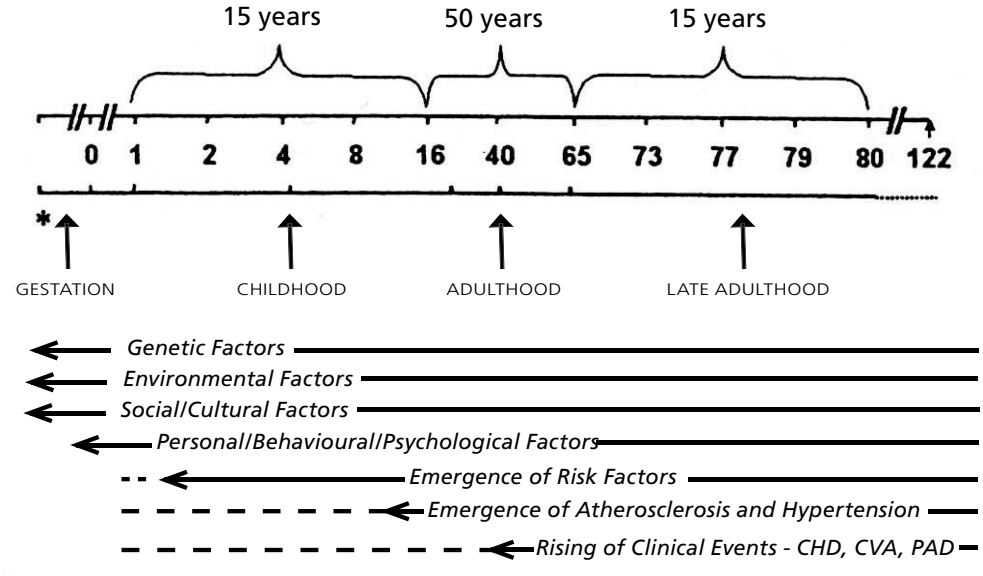

Figure 1-1: Lifetime factors influencing heart and vascular health.
**: conception; CHD: coronary heart disease; CVA: cerebro vascular accident; PAD: peripheral artery disease.*

Development of atherosclerosis

The complex chronic 'disease' of the arteries, which eventually may lead to clinical CVD, is called atherosclerosis. Atherosclerosis includes thickening and hardening of the arterial walls and may be initiated early in life.[10] At the start, healthy tissue is injured due to exposition to a variety of damaging factors. Endothelial dysfunction results in fatty streaks, which are formed by an increase of intracellular lipids, smooth muscle cells and multiplying macrophages. This initiation of potentially reversible fatty streaks already

occurs in childhood and adolescence (figure 1-2).[11, 12]
When the process of damaging persists, fatty streaks increase in size and extent, resulting in so-called atherosclerotic plaques. Initially, these plaques are fibrous, covering macrophages, lipids and debris. Longstanding damage can lead to rupture of vulnerable plaques, resulting in sudden thrombus formation that further occludes the vessel lumen and leads to ischaemia of the organ involved.[13] Thus, atherosclerosis has a long preclinical phase before ischaemic symptoms appear. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study investigators first reported on the occurrence of early atherosclerotic lesions in adolescents in association with cigarette smoking and high cholesterol levels.[14]

Primary prevention in early life

Primary prevention programs in younger individuals might help to overcome the cardiovascular epidemic by postponing atherosclerotic development, and by that retarding the clinical manifestation of CVD.[15] The association of reduced growth rates in fetal life and infancy with increased death rates from CVD poses the question of what processes link the two. Raised blood pressure increases the risk of coronary heart disease and stroke, and is one obvious link because there is already good evidence that it originates in childhood.[16-19] The persistence of rank order in the distribution of blood pressure among subjects examined at time intervals – so called 'tracking' – has been repeatedly observed in longitudinal studies of children as well as adults.[20-24] Besides fetal and infant growth, genetic factors may influence the development of CVD and its risk factors from early life onwards. Gene-environment interaction may play an important role in ultimate development of CVD. Regarding early environmental factors, it is important to gain insight into how parental cardiovascular risk factors influence the cardiovascular risk profile of their offspring.

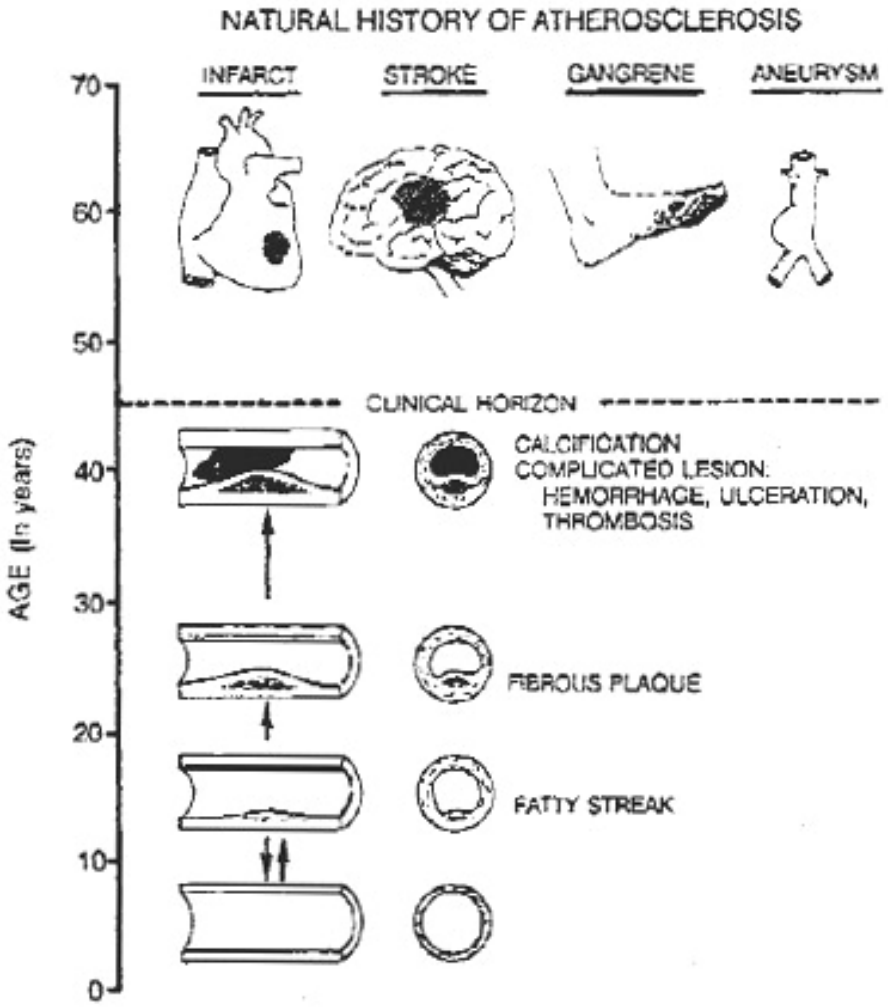

Figure 1-2: Natural history of atherosclerosis, showing progressive arterial occlusion and resultant health effects. Reproduced from McGill et al.(1963).

Development of overweight

In adults and children, the consequences of a decrease in physical activity and an increase in the intake of calories are more and more visible over the past years. At this time, 36% of the Dutch population is overweight and 12% is obese.[25] The United States of America still show the highest percentages of overweight and obese subjects, 56 % and 22% respectively. Data from the US Centers for Disease Control and Prevention (CDC) (see figure 1-3) show the dramatic increase in overweight children during the past 25 years: the proportion of overweight 6-11 year olds doubled and the proportion of obese 6-11 year olds rose eightfold.[26]
Overweight appears to influence the cardiovascular risk profile already at young ages. Over 60% of overweight children have at least one additional risk factor for CVD, such as hypertension, hyperlipidemia or hypercholesterolemia.[27] Enduring overweight is thought to be related to the development of an unfavorable cardiovascular risk profile in adulthood.

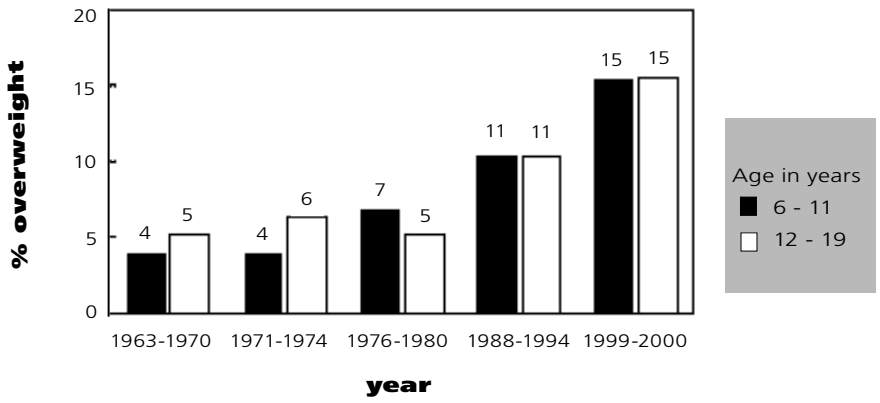

Figure 1-3: Prevalence of overweight in children and adolescents aged 6-19 years in the United States.
Overweight is defined as body mass index at or above age- and gender-specific 95th percentile body mass index cut-off points from the 2000 CDC Growth Charts, United States. Reproduced from CDC.[26]

Outline of this thesis

Identification of determinants of cardiovascular risk factors in childhood and adolescence might create a basis for targeted early preventive action. Only few long-term prospective population-based studies are conducted among children.[28-32] The Epidemiological Preventive Study of Zoetermeer (the EPOZ study) is a unique prospective study on cardiovascular risk factors for its many repeated measurements of body mass index, blood pressure and cholesterol from childhood into adulthood during a follow-up of 27 years. The study aims at gaining more insight into the determinants of early atherosclerosis, cardiovascular risk factors and the development of overweight in young adulthood. In chapter 2 of this thesis, the rationale and the design of the EPOZ study are described in detail. Chapter 3 covers the impact of parental blood pressure and body mass index on offspring cardiovascular risk and vascular damage. Chapter 4 focuses on childhood determinants of eventual vascular damage in young adulthood. Chapter 5 discusses the consequences of an insulin-like growth factor (IGF)-1 gene polymorphism on birth size, body mass index and atherosclerosis and describes the role of birth size in the development of levels of cholesterol from childhood into adulthood. Finally, in the general discussion (chapter 6), the main findings of this thesis are considered in the context of current scientific knowledge. Furthermore, relevant methodological aspects are discussed, and suggestions are made for future research in this field.

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THE EPOZ STUDY: RATIONALE AND DESIGN

2

Introduction

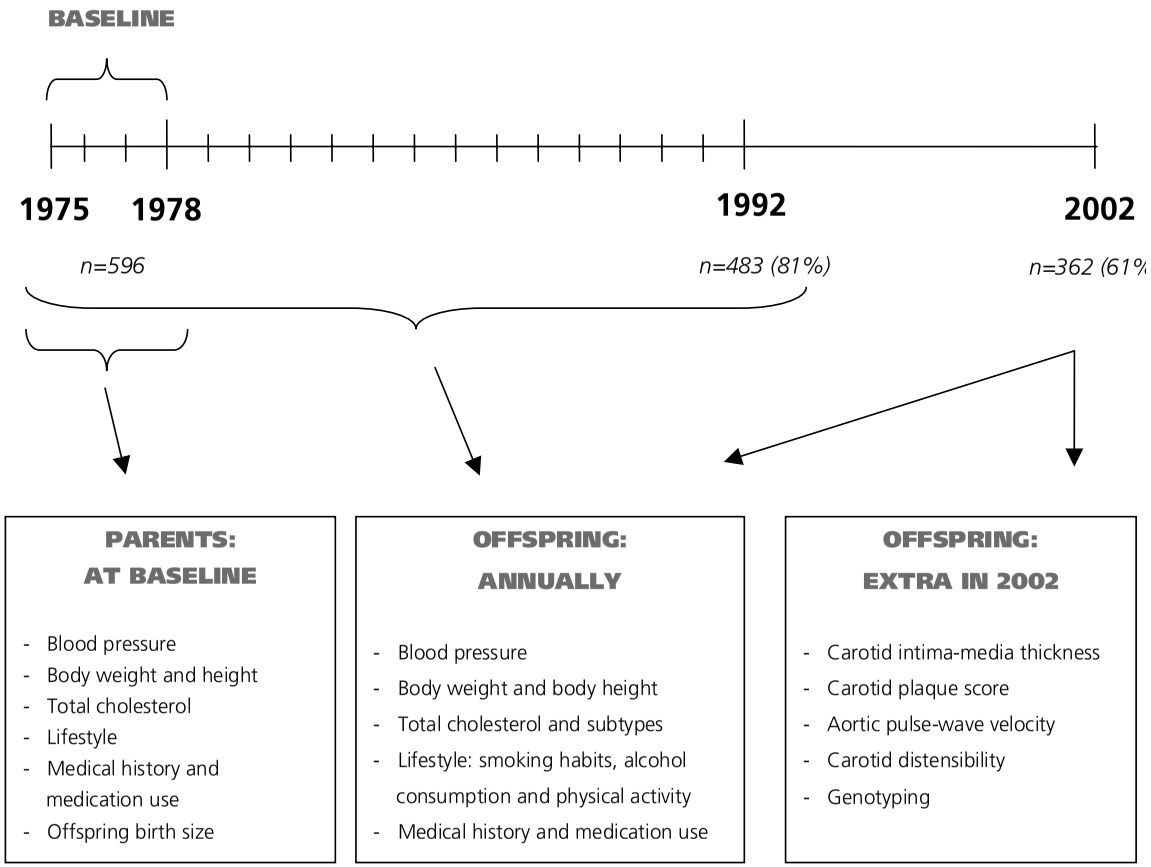
Few prospective population-based studies have been conducted among children.[1-4] Knowledge on the role of risk factors measured already during childhood in the development of adult disease could be important concerning early primary prevention. Especially cardiovascular morbidity and mortality remains a huge health problem in the industrialized countries and is rapidly increasing in the rest of the world. Intervention at the level of the known cardiovascular risk factors, especially at young ages, might be the only solution to the cardiovascular disease epidemic. For this purpose, the predictive value of risk indicators needs to be established at young ages. The Epidemiological Preventive Study of Zoetermeer (EPOZ study) was started in 1975. All persons aged 5 years or over and living at the same address in 2 districts of the town of Zoetermeer, the Netherlands constituted the heart of this study. Subsequently, this study was continued prospectively in 5-19 year olds. In 2002, measurements of vascular damage were added to the usual measurements of cardiovascular risk factors (EPOZ 2002 study) (figure 2-1). The objective of the EPOZ 2002 study was to evaluate the early determinants of cardiovascular risk factors, subclinical vascular damage, and overweight in healthy young adults.

Methods

Study population and selection procedure

Between April 15th 1975 and June 15th 1978, all families with children aged between 5-19 years who were living in two districts in the Dutch town of Zoetermeer (one urban district, Palenstein and one rural district, Dorp), were invited to participate in a cross-sectional population-based study on risk-indicators for chronic diseases (EPOZ study). Zoetermeer is a suburban residential community of at that time about 55,000 inhabitants which is situated near The Hague in the Netherlands. Ultimately, of the potential 13,461 persons drafted, 10,533 persons participated at baseline (response 78,2%). Non-response (21,8%) was mainly caused by primary refusal (15%), migration (2%) en objections on religious grounds (1.5%). Non-response was highest among the 15-19 year olds and among elderly (aged 65+). Women were somewhat more willing to participate than men and primary attendance was 5% higher in the urban district compared to the

Figure 2-1: Measurements in the EPOZ follow-up study



rural district. Of all persons aged 5-19 years, 4,649 (82%) took part in the EPOZ-study. From this group, a random sample of 596 children was selected for annual follow-up in a study on the natural history of cardiovascular risk factors and their determinants. After inclusion, participants were invited annually to visit the research center in Zoetermeer in the same month of the year, at the same time of the day between 17.00-21.00 hours. Between 1975 and 1993, participants visited the research center annually. In 2002, all subjects were invited for an examination that included measurements of atherosclerosis. In total, 362 persons participated in this examination. The median number of visits is 15 (range 2-19). Median follow-up time for the EPOZ 2002 study is 23 years. Response for the majority of visits gradually declined to 83% in 1993. For the atherosclerosis measurements in 2002, the response was 61%. The EPOZ study was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam. All participants gave written informed consent at baseline and in 2002.

Cardiovascular risk factors

Blood pressure was measured using a Hawksley random-zero sphygmomanometer (Lancing, Sussex) according to a standardized protocol[5] on the left brachial artery of the participant sitting after a resting period of 15 minutes. A cuff of 23 cm by 10 or 14 cm was used, depending on the arm circumference. The largest cuff was usually used in a child over 10 years of age. Diastolic blood pressure was taken at the 5th Korotkoff phase. The mean of two consecutive measurements was used in the analyses. Between 1975 and 1993 the same research assistant performed all blood pressure measurements. In 2002 three research assistants performed the measurements.

Body height was measured to the nearest 0.1 centimetres using a Seco stadiometer. The measurements were taken without shoes in upright position, with the heels touching and with the head in Frankfurt plane.[6] Body weight was measured to the nearest 0.1 kg using a Seco digital balance. These measurements were taken with the participants standing in wearing only light underwear and no shoes. BMI was calculated as body weight(kg)/body height(m)^2.

Information on smoking and alcohol consumption, medical history and medication use was obtained through questionnaires. In addition,

women were asked about use of oral contraceptives, menstrual cycle and pregnancies.

Laboratory analysis

Blood samples were drawn by antecubital venipuncture, applying minimal stasis, using a 21 gauge Butterfly needle with tube (Surflo winged infusion set, Terumo).

Cholesterol

Serum total cholesterol at baseline was measured with an automated enzymatic method[7] and from 1983, with a modified reagent (Syswell, CHOD/PAP High Performance, Boehringer Mannheim, Germany). In the transition period both reagents have been used simultaneously, obtaining an excellent correlation ($r>0.99$). The overall coefficient of variation was 2.5% at baseline and 2.3% during follow-up. Cholesterol determinations at follow-up were performed on serum samples stored at -20°C for up to 4 years. Repeated measurements performed by our laboratory in frozen serum showed no significant changes up to 4 years after sampling compared to frozen samples measured within one week after venipuncture. The standard deviation of these duplicate serum cholesterol measurements did not exceed 3.0% in all instances and did not show a significant drift. In 2002, serum total cholesterol was determined by an automated enzymatic procedure using Hitachi 911 Roche CHOD-PAP reagent kit. A correlation study in 201 samples between both methods (Syswell versus Hitachi) showed a mean decrease of 1.2% ($y=0.996x-0.0519$).
Measurements of high-density lipoprotein (HDL)-cholesterol were started in 1979 and low-density lipoprotein (LDL)-cholesterol in 1984. HDL- and LDL-cholesterol were measured by the same method after precipitation. For HDL-cholesterol we used the phosphotungstate method according to Burstein[8] with a minor modification as described by Grove.[9] LDL-cholesterol measurement was carried out with polyvinylsulphate (Boehringer Mannheim, FRG). In 2002, HDL-cholesterol was measured with the Hitachi 911 Roche direct HDL-cholesterol assay using PEG-modified enzymes and dextran sulphate. For HDL-cholesterol measurements, a correlation study between the manual Syswell method and the automated Hitachi 911 was carried out in 96 persons. Compared to the old method, HDL-cholesterol levels increased

with approximately 7% measured with the Hitachi 911 (y=1.0684x+0.0082). In 1994, shortly after the Hitachi 911 method was introduced, Roche introduced the first generation homogenous liquid HDL-cholesterol en by this mean HDL-cholesterol level decreased by 3% (y=0.9451x+0.0356). Summarizing, HDL-cholesterol values measured in 2002 are on average 5-6% higher compared to HDL-cholesterol levels in 1975.
All measurements were carried out in the laboratory of the Department of Epidemiology & Biostatistics at the Erasmus MC, Rotterdam, the Netherlands, which from 1978 participated in the lipid standardization program of the World Health Organization (WHO) Regional Lipid Reference Centre in Prague, Czechoslovakia (Dr. D. Grafnetter), and from 1979 in the Dutch National Cholesterol standardization program (KCA foundation), initiated in analogy to the program of the CDC Lipid Standardization Laboratory in Atlanta. In addition, during the baseline period quality control was indirectly checked on the CDC protocol by monthly comparison with cholesterol determination using the Abell-Kendall method (Gaubius Institute, TNO, Leiden).[10] Both accuracy and precision of total cholesterol and HDL-cholesterol measurements were within acceptable limits (CDC/WHO) over the entire period. All automated analyses were initially carried out on a Technicon Auto Analyzer-II system (Technicon Instruments, Tarrytown, New York, USA) and from 1989 on a Kone Specific Analyzer (Kone Instruments, Espoo, Finland) using frozen (-20°C) serum samples. From 1987, HDL_2-cholesterol and HDL_3-cholesterol subfractions in serum were assayed as described by Gidez et al[11] with slight modifications and separated using stepwise precipitation of apolipoprotein B containing lipoproteins with heparin/Mn^{2+} in two steps and HDL_2 with dextran-sulphate.

Insulin-like growth factor-1 polymorphism

DNA was isolated for insulin-like growth factor (IGF)-1 genotyping at the last visit in 2002. Polymerase chain reaction was performed using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine (CA) repeat 1 kb upstream of the human IGF-1 gene.[12] 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'-GCTAGCCAGCTGGTGTTATT-3'), 0.25 mmol/l 2'-dNTP, 2.2 mmol/l $MgCl_2$, 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 labelled 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).

Parents

Parental data of the participants was obtained at baseline (1975-1978). Body weight and height, systolic blood pressure, total cholesterol and HDL cholesterol were measured using the same protocols as in the offspring. Parental information on alcohol consumption, smoking, medication use and medical history was obtained through questionnaires filled in by the parents.

Collecting birth data

To obtain birth data, a questionnaire was sent to the parents of the children at baseline between 1975 and 1978. Questions were asked about birth weight (gram), birth length (cm), placental weight (gram), gestational age, complications during pregnancy and whether or not the mother had smoked during pregnancy. In 2002, birth weight was asked again from the participants themselves via a questionnaire. In total, birth weight was known in 371 subjects (62%).

Parameters of atherosclerosis

Common carotid intima-media thickness (IMT)

Ultrasound principle and image

A transducer sends ultrasonic waves into human tissue in a pulsed manner. Part of the waves is absorbed by the various components of the tissue and part of them is reflected. From the characteristics of the reflected waves, a two dimensional (2-D) image of various structures of the tissue underlying

the transducer is constructed. When differences in acoustic impedance exist between various anatomic tissues, reflection occurs and the structures may be seen separately on the image. The acoustic impedance is determined by the density and the flexibility of the tissue. Since calcified tissue has high density and low flexibility it will reflect most incoming ultrasound waves and looks 'white' on the scan while blood seems 'black' on the scan because it hardly reflects any wave due to low density and high flexibility. When ultrasound waves travel through the human body, reduction of their intensity occurs due to absorption, scattering and reflection. Hence, echoes coming from deep structures will tend to be smaller in amplitude than those coming from surfaces close to the transducer. To compensate for this effect, most ultrasonic instruments use a time gain compensator to amplify distant echoes more than close echoes. Whether different structures can be identified on the ultrasound image depends on the resolution of the equipment used. To be able to distinguish between two structures that are both parallel to the sound beam, the axial resolution is most important. When the frequency of the transducer increases, the axial resolution will also increase. However, high frequencies limit the ability to visualize deeper structures. A transducer of 7.5 MHz appears to be a good compromise between the resolution and depth for the assessment of carotid IMT, resulting in an axial resolution of about 0.3-0.4 mm.[13]
On a longitudinal 2-D image of the carotid artery, the near wall and far wall are displayed as two bright white lines separated by a hypo-echogenic space, representing the lumen filled with blood (figure 2-2). The three layers (tunica intima, tunica media and tunica adventitia) reflect ultrawave sounds separately, as they differ in composition (see this chapter: Parameters of arterial stiffness - the vascular wall). The distance of the leading edge of the first bright line (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) indicated the IMT of the far wall. Correspondingly, IMT of the near wall was measured.

IMT as surrogate marker of atherosclerosis and CVD

Pathology studies have demonstrated that levels of traditional risk factors are associated with the extent and severity of atherosclerosis. However, at every level of risk factor exposure, there is substantial variation in the amount of atherosclerosis, presumably related to genetic susceptibility and the influence of other risk factors. Therefore, there has been interest in a technique that can both measure and monitor atherosclerosis and reflect the pathological

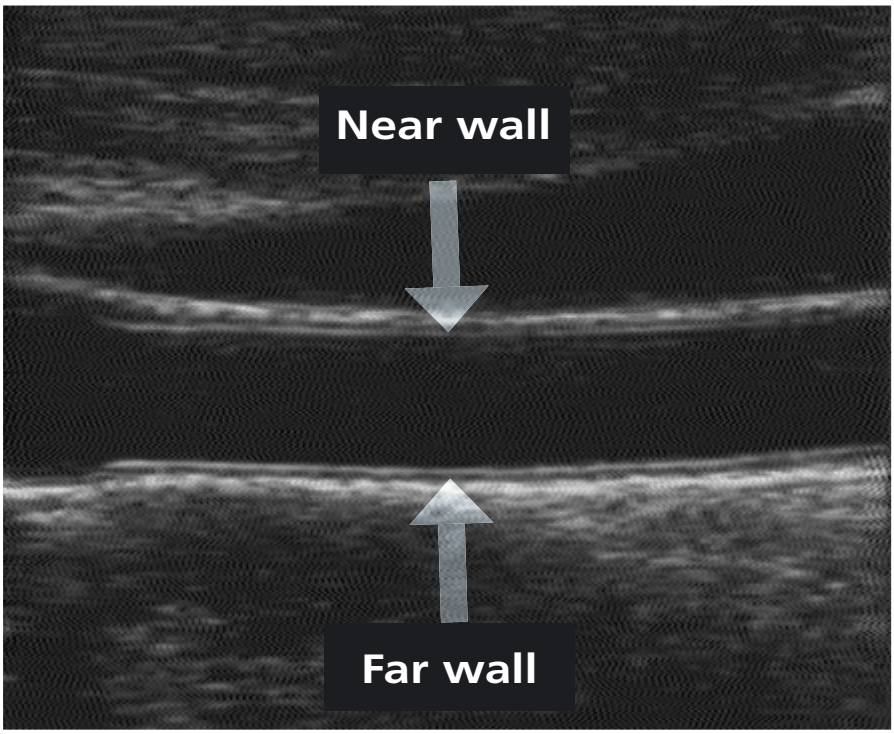


Figure 2-2: Longitudinal 2-D ultrasound image of the distal common carotid artery

endpoint of CHD risk factors. As the carotid arteries can be well visualized by ultrasonography, several population-based studies have used ultrasonographic measurements of the thickness of the carotid wall as a technique to identify and monitor subclinical atherosclerosis and to assess the predictive value for coronary artery disease. Chambless and colleagues in the Atherosclerosis Risk in Communities (ARIC) Study, related IMT measurements at baseline in 7,289 normal, healthy adults (aged 45 to 64 years) free from clinical coronary artery disease, to future coronary events during a four to seven year follow-up.[14] There was a positive association between mean carotid IMT and coronary artery heart disease. The strength of the relationship was reduced when controlled for major risk factors, but remained high in those whose baseline carotid IMT was highest. O'Leary

and colleagues studied an older population (age 65 and older) which addressed the association between IMT and the incidence of new myocardial infarction or stroke in 4,476 persons without clinical cardiovascular disease during a median follow-up period of 6.2 years.[15] For patients in the highest quintile of IMT at baseline, the accumulative risk for an event was 25% and 5% for patients in the lowest quintile of IMT. The yearly incidence of myocardial infarction or stroke increased with increasing quintiles for each of the measures of IMT. After adjusting for other risk factors the association between IMT and risk of a major event was 3.9 times higher for patients in the highest quintile. Hodis and colleagues followed 146 men aged 40 to 59 who had previous coronary artery bypass surgery.[16] IMT was measured every 6 months throughout a two-year study period and angiograms were performed at baseline and at two years. Average follow-up was 8.8 years. For each 0.03-mm increase per year in carotid IMT, the relative risk for myocardial infarction was 2.2 and the relative risk for any coronary event was 3.1. This study also showed that LDL cholesterol lowering drugs significantly reduce the common carotid artery IMT.

A limitation of carotid artery IMT as a surrogate for coronary artery disease is that it does not accurately assess the total atherosclerotic burden and therefore cannot predict the severity of coronary artery disease or distinguish patients with one-vessel, two-vessel, or more coronary artery disease. Given these limitations, more recent research has used the combined carotid artery IMT and femoral artery IMT measurements to more accurately determine atherosclerotic burden of the coronary arteries. A recent non-randomized study of 366 adults with known coronary artery disease was conducted to determine whether the addition of femoral artery IMT to carotid artery IMT would permit distinctions between subgroups, divided according to the severity of their underlying coronary atherosclerosis.[17] This study shows that the combined assessment of both carotid and femoral arteries may add to the ability to accurately estimate the total atherosclerotic burden and thus predict coronary artery disease severity. Further study is required before conclusions can be drawn concerning whether combined carotid/femoral artery assessment improves the health outcomes of patients with known or suspected coronary artery disease.

Thus, findings in the recent literature support the current policy on ultrasonographic measurement of the carotid IMT for assessing subclinical atherosclerosis.

IMT MEASURMENT IN THE EPOZ STUDY

The reading protocol that was used for the ultrasound measurements in the EPOZ study was developed within the Rotterdam Study.[13] B-mode ultrasonography of both the left and right carotid artery was performed with a 7.5 MHz linear array transducer using a Duplex scanner (ATL Ultramark IV, Advanced Technology Laboratories, Bethel, Washington, USA). The subject is in supine position with the head turned approximately 45 degrees in opposite direction. The ultrasound examination starts at the right carotid artery. An initial ultrasound scan is made showing a longitudinal view of the common carotid artery, the carotid bifurcation and the internal carotid artery. Then, a careful search is performed for the intima-lumen interface and the media-adventitia interface of the far wall of the distal common carotid artery. When an optimal longitudinal image is obtained, it is frozen on the R wave of the electrocardiogram. This procedure is repeated three times for three optimal 2-D images of the distal common carotid artery. For the bifurcation and the internal carotid artery one image showing the site with the largest distance between lumen-intima interface and media-adventitia interface, is frozen on the R wave of the electrocardiogram. The initial ultrasound scan and the frozen images are recorded on VHS videotape. The actual measurements of lumen diameter and IMT are performed offline using a procedure and additional dedicated software, that has been closely adapted from the Wallenberg Laboratory for Cardiovascular Research, Gothenburg, Sweden.[18] A frozen image that was stored on videotape is digitised and displayed on the screen of a Laser 286/2 personal computer using a frame grabber (VP 1400-KIT-512-E-AT, Imaging Technology). After calibration, two vertical lines are drawn on the digitised image, using a graphic XY tablet and a mouse (Summagraphics M II 1201). The distance between the two lines is set on 10 mm. The first vertical line is placed at the beginning of the dilatation of the distal common carotid artery, which serves as a reference point for the start of the measurement. With a cursor, the intima-lumen interface at the near wall and the lumen-intima interface and the media-adventitia interface at the far wall of the distal common carotid artery are marked over a length of 10 mm. Computer software calculates the mean values as well as maximal values for lumen diameter and the IMT. The average of the lumen diameter and the IMT of each of the 3 frozen images are calculated. For each subject a mean lumen diameter and a mean maximal IMT ((left + right)/2) are taken as a measure for current lumen diameter and wall thickness of the distal common carotid artery, respectively.

A similar procedure is followed for the measurement of the IMT of the carotid bifurcation and the internal carotid artery. As in these segments clear interfaces of 10 mm are not often seen, IMT is measured from the frozen images showing the site of the maximal distance between lumen-intima interface and media-adventitia interface.

Carotid Plaques

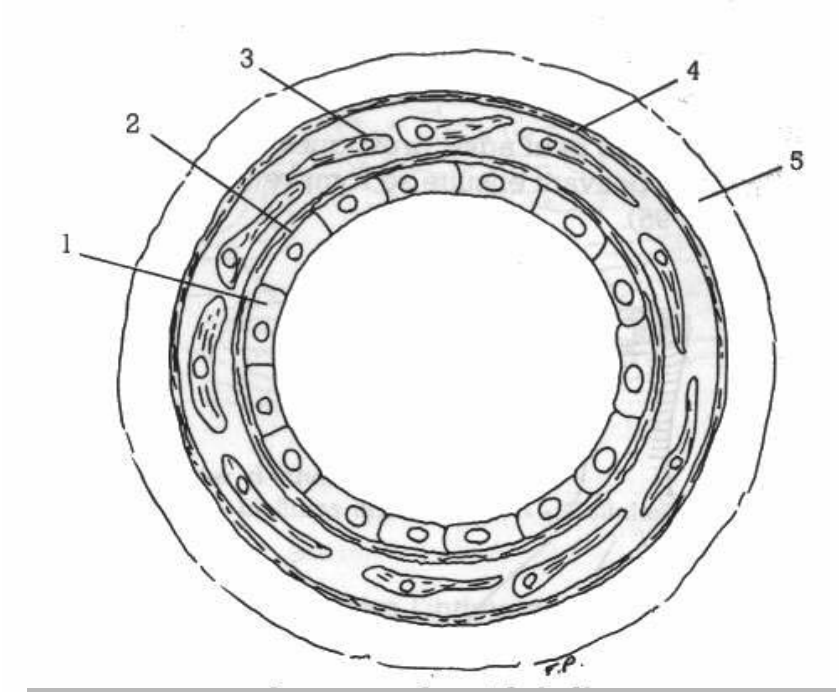
The presence of plaques in the carotid artery was assessed by evaluating the ultrasonographic images of the common, internal and bifurcation site of the carotid artery for the presence (yes/no) of atherosclerotic lesions. Plaques were defined as focal thickenings of the vessel wall of more than 50% relative to adjacent segments with protrusion into the lumen composed of either only calcified deposits or a combination of calcification and noncalcified material. Subsequently, the focal lesions were scored for presence of calcification and acoustic shadowing, homogeneity and size.

Parameters of arterial stiffness

The vascular wall

The arterial wall consists of 3 layers, from the lumen site to the outer site tunica intima, tunica media and tunica adventitia respectively (figure 2-3). Demarcation between the tunica intima and media is provided by te lamina elastica interna, a structure that consists of a fenestrated membrane of elastin lined on te intimal side by a coarse fibrous network. The lamina elastica externa demarks the tunica adventitia.
The inner layer of an arterial wall, the tunica intima, consists of vascular endothelium. The outer layer of an arterial wall, the tunica adventitia, is a region of collagen and some elastin tissue that merges with the surrounding connective tissue. The mid layer, the tunica media, consists of elastin and collagen fibers and smooth-muscle cells and forms the largest part of the arterial wall. The tunica media is the principal determinant of the mechanical properties of the arterial wall. The composition of the tunica media changes along the arterial tree. In the proximal aorta elastin is the principal

component while in the peripheral arteries collagen dominates. Because elastin is much more elastic than collagen, the arteries become stiffer with increasing distance from the heart.[19]



1 tunica intima
2 lamina elastica interna
3 tunica media
4 lamina elastica externa
5 tunica adventitia

Figure 2-3: Schematic view of an arterial wall in cross-section.

With increasing age, arteries stiffen as in the tunica intima, the endothelial cells become more irregular in size and shape, the subendothelial layer thickens and in the tunica media elastic fibers degenerate with an increase in collagenous fibers and calcium deposition. Thus, an artery is a viscoelastic tube, whose diameter varies with pulsating pressures due to contraction of the heart (figure 2-4). In addition to their conduit function, arteries also perform a cushioning function that transforms the pulsatile flow generated

by contraction of the left ventricle into steady flow at the periphery, enabled by the viscoelastic properties. These viscoelastic properties are frequently described in terms of compliance, distensibility or stiffness, which is the inverse of distensibility. The propagation velocity is determined by the elastic and geometric properties of the arterial wall and the characteristics of the fluid (blood density).[20] When the elastic vessel wall properties of the arterial system decrease and the artery becomes stiffer, the high pressure generated by the heart is no longer cushioned. This results in a higher proportion of the stroke volume that is forwarded directly to the peripheral circulation and by that to increased systolic blood pressure. The higher the stiffness, the lower the distensibility and the higher the velocity.

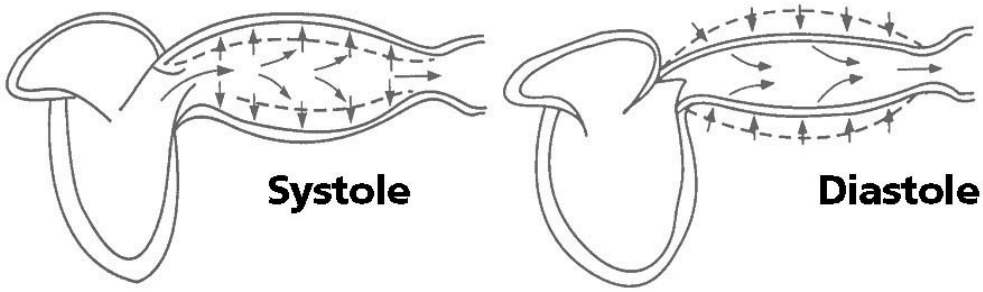


Figure 2-4: The effect of the pulse wave of the blood on the arterial wall. *Reproduced from www.complior.com.*

In conclusion, arterial stiffness plays a potential etiologic role in cardiovascular disease and may help to recognize arterial changes. Arterial stiffness may constitute an early risk marker.
The past decade, accurate methods to non-invasively measure arterial stiffness have become available. Two of the most frequently used methods are the measurement of pulse wave velocity over a certain part of the arterial tree and the measurement of changes in arterial diameter due to changes in arterial pressure over the cardiac cycle at one specific point in the arterial tree.

Aortic pulse wave velocity (PWV)

PWV is a simple noninvasive, accurate and highly reproducible method to assess arterial stiffness[21,22] that has been used for a long time.[23] It can be performed quickly (within 10 minutes) with relatively inexpensive equipment. In comparison to other methods for measuring arterial stiffness, using ultrasound or even magnetic resonance imaging (MRI), PWV requires a relatively simple computer with a software application and two connected tonometers. The measurement is easy to perform and does not require extensively trained personnel. Another advantage of carotid-femoral PWV is that it measures arterial stiffness over a large part of the arterial tree, thereby providing a measure of general stiffness of that part of the arterial tree. Other methods, which measure arterial stiffness at one specific point in the arterial tree, could have the disadvantage of that specific point not being representative of a larger arterial area.
One limitation of carotid-femoral PWV is that it combines measurement of elastic arteries (proximal aorta) with more muscular arteries (distal aorta, iliac artery and femoral artery), making it impossible to evaluate differences in elastic properties or differences in determinants of the process of arterial stiffening between elastic arteries and more muscular arteries.

PWV measurement in the EPOZ study

Participants were instructed to refrain from smoking, from drinking coffee, tea, alcohol and taking pain-medication on the day of measurement, and from drinking alcohol on the day before measurement. Carotid-femoral PWV was measured with subjects in supine position. Before measurement of PWV, blood pressure was measured twice with a sphygmomanometer after five minutes of rest and the mean was taken as the subject's reading. Mean arterial pressure (MAP) was calculated by the following formula: diastolic blood pressure + ⅓*(systolic blood pressure – diastolic blood pressure). Carotid-femoral PWV was assessed using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France)[21] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery (figure 2-5). The distance travelled by the pulse wave between the carotid artery and the femoral artery was 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 (b).[24] PWV was calculated as the ratio between the distance travelled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. The average of 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses. One limitation of the method used is a possible higher short-term variability as the PWV measurements are not performed on the same pressure waves.

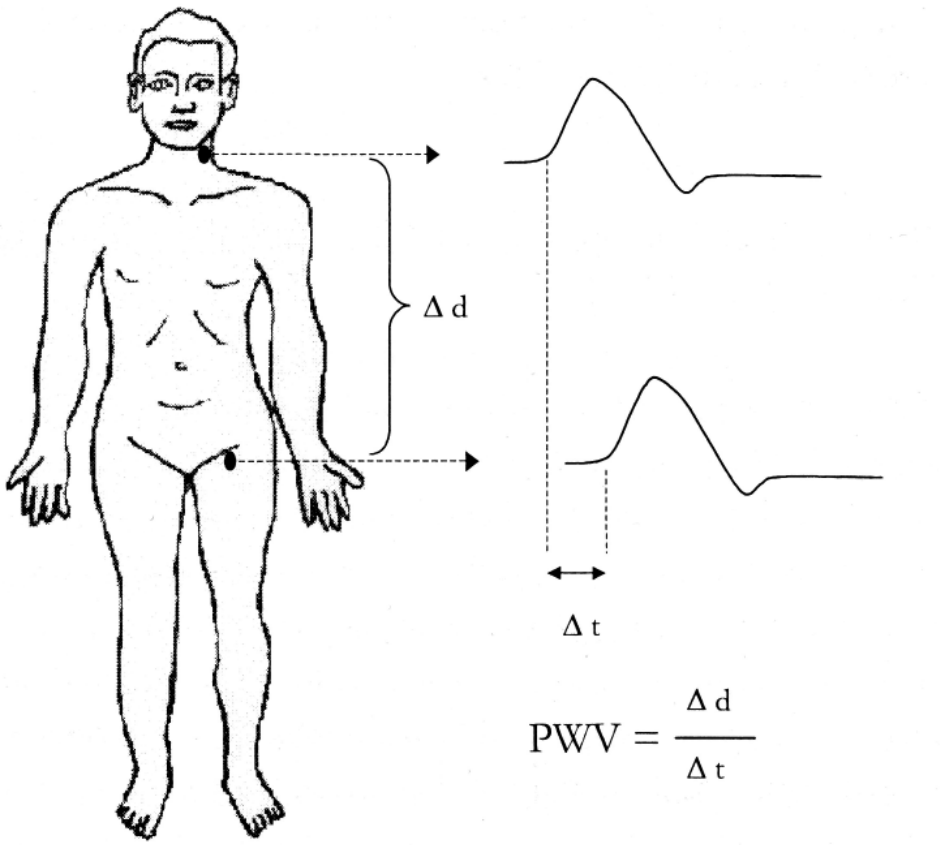
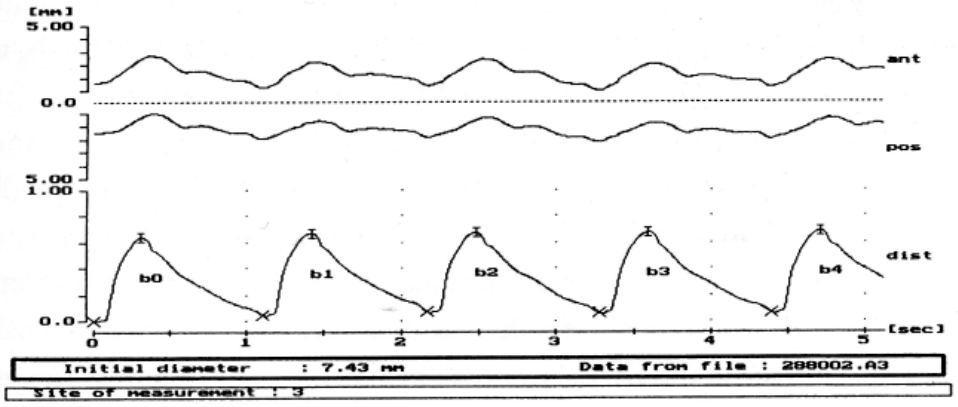


Figure 2-5: Measurement of aortic pulse wave velocity.
Reproduced from: Popele, NM van; Causes and consequences of arterial stiffness. An epidemiological approach (Thesis).[24]

Carotid distensibility

Common carotid distensibility was assessed with the subjects in supine position. The vessel wall motion of the right CCA was measured by means of a Duplex scanner (Ultramark IV, ATL, Bethel, Washington, USA) connected to a vessel wall movement detector system.[25,26] After five minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasonography. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two-selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings (figure 2-6).



beat	dist (µm)	diam (mm)	dist (%)	dA/A (%)	RR-int (ms)	rise-time (ms)	ECG-10% (ms)
0	637	7.43	8.58	17.88	1100	143	97
1	634	7.47	8.49	17.71	1065	145	91
2	612	7.49	8.16	17.00	1110	142	96
3	624	7.48	8.34	17.38	1115	143	91
4	630	7.48	8.42	17.54	---	146	93
mean	627	7.47	8.40	17.50	1098	144	94
stdev	10	0.02	0.16	0.34	23	2	3

Figure 2-6: Measurement of carotid distensibility.

Blood pressure was measured twice with a Dinamap automatic blood pressure recorder (Critikon, Tampa, Florida, USA) and the mean was taken as the subjects reading. Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient (DC) was calculated according to the following equation: $DC = (2\Delta D/D)/\Delta P$ (10^{-3} / kPa).[27]

Data analysis

In most analyses, repeatedly measured blood pressure, cholesterol or body weight in the offspring were studied as outcome. As repeated measurements within children are non-independent observations, we used unbalanced repeated measurement analysis to take this correlation into account. With this method, we investigated the pattern of the outcome in the offspring over time and the effect of covariates on these patterns. Intercept and age were mostly used as random effects. In the analyses with parameters of vascular damage as outcome, univariate and multivariate linear regression models were used. The relationships between cardiovascular risk factors in offspring or parents and the dichotomous variable for presence of plaques were evaluated by univariate and multivariate logistic regression analysis. Subgroup analysis was performed only when the added interaction term was significant in the multivariate model and if a biologically plausible mechanism was present.

In the analysis with PWV as the outcome variable and cardiovascular risk factors as independent variables, failure to correct for blood pressure could result in residual confounding: on the one hand PWV is related to wall elasticity and by that to distending pressure,[28] while on the other hand blood pressure is related to several cardiovascular risk factors such as body mass index, waist circumference, HDL-cholesterol and insulin. Mean arterial pressure (MAP) 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.[29] Besides blood pressure, also heart rate is an important factor in the intraindividual variation of PWV.[30]

All statistical analyses were performed by using the Statistical Analysis System (SAS), with the Proc Mixed module for unbalanced repeated measurement analysis,[31] the Proc GLM module for linear regression analysis

and the Proc Logistic module for logistic regression analysis.

Conclusion

The EPOZ-study is a cohort study in children, followed from childhood into adulthood, which aimed to provide data on early determinants of vascular damage. Together with knowledge from other cohort studies in children, in particular the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study,[32] the Muscatine Study[33] and the Bogalusa Heart Study,[34] the EPOZ study aimed to enhance our understanding of the underlying mechanism of atherosclerosis and CVD. Such knowledge is needed for the possible new paradigm of the 21st century, namely shifting the diagnosis and treatment of atherosclerosis to children and young adults.

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FAMILIAL CARDIOVASCULAR DISEASE RISK

3

3.1

Families and the natural history of blood pressure: a 27-year follow-up study

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Abstract

BACKGROUND

Previous studies on familial aggregation of blood pressure have reported data on family history of hypertension. Data on actual parental blood pressure levels and the subsequent natural history of blood pressure in their offspring are scarce.

METHODS

In a population based study with 596 children aged 5-19 years, cardiovascular risk factors were measured annually from 1975 until 2002. Parental data were obtained at baseline. Repeated blood pressure measurements were studied as a function of tertiles of age-adjusted blood pressures measured in their parents at baseline.

RESULTS

Systolic blood pressure during follow-up was higher in offspring whose parents were both in the highest tertile compared to children whose parents were not in the highest tertile (difference 2.7 mmHg, 95% confidence interval: 0.2,5.2). Having both parents in the highest tertile of diastolic blood pressure resulted in a substantially higher diastolic blood pressure ranging from 1.9 mmHg at age 15 to 8.5 mmHg at age 45. These differences were adjusted for age, gender, body mass index, total serum cholesterol, smoking habits and alcohol consumption.

CONCLUSION

Actual parental blood pressure shows to be an important predictor of blood pressure development from childhood into young adulthood. This is important when constituting cardiovascular risk profiles for children and young adults.

Introduction

Prospective studies have shown that cardiovascular disease aggregates in families.[1,2] This is probably partly due to familial aggregation of important cardiovascular risk factors such as blood pressure and plasma cholesterol.[3,4] It is long recognized that primary hypertension has its roots in early life.[5,6] Familial aggregation of blood pressure levels is well established in cross-sectional studies in various societies[7-10] and was shown to be detectable in children at a very young age.[11]
In longitudinal studies, children with a family history of hypertension were shown to have persistently higher blood pressure levels than children without such a history over follow-up periods of up to 10 years.[12-14] Still, the usefulness of recording a positive family history in the prediction of hypertension in the individual is considered to be limited.[15,16] No data is available on young parents' actual blood pressure levels in relation to the subsequent natural history of blood pressure in their children. It is of particular interest to know whether parental blood pressure levels are predictors of their offspring's subsequent blood pressure development into young adulthood and whether this holds for the whole distribution of parental blood pressure. In young adulthood, environments are shared less than in childhood and the more stable blood pressure levels are claimed to be predictive of future hypertension and its cardiovascular sequelae.[17]
In our 27-year follow-up study, we assessed the relation between blood pressure levels measured in young parents and the subsequent course of blood pressure levels in their offspring, measured annually from childhood to young adulthood.

Methods

Study population

Families with children aged 5-19 years old who were living in 2 districts in the Dutch town of Zoetermeer were invited to participate in a cross-sectional population-based study on risk-indicators for chronic disease (Epidemiological Preventive Study Zoetermeer (EPOZ)). All participants were included between 1975 and 1978.[18] Zoetermeer is a suburban residential community of at that time about 55,000 inhabitants which is situated near The Hague in the Netherlands. Of all persons, 4,649 (82 %) took part in

the study. From this group, a random sample of 596 children was selected for annual follow-up in a study on the natural history of cardiovascular risk factors and their determinants. Between 1975 and 1993, subjects visited the research center annually in the same month of the year, preferably at the same time of the day. In 2002, all subjects were invited for an examination that included measurements of both blood pressure and measurements of atherosclerosis. The median number of visits is 15 (range 2-19). Median follow-up time for the present analyses is 23 years. The present study is based on 452 subjects with data available on blood pressures of both mother and father. Response gradually declined to 83% in 1993. In 2002 response was 61%. The Medical Ethics Committee of the Erasmus MC approved the study and all participants gave informed consent.

Measurements

Blood pressure was measured using a Hawksley random-zero sphygmomanometer (Lancing, Sussex) according to a standardized protocol[19] on the left brachial artery of a sitting subject after a resting period of 15 minutes. A cuff of 23 cm by 10 or 14 was used, depending on the arm circumference. The largest cuff was usually used in a child over 10 years of age. Diastolic blood pressure was taken at the 5th Korotkoff phase. The mean of two consecutive measurements was used in the analyses. Between 1975 and 1993 the same research assistant performed all blood pressure measurements. In 2002 three research assistants performed the measurements. Body height and weight were measured and body mass index (BMI) was calculated (weight(kg)/height^2(m^2)). At each visit non-fasting blood samples were taken. In the period 1975-1993 serum total cholesterol was determined by an enzymatic procedure using Boehringer Mannheim CHOD/PAP High Performance. The latest serum total cholesterol measurements were determined by an automated enzymatic procedure using Roche CHOD-PAP reagent kit. Information on smoking habits and alcohol use, medical history and medication use was obtained through questionnaires. In addition, women were asked about use of oral contraceptives, menstrual cycle and pregnancies. Parental data of the subjects were obtained at baseline. In parents, blood pressure was measured similarly using a Hawksley random-zero sphygmomanometer (Lancing, Sussex) according to a standardized protocol.[19] Total cholesterol was determined by an enzymatic procedure using Boehringer Mannheim CHOD/PAP High Performance. Parental information on smoking habits, alcohol

consumption, medical history and use of antihypertensive medication was obtained through questionnaires.

Data analysis

Parental blood pressure level was examined as a determinant of offspring blood pressure levels over time. Tertiles of age-adjusted blood pressure distributions were made separately for fathers and mothers using linear regression. The highest tertile included parents using anti-hypertensive medication. A categorical variable indicating for each child the number of parents (none, one or both) in the highest tertile of blood pressure was used as the determinant in the subsequent analysis. Repeatedly measured blood pressure levels in offspring were studied as outcome. As repeated blood pressure measurements within children are non-independent observations we used unbalanced repeated measurement analysis. The relation between blood pressure and age was modelled using fractional polynomials.[20] Intercept and age were used as random effects. The same modelling was used to obtain mean blood pressure in offspring by parental blood pressure adjusted for offspring gender, age, height, BMI, total cholesterol, smoking habits and alcohol consumption. An interaction term age offspring*parental blood pressure was added to evaluate if the relation between parental blood pressure and offspring blood pressure changed with increasing offspring age. The model included repeated blood pressure measurements in offspring as the dependent variable and an indicator for parental blood pressure tertile as well as the above-mentioned factors as independent variables. To evaluate the impact of paternal and maternal blood pressure separately, offspring blood pressure level was analysed according to continuous blood pressure levels in respectively fathers and mothers. The interaction terms gender*paternal systolic blood pressure, gender*maternal systolic blood pressure, gender*paternal diastolic blood pressure and gender*maternal diastolic blood pressure were added to assess the paternal and maternal effects on sons and daughters separately. All statistical analyses were performed by using the Statistical Analysis System (SAS), with the Proc Mixed module for unbalanced repeated measurement analysis.[21]

Results

The mean age, median years of follow-up and the levels of risk factors at baseline of both parents and offspring are shown in table 3-1. Mean baseline systolic blood pressure of 362 subjects seen in 2002 and of 183 subjects not seen in 2002 was 113.3 mmHg respectively 114.8 mmHg (p=0.26). The final model included age and age$^{-½}$.
Figure 3-1 reflects the association between parental systolic blood pressure and systolic blood pressure of the offspring. Offspring with both parents in the highest blood pressure tertile at baseline had persistently higher systolic blood pressure levels throughout the 27-year follow-up as compared to those with only one or no parents in the highest tertile. Differences between the highest and the lowest parental tertiles were statistically significant. The

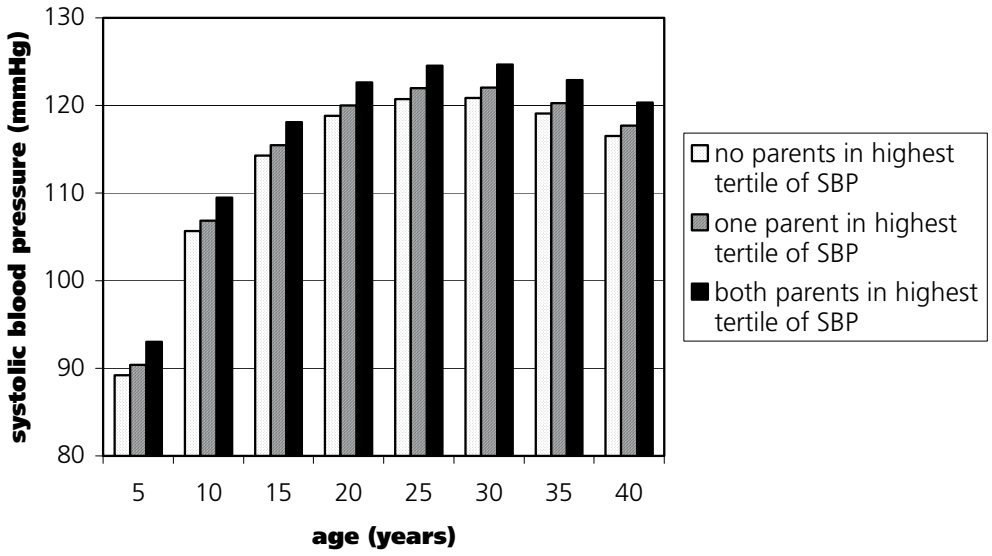
Table 3-1 Characteristics of parents and offspring at first visit.

Variable	Mother n=452	Father n=452	Female offspring n=219	Male offspring n=233
Age (years)	41.9 (7.4)	44.8 (7.9)	12.9 (4.1)	13.3 (4.2)
Height (cm)	164.5 (6.0)	176.1 (6.8)	152.1 (17.6)	157.9 (22.2)
Weight (kg)	65.9 (10.2)	76.4 (10.1)	44.4 (15.3)	47.3 (18.0)
Total cholesterol (mmol/l)	5.7 (1.1)	5.9 (1.1)	4.8 (0.7)	4.6 (0.8)
Systolic blood pressure (mmHg)	125.7 (16.6)	128.6 (15.3)	112.9 (13.2)	115.5 (16.5)
Diastolic blood pressure (mmHg)	79.3 (11.7)	79.4 (11.3)	67.2 (10.4)	67.7 (10.1)
Body mass index (kg/m²)	24.3 (3.6)	24.6 (2.8)	18.5 (3.2)	18.1 (2.7)
% hypertensive*	22.8	26.8		
Anti-hypertensive drugs (%)	2.6	2.0		
Median follow-up (years)			23.2	23.3

Values are means (standard deviations) unless otherwise indicated.
** using antihypertensive medication or having systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg.*

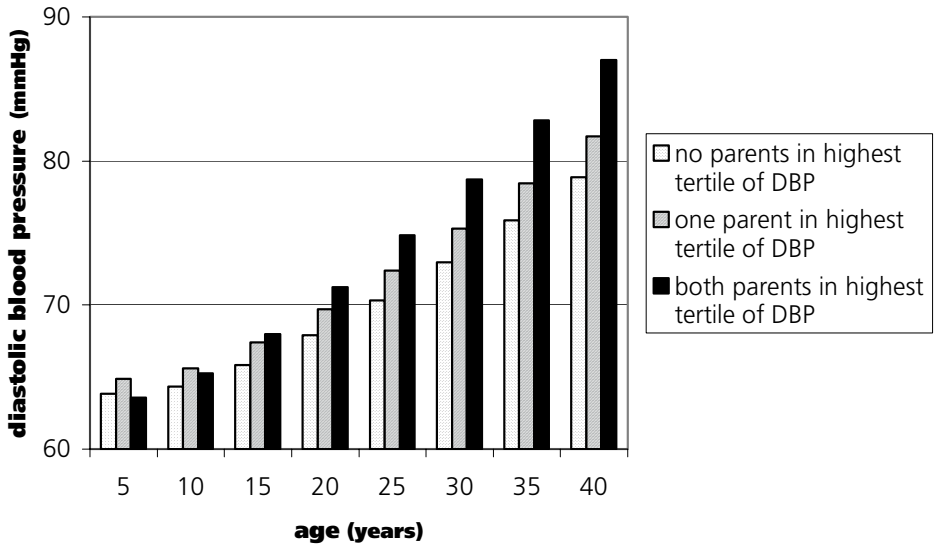
difference for systolic blood pressure was estimated at 2.72 mmHg (95% confidence interval 0.21,5.24). The coefficient for the interaction term age offspring*parental systolic blood pressure was not statistically significant (p=0.67), indicating that parentally determined systolic blood pressure differences in offspring did not vary with increasing offspring age.
Figure 3-2 shows the positive association between parental diastolic blood pressure and diastolic blood pressure of offspring. The coefficient of the interaction term age offspring *parental diastolic blood pressure was highly statistically significant (p=0.006). Having both parents in the highest tertile of diastolic blood pressure resulted in a substantially higher diastolic blood pressure in the offspring, from 1.94 mmHg at age 15 to 8.47 mmHg at age 45 and 11.74 mmHg at age 60.

Figure 3-1 Systolic blood pressure development of children according to parental systolic blood pressure.



Values based on a repeated measurement regression model adjusted for offspring age, gender, standardized height, body mass index, total cholesterol, smoking habits and alcohol consumption. SBP = systolic blood pressure.

Figure 3-2 Diastolic blood pressure development of children according to parental diastolic blood pressure.



*Values based on a repeated measurement regression model adjusted for offspring age, gender, standardized height, body mass index, total cholesterol, smoking habits, alcohol consumption and the interaction term age*parental DBP.*
DBP = diastolic blood pressure.

The systolic blood pressure of the offspring raises 0.09 mmHg (0.04,0.13) per mmHg increase of maternal systolic blood pressure and diastolic blood pressure raises 0.04 mmHg (-0.01,0.08) per mmHg increase of maternal diastolic blood pressure. No difference in effect of maternal blood pressure was seen in sons and daughters (p-values of interaction terms gender*maternal systolic blood pressure and gender*maternal diastolic blood pressure 0.38 respectively 0.50). For paternal systolic blood pressure the interaction term with gender was borderline significant (p=0.07), being a 0.14 mmHg per mmHg increase in systolic blood pressure in sons (0.07,0.22) and a 0.02 mmHg increase per mmHg in daughters (–0.05,0.09). The effect

of paternal diastolic blood pressure was significantly different for sons and daughters (p-value interaction term gender*paternal diastolic blood pressure 0.02), being a 0.16 mmHg increase per mmHg for sons (0.10,0.23) and a 0.08 mmHg per mmHg increase for daughters (0.01,0.14).

Discussion

In this longitudinal study, we found that actual parental blood pressure is a strong determinant of the natural history of blood pressure in their offspring from childhood into young adulthood. The association was found for the highest tertile of blood pressure levels of the parents and not only for parental hypertension.

Before we discuss our findings, some methodological issues need to be considered. The group selected for follow-up was a random sample from the youngsters who participated in the baseline study. Blood pressure values among those lost to follow-up and those not lost to follow-up were similar. Therefore, we do not think that loss-to-follow-up has affected our results. Moreover, only small numbers of children were lost in a strict sense; most only had some missing values. Measurements of blood pressure include within-subject variability and measurement error. The large number of measurements of blood pressure that were performed in each individual enhances more accurate estimation of subject's true underlying blood pressure levels at every age.

Cardiovascular risk factors including high blood pressure are known to aggregate in families,[9,11-14] with studies showing the variation in blood pressure determined by both genetic and environmental influences.[14,22-26] A relation between a positive family history of hypertension and blood pressure in offspring from childhood to young adulthood has been shown,[12,13] including in previous reports of the EPOZ study in children.[19,27] A genetic basis for the differences found in our study is supported by the fact that familial differences are not only present at a young age when environments are shared, but persist into young adulthood when environments become more different. Familial aggregation is also found for other important cardiovascular risk factors such as total serum cholesterol and low-density lipoprotein cholesterol,[28] smoking habits,[29] diabetes mellitus[30] and obesity.[31] Our association remained after control for these factors, suggesting that the findings of this study are independent of the familial aggregation of those

other factors.
It is well established that there is a positive relation between initial level of blood pressure and subsequent rise of blood pressure in adulthood,[32] a phenomenon called horse-racing.[33] This would indicate that the familial differences found in our study might be expected, if anything, to further increase in later life. Furthermore, is has been shown in adulthood (> 25 years of age) that prolonged differences in blood pressure have a substantial impact on heart disease risk.[34] For example, differences in systolic blood pressure of 9, 14 and 19 mmHg were reported to be associated with subsequent 34%, 46% and 56% differences in 10-year stroke risk and 21%, 29% and 37% differences in 10 year coronary heart disease risk, respectively. The longitudinal association between high blood pressure in childhood and cardiovascular disease much later in life has been shown in cross-sectional and short-term longitudinal studies only. However, recent long-term follow-up studies showed a significant relationship between blood pressure in childhood and levels of atherosclerosis in young adulthood.[39,40] The familial differences in youngsters found in our study, amounting to 2.72 mmHg systolic and 8.47 mmHg diastolic blood pressure at 45 years of age, may lead to material differences in risk of getting stroke and coronary heart disease in the far future. It should be added that blood pressure is not the only cardiovascular risk factor that shows familial aggregation over time. We have shown previously in this cohort that increased parental lipid levels are associated with persistently and substantially higher lipid levels in their offspring.[28] Familial aggregation of multiple cardiovascular risk factors already present at an early age seems to persist over time in the offspring, as described here. Moreover, it has been reported that clusters of cardiovascular risk factor levels show tracking in unselected young populations.[41] Our findings add to the current knowledge in several ways. The impact of parental blood pressure was present over the whole distribution of parental blood pressure levels, meaning the lower the parental blood pressure the lower the blood pressure levels of the offspring. Furthermore, our data suggest that the impact of parental blood pressure starts at an early age and is strong and long lasting. Cardiovascular high-risk profiles in the young may be better predictors of elevated risk profiles in adulthood and eventually of cardiovascular endpoints than single risk factors. Because our data show that on a group level parental blood pressure characterizes offspring blood pressure throughout youth into young adulthood, actual parental blood pressure levels may play an important role in assessing cardiovascular risk in

childhood.

Conclusion

Actual parental blood pressure shows to be an important predictor of blood pressure development from childhood into young adulthood. This is important when constituting cardiovascular risk profiles for children and young adults.

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3.2

Parental body mass index and overweight and vascular damage in the offspring: a 27-year follow-up study

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Abstract

BACKGROUND

The dramatic increase in the prevalence of childhood obesity may have implications for children's current and future cardiovascular health. Family dynamics may play an important role in the development of childhood obesity and subsequent vascular changes in adulthood. Objective of this study is to determine the association between parental body mass index (BMI) and the subsequent development of BMI, cardiovascular risk profile and vascular changes in their children.

METHODS

During a follow-up of 27 years, cardiovascular risk factors were measured annually in 596 children aged 5-19 years at baseline. In 2002, measurements of carotid intima-media thickness were added. In their parents, cardiovascular risk factors were measured at baseline. Repeated measurements of BMI, cholesterol and systolic blood pressure and a single measurement of atherosclerosis in children were studied as a function of parental BMI. Overweight was defined as age, sex and height adjusted weight ≥ 1 standard deviation.

RESULTS

Offspring with both parents in the highest tertile of BMI had a 3.0 kg/m² (2.0-3.9) higher BMI and a 14 times higher risk of becoming overweight (odds ratio 14.4 (9.1-22.8)) compared to offspring with both parents in the lowest tertile. High parental BMI was associated with clustering of cardiovascular risk factors and increased carotid intima-media thickness in offspring, independent of childhood BMI.

CONCLUSION

Parental BMI has a strong and lasting impact on the BMI and cardiovascular risk profile of the offspring, already at young ages. Parental BMI predicts vascular changes in young adulthood. The results underline the importance of early prevention of excessive weight gain and the necessity of involving the parents in fighting the obesity epidemic.

Introduction

The prevalence of overweight and obesity has strongly increased in adults as well as in children.[1-5] Obesity, once established, is difficult to treat. It has been proposed that early life prevention is the only effective solution to the problem of adult obesity.[6] Already at young ages family dynamics may play an important role in the development of obesity.[7] The importance of parental obesity in predicting children's risk of obesity in adulthood has been shown in a retrospective cohort study.[8] In this study, Whitacker et al showed that parental obesity more than doubles the risk of adult obesity among both obese and nonobese children less than 10 years of age. The impact of parental body mass index (BMI) on the manifestation of overweight has been shown in cross-sectional studies as well.[4,9,10] Few studies however, used actual measurements of parental BMI and only few studies used repeated measurements of BMI in offspring from childhood into adulthood. Most studies looked at familial aggregation of obesity using cut-offs for BMI. Furthermore, little is known about the impact of parental body weight and body weight in childhood on vascular risk in early life.
The present study was designed to examine the association between measurements of parental BMI and BMI and cardiovascular risk factors in offspring during a 27-year follow-up period. We also evaluated the relation between parental BMI and the presence of atherosclerosis in adult offspring.

Methods

Study population

Families with children aged 5-19 years who were living in two districts in the Dutch town of Zoetermeer were invited to participate in a cross-sectional population-based study on risk-indicators for chronic diseases (Epidemiological Preventive Study Zoetermeer (EPOZ)). Zoetermeer is a suburban residential community of at that time about 55,000 inhabitants which is situated near The Hague in the Netherlands. All families were included between 1975 and 1978.[11] Of all persons aged 5-19 years, 4,649 (82 %) took part in the study. From this group, a random sample of 596 children was selected for annual follow-up in a study on the natural history of cardiovascular risk factors and their determinants. After inclusion, participants were invited annually to visit the research center in Zoetermeer

in the same month of the year, at the same time of the day between 17.00-21.00 hours. Between 1975 and 1993, participants visited the research center annually. In 2002, all subjects were invited for an examination that included measurements of atherosclerosis. In total, 362 persons participated in this examination. The median number of visits is 15 (range 2-19). Median follow-up time for the present analyses is 23 years. Response for the majority of visits gradually declined to 83% in 1993. For the atherosclerosis measurements in 2002, the response was 61%. The EPOZ study was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam. All participants gave written informed consent at baseline and in 2002.

Measurements

Body height was measured to the nearest 0.1 centimetres using a Seco stadiometer. The measurements were taken without shoes in upright position, with the heels touching and with the head in Frankfurt plane.[12] Body weight was measured to the nearest 0.1 kg using a Seco digital balance. These measurements were taken with the participants standing in wearing only light underwear and no shoes. BMI was calculated as body weight(kg)/body height(m)^2. Blood pressure was measured using a Hawksley random-zero sphygmomanometer according to a standardized protocol on the left brachial artery of the sitting participant after a resting period of 15 minutes.[13] The mean of two consecutive measurements was used in the analyses. At each visit non-fasting blood samples were taken. In the period 1975-1993, serum total cholesterol was determined by an enzymatic procedure using Boehringer Mannheim CHOD/PAP High Performance, high-density lipoprotein (HDL) cholesterol was measured similarly after precipitation of the non-HDL-cholesterol fraction.[14] In 2002, serum total cholesterol was determined by an automated enzymatic procedure using Roche CHOD-PAP reagent kit. HDL cholesterol was measured with the Roche direct HDL cholesterol assay using PEG-modified enzymes and dextran sulphate. Information on smoking and alcohol consumption, medical history and medication use was obtained through questionnaires. Parental data of the participants were obtained at baseline. Body weight and height, systolic blood pressure, total cholesterol and HDL cholesterol were measured using the same protocols as in the offspring. Parental information on alcohol consumption, smoking, medication use and medical history was obtained through questionnaires filled in by the parents.

In 2002, IMT was measured by recording of ultrasonographic images of both

the left and right carotid artery, using a 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Technology Laboratories, Bethel, Washington, USA). The lumen-intima interface and the media-adventitia interface of the near and far wall of the distal common carotid artery were measured off-line. The protocol has been described in detail elsewhere.[15] The common carotid IMT was determined as the average of near and far wall measurements of both left and right side.

Statistical analysis

The first aim was to analyse parental BMI as a determinant of offspring BMI over time. To that end, an unbalanced repeated measures model was used with repeated measurements of offspring BMI as dependent variable and an independent variable indicating both parents being in the highest, the middle, or the lowest tertile of BMI. Each offspring BMI response was assumed to have a linear relation with age and to have its own random intercept and slope. The independent variable was constructed using tertiles of age-adjusted BMI distributions that were made separately for fathers and mothers. An interaction term parental BMI*age offspring was used to evaluate a possible change in the relation between parental BMI and offspring BMI with increasing offspring age. Using logistic regression we evaluated the relation between parental BMI and overweight in the offspring. Overweight was defined as age, sex and height adjusted weight ≥ 1 standard deviation (SD). The same model was used for evaluating the effect of maternal and paternal BMI separately. In all models we adjusted for offspring gender, age, total cholesterol, smoking habits and alcohol consumption. A repeated measures linear regression model was used to evaluate the relation between parental BMI and cardiovascular risk factors in the offspring. At each visit, a cardiovascular risk score was calculated for each person based on systolic blood pressure, total cholesterol and BMI level, ranging from 0 (none of the variables in the highest tertile) and 3 (all variables in the highest tertile). The models included repeated risk scores in offspring as the dependent variable and the categorical variable indicating parental BMI status as the independent variable. Adjustments were made for age, gender, smoking habits and alcohol consumption of the offspring. The impact of parental BMI on carotid IMT in the offspring was studied using multiple linear regression analysis. The model included offspring IMT as the dependent variable and parental BMI as the independent variable and adjustments were made for age, gender, total cholesterol, smoking habits

and alcohol consumption. All statistical analyses were performed using the Statistical Analysis System (SAS), with the Proc Mixed module for unbalanced repeated measurement analysis.[16]

Results

The mean age, median years of follow-up and the levels of risk factors of both parents and offspring are shown in Table 3-2. Mean baseline levels of BMI of the offspring seen at baseline, in 1992 and in 2002 were 18.4 kg/m², 18.3 kg/m² and 18.6 kg/m², respectively. Offspring with both parents in the highest BMI tertile at baseline had persistently higher BMI throughout the 27-year follow-up as compared to those with both parents in the middle or lowest tertile (Figure 3-3). Having both parents in the highest tertile of BMI was associated with an average 2.97 kg/m² (95% confidence interval 2.04-3.89) higher BMI in offspring in comparison with having both parents in the lowest tertile. Having both parents in the middle tertile resulted in a 1.38 kg/m² (0.32-2.43) higher BMI compared to having both parents in the lowest tertile. There was no interaction between the effects of parental BMI and age of the offspring (p=0.30). Thus, the relation between parental and offspring BMI did not depend on offspring age. Adjustment for total cholesterol, smoking and alcohol use of offspring did not substantially change the estimates. Male offspring with both parents in the highest tertile had a 2.22 kg/m² (1.07-3.37) higher BMI than male offspring with both parents in the lowest tertile. The difference between male offspring with both parents in the middle tertile of BMI and those with both parents in the lowest tertile was 0.84 kg/m² (–0.48-2.15). In female offspring, corresponding differences in BMI amounted to 3.72 kg/m² (2.27-5.18) and 1.94 kg/m² (0.28-3.61) respectively. The difference in effect of parental BMI between boys and girls was not statistically significant (p=0.10). Offspring with a father in the highest tertile had a 1.66 kg/m² (1.11-2.21) higher BMI than offspring with a father in the lowest tertile. Offspring with a mother in the highest tertile had a 1.19 kg/m² (0.64-1.73) higher BMI than offspring with a mother in the lowest tertile. The relation between parental BMI and risk of becoming overweight in the offspring is shown in table 3-3.
Figure 3-4 shows the relation between parental BMI and cardiovascular risk in the offspring. Children with both parents in the highest tertile of BMI had a 0.65 (0.45-0.86) higher cardiovascular risk score compared to children with

Table 3-2 Characteristics of parents and offspring.

Variable	Father *baseline visit* n=466	Mother *baseline visit* n=494	Male offspring *baseline visit* n=193	Female offspring *baseline visit* n=166	Male offspring *last visit (2002)* n=193	Female *offspring* *last visit (2002)* n=166
Age (years)	44.8 (8.0)	41.9 (7.6)	14.4 (5.9)	13.5 (5.8)	38.0 (4.4)	36.9 (4.5)
Height (cm)	176.0 (7.1)	164.5 (6.0)	160.4 (21.8)	151.8 (17.5)	182.2 (6.8)	169.0 (7.4)
Weight (kg)	76.2 (10.3)	65.8 (10.1)	49.7 (18.5)	44.7 (16.3)	85.1 (13.6)	73.0 (15.1)
Body mass index (kg/m^2)	24.6 (2.8)	24.3 (3.6)	18.5 (2.9)	18.7 (3.6)	25.6 (3.6)	25.5 (4.7)
Total cholesterol (mmol/l)	6.0 (1.1)	5.7 (1.1)	4.6 (0.9)	4.8 (0.8)	5.2 (1.1)	4.9 (0.9)
Systolic blood pressure (mmHg)	128.5 (15.6)	125.6 (16.7)	116.4 (16.3)	113.0 (13.2)	124.2 (13.9)	115.5 (11.7)
Diastolic blood pressure (mmHg)	79.4 (11.3)	79.3 (11.8)	68.8 (10.6)	67.9 (9.7)	82.5 (9.2)	76.4 (8.9)
Anti-hypertensive drugs (%)	2.6	2.0			2.6	1.2
Smoking (%): current					39.9	28.9
past					23.8	33.1
Alcohol consumption (%)					86.5	75.9
Intima media thickness (mm)					0.72 (0.08)	0.69 (0.07)
Median follow-up (years)					23.3	23.2

Values are means (SD), unless otherwise indicated

both parents in the middle or lowest tertile. Neither age nor gender effects were present (interaction terms respectively p=0.81 and p=0.94). The effects of father's BMI and mother's BMI on cardiovascular risk were similar. Offspring with paternal BMI in the highest tertile had a 0.036 mm (0.015-0.057) higher IMT compared to offspring of fathers with a BMI in the lowest tertile (figure 3-5). Offspring's IMT increased with 0.005 (0.002-0.008) mm per 1 kg/m² increase in their father's BMI (p=0.001). This association was independent of offspring BMI as the IMT still increased when adding offspring BMI to the latter model (0.004 (0.001-0.008) mm per 1 kg/m² increase in paternal BMI (p=0.004)). Offspring with maternal BMI in the highest or middle tertile did not differ in IMT with offspring of mothers with a BMI in the lowest tertile (differences were −0.002 mm (-0.023-0.019) and −0.012 mm (-0.033-0.009), respectively).

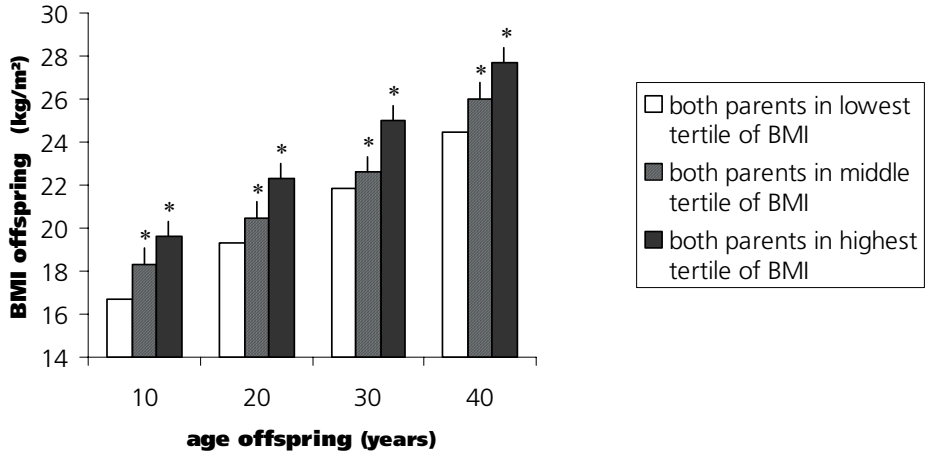
Discussion

In the present 27-year follow-up study, we found that the BMI of parents is strongly related to the BMI and cardiovascular risk of their offspring from childhood well into adulthood. Notably, we found an association between parental BMI and vascular changes in adult offspring.

Before interpreting our results, some methodological issues need to be discussed. During the follow-up period of 27 years, a single research assistant performed the vast majority of the average of 15 measurements per participant, reducing measurement variation. The large number of measurements of BMI that was performed in each individual further enhanced more accurate estimation of person's true underlying levels at every age. The population selected for follow-up was a random sample from the youngsters who participated in the baseline study. As BMI values at baseline were similar among those who were and those who were not lost to follow-up, we do not think that selective loss-to-follow-up has affected our results. We used measurement of IMT which is a recognized measure of atherosclerosis.[15,17-20] Ultrasonographic measurement of carotid IMT correlates well with pathological measurements[21] and is an established risk indicator of cardiovascular disease.

The importance of family dynamics for the development of childhood obesity is suggested by several findings. From previous studies it is known that parental obesity is associated with body mass index in the offspring.

Figure 3-3 Offspring body mass index according to parental body mass index at different ages.



Values (standard error) based on a repeated measurement regression model adjusted for age, gender, total cholesterol, smoking and alcohol consumption of offspring and interaction of age with parental body mass index; BMI=body mass index.
**p-value < 0.05, compared to offspring with both parents in the lowest tertile of BMI (reference).*

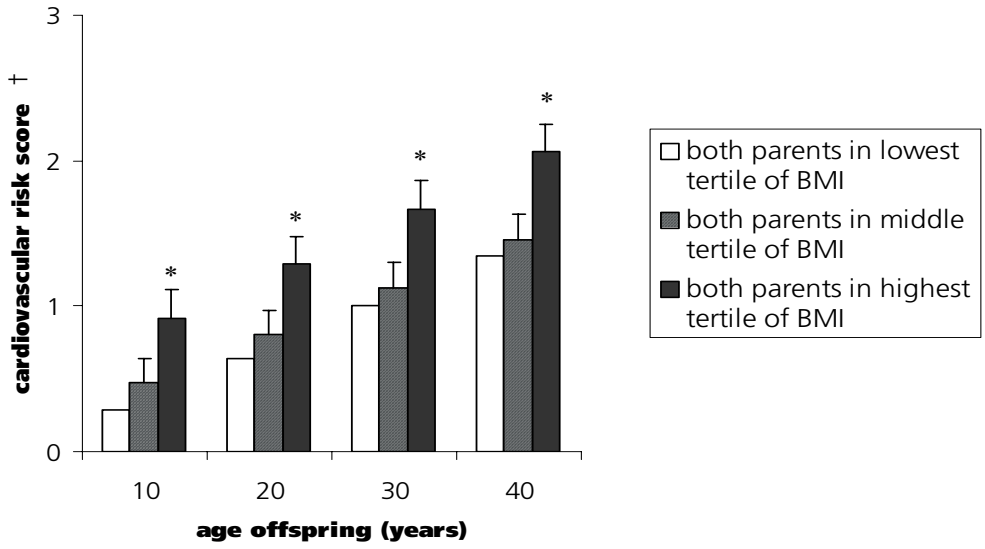
Table 3-3 Parental body mass index and risk of overweight in offspring.

Category of offspring	OR *	95% CI
both parents in highest tertile of BMI *	14.4	9.1 – 22.8
both parents in middle tertile of BMI *	3.1	2.2 – 4.1
father in highest tertile of BMI *	3.0	2.5 – 3.6
mother in highest tertile of BMI *	1.8	1.5 – 2.2

*Overweight: age, sex and height adjusted weight ≥ 1 standard deviation; OR: odds ratio; CI: confidence interval; BMI: body mass index. Values are from logistic regression models adjusted for offspring gender, age, total cholesterol, smoking habits and alcohol consumption. *Reference category is offspring with a father and/ or a mother in the lowest tertile of BMI.*

Whitacker et al showed that parental obesity more than doubles the risk of adult obesity among both obese and nonobese children under 10 years of age.[8] The impact of parental BMI on the manifestation of overweight in the offspring has also been shown in cross-sectional studies.[4,9,10,22] Furthermore, it is known that obese children tend to become obese adults. Of children who were overweight at around 12 years of age some 80% were still obese at around 30 years.[23] Our findings add to the current knowledge in several ways. The impact of parental BMI was present over the whole range of

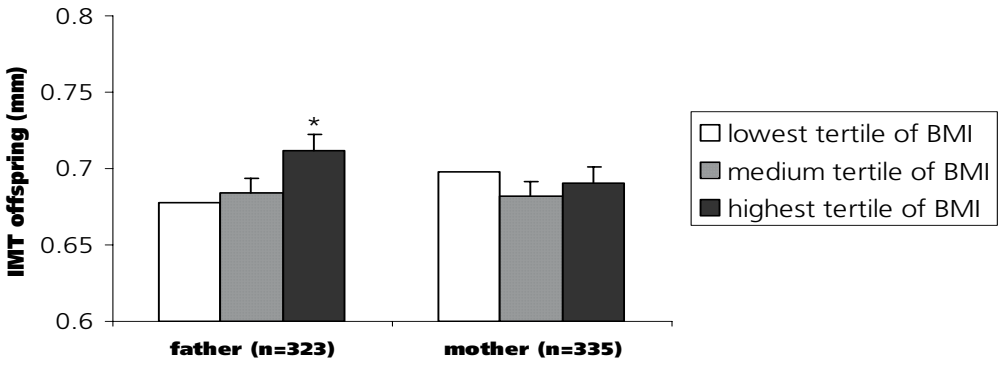
Figure 3-4 Clustering of cardiovascular risk factors in offspring according to parental body mass index.



*Values (standard error) based on a repeated measurement regression model adjusted for age, gender, smoking and alcohol consumption of offspring and with the interaction age offspring*parental body mass index; BMI=body mass index.*
**p-value < 0.05, compared to offspring with both parents in the lowest tertile of BMI (reference).*
† Cardiovascular risk score is based on systolic blood pressure, total cholesterol and BMI and ranges from 0 (none of the variables in the highest tertile) to 3 (all variables in the highest tertile).

parental BMI levels, meaning the lower the parental BMI the lower the child's BMI. Furthermore, our data suggest that the impact of parental BMI starts at an early age, is strong and long lasting, and is also associated with increased cardiovascular risk. Finally, our data showed a relation between parental BMI and vascular changes in young adulthood, independent of childhood BMI. Data on the cardiovascular consequences of high parental BMI are scarce. In previous studies we showed BMI development in adolescence to be related to young adult arterial wall thickness.[24] Two longitudinal studies have recently shown that BMI measured in childhood and adolescence predict carotid IMT in young adulthood.[25,26] However, this association was not found in the Thousand Families cohort Study.[27] In the present study we found that paternal BMI was related to offspring IMT and the association between parental BMI and childhood BMI could only partly explain this effect. We have no explanation for the restriction of the latter association to BMI of the fathers.

Figure 3-5 Carotid intima-media thickness in adult offspring according to parental body mass index.



Values (standard error) based on a linear regression model adjusted for offspring age, gender, total cholesterol, smoking habits and alcohol consumption.
**p-value < 0.05, compared to offspring with a father in the lowest tertile of BMI.*
BMI = body mass index; IMT = intima-media thickness.

There is growing agreement that the environment, rather than biology, is driving the current obesity epidemic.[28,29] Biology clearly contributes to individual differences in weight and height as more than 430 genes, markers, and chromosomal regions have been associated or linked with human obesity phenotypes.[30] Twin studies and earlier observational studies also revealed an inherited susceptibility to obesity.[30-32] However, the rapid excess weight gain and dramatic increase in the prevalence of overweight and obesity that has occurred over the past decades in both children and adults[1-5] is more likely the result of at least a strong interaction with the changing environment. In our study, adjustment for the life style factors smoking and alcohol consumption did not substantially change the estimates. As we lack information on physical activity and nutrition, it is not possible to discriminate between effects of environment and biology. Once there is manifest obesity it is very difficult to treat, also in youngsters,[33] and there is growing consensus that intervention at early ages may be the only effective preventive approach.[6,7] Knowledge about familial clustering of body weight is of crucial importance for primary prevention at an early age. First, our study shows that familial weight clustering has a strong and long-lasting impact on the BMI in children. Second, the impact of parental BMI was present over the whole range of parental BMI levels, meaning the lower the parental BMI the lower the child's BMI. Finally, parental BMI does not only affect the current health or social status but is also a predictor of future vascular damage in adult offspring and thereby the risk of manifest cardiovascular disease in later life. Therefore, the family drive of BMI may have strong implications for children's current and future cardiovascular health.

Conclusion

Parental body mass index has a strong and lasting impact on the body mass index and cardiovascular risk of the offspring, already at young ages. Furthermore, parental body mass index is associated with vascular changes in young adulthood. The results from our study underline the importance of early prevention of excessive weight gain and the necessity of involving the parents in fighting the obesity epidemic.

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CHILDHOOD DETERMINANTS OF CARDIOVASCULAR DISEASE RISK

4

4.1

Cardiovascular risk factors in childhood and vascular damage in adulthood: a 27-year follow-up study

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Abstract

Background

It has been suggested that the proper strategy for cardiovascular disease prevention should target children and young adults. Accurate identification of those at highest risk at a younger age can only be based on knowledge of the predictive ability of childhood cardiovascular risk factors for vascular damage at later age. Objective is to determine the associations of repeated childhood measurements of body mass index, systolic blood pressure and serum total cholesterol with atherosclerosis and arterial stiffness in young adulthood.

Methods

During a follow-up of 27 years, cardiovascular risk factors were measured repeatedly in 596 children aged 5-19 years at baseline. In 2002, measurements of atherosclerosis (carotid intima-media thickness (IMT) and plaques) and arterial stiffness (femoral-carotid pulse wave velocity (PWV) and carotid distensibility) were added and the vascular measures were studied as functions of repeated measurements of body mass index, systolic blood pressure and total cholesterol. Adjustments were made for age, gender, social economic status, smoking habits, alcohol consumption and alternately mean arterial pressure, heart rate, body mass index and total cholesterol.

Results

Body mass index, systolic blood pressure and total cholesterol levels measured both in adulthood and childhood predicted carotid intima-media thickness and carotid plaques in adulthood. Relative risks of plaques were largest for cardiovascular risk factors measured 20 years earlier in childhood (odds ratio's per 1 SD increase in body mass index, systolic blood pressure and total cholesterol, respectively 1.5 (1.1-1.9), 1.4 (1.3-1.5) and 1.6 (1.2-2.2). Carotid-femoral pulse-wave velocity was only predicted by systolic blood pressure and total cholesterol, measured concurrently. Carotid distensibility was solely predicted by systolic blood pressure, measured both in childhood and in adulthood.

Conclusion

We found that repeated childhood measurements of body mass index, systolic blood pressure and serum total cholesterol were strongly related to atherosclerosis in young adulthood. Childhood systolic blood pressure was associated with future arterial stiffness. These findings underline the

importance of targeting children in delaying atherogenesis and lowering the risk of its cardiovascular sequelae with aging.

Introduction

It has been suggested that the proper strategy for tackling cardiovascular disease should target children and young adults.[1] Information about risk factors for the development of early atherosclerosis has been derived mainly from autopsy studies.[2,3] In the Bogalusa autopsy study, the number of plaques in the aorta and coronary artery wall increased significantly with age among children and young adults and was associated with traditional coronary risk factors. Furthermore, a higher number of risk factors predicted more extensive lesions.[3] These observations have been extended by ultrasound studies relating childhood risk factors to carotid intima-media thickness (IMT) in young adults.[4-7] Data on the association between childhood cardiovascular risk factors and arterial stiffness in young adulthood are scant.[8] Knowledge of the predictive value of cardiovascular risk factors for premature atherosclerosis and arterial stiffness in adulthood, may lead to identification of those at highest risk of cardiovascular disease at a young age.
The aim of our 27-year follow-up study was to determine the relationship of body mass index (BMI), systolic blood pressure and total cholesterol measured from childhood into adulthood with measures of atherosclerosis and arterial stiffness in adulthood.

Methods

Study population

Families with children aged 5-19 years who were living in two districts in the Dutch town of Zoetermeer were invited to participate in a population-based study on risk-indicators for chronic diseases (Epidemiological Preventive Study Zoetermeer (EPOZ)). Zoetermeer is a suburban residential community of at that time about 55,000 inhabitants which is situated near The Hague in the Netherlands. All families were included between 1975 and 1978.[9] Of all persons aged 5-19 years, 4,649 (82 %) took part in the study. From this group, a random sample of 596 children was selected for annual follow-

up in a study on the natural history of cardiovascular risk factors and their determinants. Between 1975 and 1992, participants were invited annually to visit the research center in Zoetermeer in the same month of the year, preferably at the same time of the day. In 2002, all subjects were invited again for an examination that included measurements of atherosclerosis and arterial stiffness. In total, 362 subjects participated in this examination. The median number of visits is 15 (range 2-19). Median follow-up time for the present analyses is 23 years. Response for the annual visits gradually declined to 83% in 1992. For the atherosclerosis measurements in 2002, the response was 61%.

Risk factor assessment

Body height was measured to the nearest 0.1 centimetres using a Seco stadiometer. The measurements were taken without shoes in upright position, with the heels touching and with the head in Frankfurt plane.[10] Body weight was measured to the nearest 0.1 kg using a Seco digital balance. These measurements were taken with the participants standing and wearing only light underwear and no shoes. BMI was calculated as body weight(kg)/body height(m)^2. Information on smoking and alcohol consumption, medical history and medication use was obtained through annual questionnaires. At all visits, blood pressure was measured using a Hawksley random-zero sphygmomanometer (Lancing, Sussex) according to a standardized protocol[11] on the left brachial artery of a sitting subject after a resting period of 15 minutes. The mean of two consecutive measurements was used in the analyses. Between 1975 and 1992 the same research assistant performed all blood pressure measurements. At each visit non-fasting blood samples were taken. In the period 1975-1992, serum total cholesterol was determined by an enzymatic procedure using Boehringer Mannheim CHOD/PAP High Performance.[12] In 2002, serum total cholesterol was determined by an automated enzymatic procedure using Roche CHOD-PAP reagent kit. Finally, at the last visit, additional measurements of vascular status were performed.

Indicators of atherosclerosis and arterial stiffness

All measurements were taken with the subjects in supine position. First, IMT was measured by recording of ultrasonographic images of both the left and right carotid artery, using a 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Technology Laboratories, Bethel, Washington, USA). Three

optimal longitudinal images of the intima-lumen inter interface and the media-adventitia interface of the far wall and the near wall of the distal common carotid artery were frozen on the R wave of the electrocardiogram and stored on videotape. The actual measurements of lumen diameter and IMT were performed off-line using a procedure and additional dedicated software, that has been closely adapted from the Wallenberg Laboratory for Cardiovascular Research, Gothenburg, Sweden.[13] A frozen image that was stored on videotape is digitised and displayed on the screen of a Laser 286/2 personal computer using a frame grabber (VP 1400-KIT-512-E-AT, Imaging Technology). The protocol has been described in more detail elsewhere.[14]
The common carotid intima-media thickness was determined as the average of the maximal near and far wall measurements of both left and right side.
The amount of plaques in the carotid artery was assessed by evaluating the ultrasonographic images of the common, internal and bifurcation site of the carotid artery for the presence of atherosclerotic lesions. Plaques were defined as a focal thickening relative to adjacent segments with protrusion into the lumen composed of either only calcified deposits or a combination of calcification and noncalcified material.
Carotid-femoral pulse-wave-velocity (PWV) was assessed using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France)[15] that computed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery. Distances from the carotid sampling site to the suprasternal notch and from the suprasteranal notch to the femoral artery were measured using a pair of compasses.[16] PWV was calculated as the ratio between the distance travelled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. The average of 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses. Before measurement of PWV, blood pressure was measured twice with a sphygmomanometer after five minutes of rest and the mean was taken as the subjects reading. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + ⅓*(systolic blood pressure – diastolic blood pressure).
Finally, the vessel wall motion of the right common carotid artery was measured by means of a Duplex scanner (Ultramark IV, ATL, Bethel, Washington, USA) connected to a vessel wall movement detector system.[17,18] After five minutes rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasonography. The displacement of the arterial walls was obtained by processing the radio

frequency signals originating from two-selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. During the common carotid distensibility measurement, blood pressure was measured twice with a Dinamap automatic blood pressure recorder (Critikon, Tampa, Florida, USA) and the mean was taken as the subjects reading. Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient (DC) was calculated according to the following equation: $DC = (2\Delta D/D)/\Delta P$ (10^{-3} / kPa).[19]

Data analysis

All measurements of atherosclerosis and arterial stiffness were studied as functions of repeated measurements of BMI, systolic blood pressure and total cholesterol. BMI was first standardized by age and gender.[20] Systolic blood pressure and total cholesterol were standardized by age. Next, the means of standardized levels of BMI, systolic blood pressure and total cholesterol were calculated separately for four time-periods (16-20 years, 11-15 years and 6-10 years before and concurrent to the measurement of vascular status). These means were used to compute tertiles of the risk factor variables for each of the four time-periods. Carotid IMT was studied as a function of the tertiles of BMI, systolic blood pressure and total cholesterol in each period, adjusted for age, gender, social economic status, smoking habits and alcohol consumption. With logistic regression analysis, we examined the relation of standardized mean levels of BMI, systolic blood pressure and total cholesterol at the different points in time and the presence of plaques in the carotid arteries, adjusted for the variables as described for the analysis of carotid IMT. The relations of mean standardized BMI, mean standardized systolic blood pressure and mean standardized total cholesterol in each period with carotid-femoral PWV and carotid distensibility were examined using multiple linear regression analysis. The analyses with BMI as independent variable were additionally adjusted for blood pressure and total cholesterol. In the analyses with systolic blood pressure and total cholesterol as independent variables additional adjustments were made for BMI. As both blood pressure and heart rate are important factors in the intraindividual variation of PWV,[21,22] we additionally adjusted for mean arterial pressure and heart rate in all analyses with PWV as outcome.

A possible age-effect in the relations of BMI, systolic blood pressure and total cholesterol with vascular damage was evaluated by adding interaction terms with age. All statistical analyses were performed using the Statistical Analysis System (SAS), with the Proc Mixed module for unbalanced repeated measurement analysis.[23]

Results

General characteristics of the study population at baseline and at the latest visit are presented in table 4-1.
Figure 4-1 shows the associations of BMI, standardized for age and sex and measured at four different time periods with IMT in 2002. There was a steady positive relationship between BMI and IMT. Persons with a concurrently measured BMI in the highest tertile had a 0.043 mm (0.024-0.062) higher IMT compared to persons with a BMI in the lowest tertile. Being in the highest tertile of BMI 6-10 years before, 11-15 years before and 16-20 years before the IMT measurement predicted respectively a 0.032 mm (0.013-0.051), 0.028 mm (0.010-0.047) and 0.025 mm (0.007-0.043) higher IMT compared to persons in the lowest tertile of BMI at these points in time. Also, those with a concurrently measured BMI in the middle tertile had a significantly higher IMT (0.024 mm (0.006-0.043)) than persons with a BMI in the lowest tertile. This association was not seen for BMI measurements in earlier time periods. Figure 4-2 shows the association between systolic blood pressure and carotid IMT. At all time periods, carotid IMT increased significantly with increasing of systolic blood pressure. Being in the highest tertile of systolic blood pressure measured concurrently, 6-10 years before, 11-15 years before and 16-20 years before the IMT measurement, predicted respectively a 0.024 (0.005-0.044) mm, 0.041 (0.021-0.061) mm, 0.036 (0.016-0.057) mm and 0.030 (0.010-0.049) mm higher IMT compared to persons in the lowest tertile of systolic blood pressure at these points in time. Figure 4-3 shows the association between total cholesterol levels and carotid IMT. The differences in carotid IMT between subjects in the highest tertile of total cholesterol compared to subjects in the lowest tertile of total cholesterol, amounted to 0.020 (0.001-0.038), 0.024 (0.005-0.042), 0.027 (0.009-0.045) and 0.027 (0.009-0.045) mm for the time-periods concurrently, 6-10 years earlier, 11-15 years earlier and 16-20 years earlier, respectively. Table 4-2 shows the relationship between BMI, systolic blood

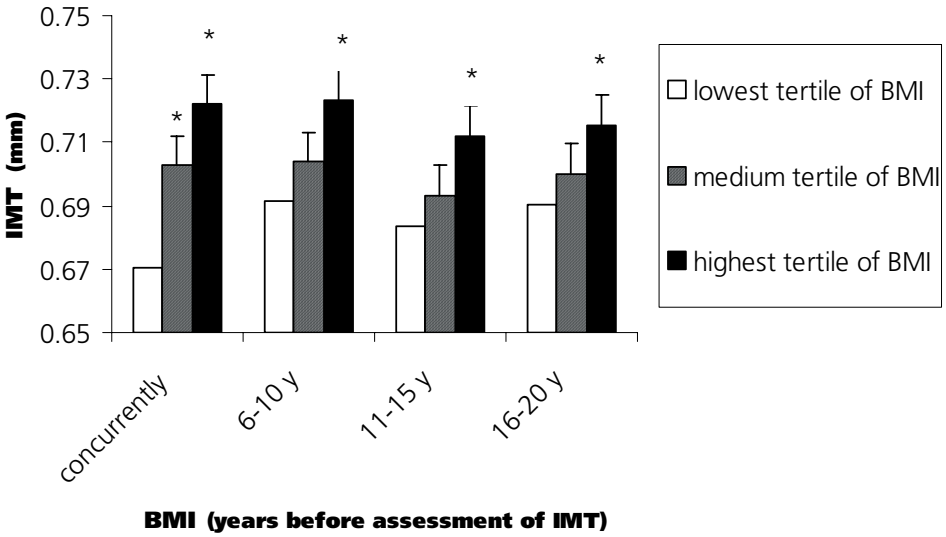
Table 4-1 General characteristics of the study population at baseline and at last visit.

Characteristic	Men (n=193)	Women (n=166)
Baseline (1975-1978)		
Age (years)	14.4 (5.9)	13.5 (5.8)
Height (cm)	160.4 (21.8)	151.8 (17.5)
Weight (kg)	49.7 (18.5)	44.7 (16.3)
Body mass index (kg/m^2)	18.5 (2.9)	18.7 (3.6)
Total cholesterol (mmol/l)	4.6 (0.9)	4.8 (0.8)
Systolic blood pressure (mmHg)	116.4 (16.3)	113.0 (13.2)
Diastolic blood pressure (mmHg)	68.8 (10.6)	67.9 (9.7)
Last visit (2001-2002)		
Age (years)	38.0 (4.4)	36.9 (4.5)
Height (cm)	182.2 (6.8)	169.0 (7.4)
Weight (kg)	85.1 (13.6)	73.0 (15.1)
Body mass index (kg/m^2)	25.6 (3.6)	25.5 (4.7)
Waist-to-hip ratio	0.90 (0.06)	0.80 (0.08)
Alcohol (n/week)	15.0 (21.0)	5.5 (8.9)
Highest education (%): elementary school	0	0.6
secondary school	23.5	31.2
lower/intermediate vocational training	50.8	49.4
higher vocational training/university	25.7	18.8
Smoking (%): current	39.9	28.9
past	23.8	33.1
Total cholesterol (mmol/l)	5.2 (1.1)	4.9 (0.9)
HDL-cholesterol (mmol/l)	1.12 (0.29)	1.37 (0.32)
Systolic blood pressure (mmHg)	124.2 (13.9)	115.5 (11.7)
Diastolic blood pressure (mmHg)	82.5 (9.2)	76.4 (8.9)
Use of antihypertensive drugs (%)	2.6	1.2
Mean arterial pressure (mmHg)	96.3 (9.8)	89.4 (9.2)
Pulse pressure (mmHg)	41.6 (10.1)	39.1 (7.9)
Heart rate (beats per minute)	65.6 (11.2)	69.2 (10.9)
Maximal carotid intima-media thickness (mm)	0.72 (0.08)	0.69 (0.07)
Carotid plaques present (%)	34.9	30.1
Carotid-femoral pulse wave velocity (m/s)	9.98 (1.50)	8.63 (1.15)
Median follow-up (years)	23.3	23.2

Values are expressed as mean (standard deviation) in case of continuous variables, and as percentages in case of categorical variables.

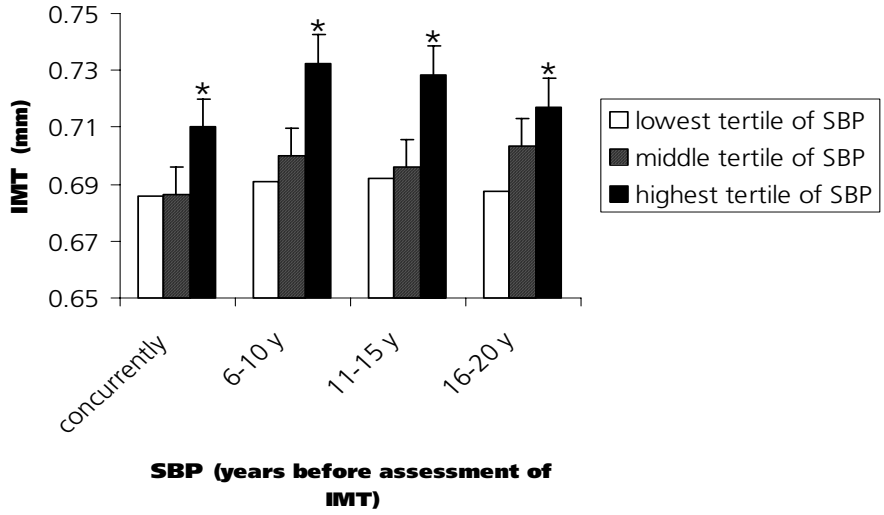
pressure and total cholesterol measured at four different time periods and the presence of carotid plaques. The earlier the risk factor is measured, the higher the risk of carotid plaques.
Table 4-3 shows the associations of BMI, systolic blood pressure and total cholesterol on both carotid-femoral PWV and common carotid distensibility. BMI was inversely associated with both PWV and carotid distensibility. With increasing systolic blood pressure, PWV increased and carotid distensibility decreased. No association was found between total cholesterol and arterial stiffness except for a positive relation between PWV and total cholesterol measured concurrently.

Figure 4-1 Carotid intima-media thickness in adulthood according to body mass index measured at different point in time.



Values (standard error) based on a linear regression model adjusted for age, gender, social economic status, smoking habits and alcohol consumption.
**p-value < 0.05, compared to the lowest tertile of BMI.*
BMI = body mass index standardized for age and gender; IMT = intima-media thickness.

Figure 4-2 Carotid intima-media thickness in adulthood according to systolic blood pressure level measured at different point in time.

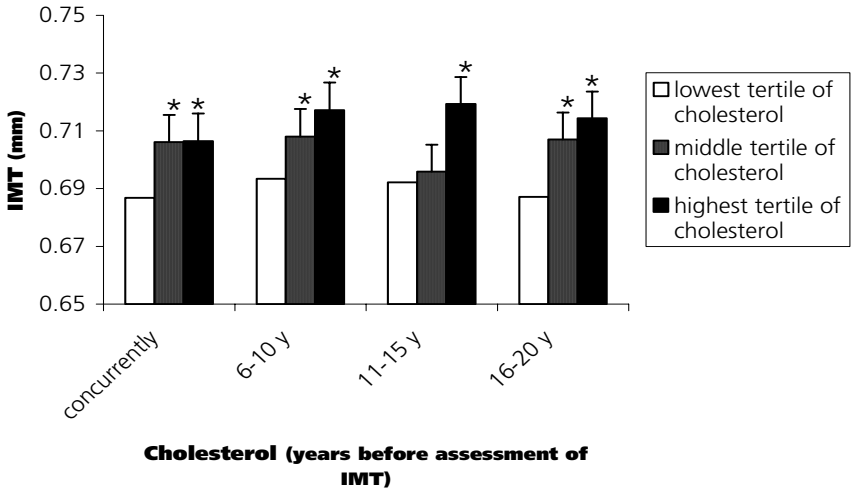


Values (standard error) based on a linear regression model adjusted for age, gender, social economic status, smoking habits and alcohol consumption.
**p-value < 0.05, compared to the lowest tertile of systolic blood pressure.*
SBP=systolic blood pressure standardized for age; IMT = intima-media thickness.

Discussion

In the present 27-year follow-up study, we found that BMI, systolic blood pressure and total cholesterol measured during childhood and young adulthood were strongly related to carotid IMT and plaques in adulthood. The association of these risk factors with carotid plaques was stronger when these risk factors were measured at a young age. Arterial stiffness was associated with BMI and total cholesterol measured concurrently, but with blood pressure measurements in both childhood and adulthood.
Before interpreting our results, some methodological issues need to be discussed. During the follow-up period of 27 years, a single research assistant performed the vast majority of the average of 15 measurements per participant, reducing measurement variation. The large numbers of measurements of BMI, systolic blood pressure and total cholesterol that were

Figure 4-3 Carotid intima-media thickness in adulthood according to total cholesterol level measured at different point in time.



Values (standard error) based on a linear regression model adjusted for age, gender, social economic status, smoking habits and alcohol consumption.
**p-value < 0.05, compared to the lowest tertile of total cholesterol.*
Cholesterol=total cholesterol standardized for age; IMT = intima-media thickness.

performed in each individual further enhanced more accurate estimation of person's true underlying levels. The population selected for follow-up was a random sample from the youngsters who participated in the baseline study. Furthermore, as values of BMI, systolic blood pressure and total cholesterol at baseline were similar among those who were and those who were not lost to follow-up, we do not think that selective loss-to-follow-up has affected our results.

We used the measurement of carotid IMT and plaques as measures of atherosclerosis.[24] Ultrasonographic measurement of carotid IMT correlates well with pathological measurements of atherosclerosis.[25, 26] Prospective epidemiological studies have shown that carotid IMT is associated with the risk for myocardial infarction and stroke, and that a decrease in IMT due to drug treatment is associated with a decrease in the risk of vascular events.[14,27-30] The presence of carotid plaques is associated with cardiovascular

Table 4-2 Relationship between body mass index, systolic blood pressure and total cholesterol measured during childhood into young adulthood and presence of carotid plaques measured in adulthood.

	OR_{BMI} * (95% CI)	OR_{SBP} † (95% CI)	OR_{CHOL} ‡ (95% CI)
Risk factor measured concurrently	1.22 (1.00-1.52)	1.08 (1.02-1.14)	1.26 (0.99-1.62)
Risk factor measured 6-10 years earlier	1.22 (0.92-1.62)	1.30 (1.22-1.39)	1.51 (1.11-2.05)
Risk factor measured 11-15 years earlier	1.31 (1.00-1.69)	1.31 (1.23-1.40)	1.57 (1.13-2.17)
Risk factor measured 16-20 years earlier	1.47 (1.13-1.91)	1.36 (1.28-1.45)	1.61 (1.16-2.24)

Values are odds ratios from logistic regression analysis adjusted for age, gender, social economic status, smoking habits and alcohol consumption.
OR: odds ratio; CI: confidence interval; BMI: body mass index standardized for age and gender; SBP: systolic blood pressure standardized for gender; CHOL: total cholesterol standardized for gender.
** odds ratio for having any plaque per 1 SD increase in body mass index; additionally adjusted for blood pressure and total cholesterol.*
† odds ratio for having any plaque per 1 SD increase in systolic blood pressure; additionally adjusted for BMI and total cholesterol.
‡ odds ratio for having any plaque per 1 SD increase in total cholesterol; additionally adjusted for BMI and systolic blood pressure.

Table 4-3 Relation of body mass index, systolic blood pressure and total cholesterol at different points in time and arterial stiffness in adulthood.

Risk factor (SD)	**Carotid-femoral PWV** (m/s)	**Carotid distensibility** (10^{-3}/kPa)
BMI (kg/m²) measured concurrently	-0.08 (-0.20, 0.05)	-1.09 (-1.66, -0.52)
BMI (kg/m²) measured 10 yrs earlier	-0.21 (-0.38, 0.04)	-0.74 (-1.51, 0.04)
BMI (kg/m²) measured 11-15 yrs earlier	-0.24 (-0.40, -0.08)	-0.59 (-1.32, 0.14)
BMI (kg/m²) measured 16-20 yrs earlier	-0.16 (-0.31, -0.00)	-0.44 (-1.13, 0.25)
SBP (mmHg) measured concurrently	0.016 (0.004, 0.028)	-0.15 (-0.20, -0.09)
SBP (mmHg) measured 10 yrs earlier	0.015 (-0.002, 0.032)	-0.08 (-0.16, -0.00)
SBP (mmHg) measured 11-15 yrs earlier	0.015 (-0.001, 0.030)	-0.04 (-0.11, 0.04)
SBP (mmHg) measured 16-20 yrs earlier	0.013 (-0.002, 0.029)	-0.09 (-0.17, -0.02)
Total cholesterol (mmol/l) measured concurrently	0.21 (0.06, 0.35)	0.15 (-0.51, 0.80)
Total cholesterol (mmol/l) measured 10 yrs earlier	-0.002 (-0.17, 0.17)	0.24 (-0.52, 0.99)
Total cholesterol (mmol/l) measured 11-15 yrs earlier	0.04 (-0.15, 0.22)	-0.41 (-1.23, 0.42)
Total cholesterol (mmol/l) measured 16-20 yrs earlier	0.10(-0.09, 0.28)	-0.57 (-1.37, 0.23)

Values are β-coefficients and 95% confidence intervals from multivariable linear regression models adjusted for age, gender, social economic status, smoking habits and alcohol consumption. The analysis with BMI are additionally adjusted for total cholesterol and mean arterial pressure. The analysis with total cholesterol is additionally adjusted for BMI and mean arterial pressure. The analysis with SBP are additionally adjusted for BMI and total cholesterol. The analysis with PWV as dependent variable are additionally adjusted for heart rate and mean arterial pressure.
Bold: p-value<0.05; SD=standard deviation; BMI=body mass index; SBP =systolic blood pressure; PWV=pulse wave velocity.

and cerebrovascular disease as well, irrespective of the side and location of the plaque.[29,31,32] We used the measurements of carotid-femoral PWV and carotid distensibility as measures of arterial stiffness. Aortic stiffness, measured through PWV is increased in the presence of a myocardial infarction with a strength of association comparable to that of atherosclerosis.[33-35] The measurement of aortic PWV allows only an estimate of the actual distance travelled by the pulse wave between the carotid artery and the femoral artery. The distances were measured in a straight line using a pair of compasses to reduce the influence of body contours. We tried to approach the true distance by subtracting the distance between the suprasternal notch and the carotid location from the distance between the suprasternal notch and the femoral site, because the pulse travels in the opposite direction.[36] This might underestimate PWV, because arteries become longer and more tortuous with age. However, as the participants were relatively young, the error will be relatively small. Common carotid artery distensibility is strongly associated with previous stroke and also, but less strongly with previous myocardial infarction.[37] By calculating the distensibility coefficient, distension of the common carotid artery is adjusted for pulse pressure measured in the brachial artery. We thereby assume that pulse pressure measured in the brachial artery is representative of pulse pressure in the carotid artery. In dogs, it has been demonstrated that pulse pressure in the brachial artery is linearly related to blood pressure in the carotid artery over a wide range of blood pressures.[38] However, it is known that the arterial pressure waves undergo transformation in the arterial tree and therefore the pulse pressure is higher in the brachial artery than in the more central vessels like the carotid artery.[39] On the other hand, non-invasive cuff-based measurement of blood pressure underestimates pulse pressure.[40] Therefore, we do not think that this difference in pulse pressure had a large effect on our measurements.

Longitudinal data on the vascular consequences of cardiovascular risk factors in childhood are scarce. In the ARYA study, it was shown that BMI development in adolescence was related to young adult arterial wall thickness.[41] Four other longitudinal studies showed that BMI, systolic blood pressure and low-density-lipoprotein cholesterol measured both in childhood and in adolescence predicted carotid IMT in adulthood.[4-7] However, these associations were not found in the Thousand Families cohort Study.[42] In the present study, we found strong relationships between BMI, systolic blood pressure and total cholesterol during childhood and young adulthood and

carotid IMT in adulthood, irrespective of the moment of measurement of the cardiovascular risk factors. The risk of carotid plaques in young adulthood was also associated with childhood and young adulthood measurements of BMI, systolic blood pressure and total cholesterol. The associations were strongest with the earliest measurements of risk factors.

As a high arterial distensibility indicates a high elasticity of the vessel, an inverse relationship was expected between the risk factors studied and carotid distensibility. We indeed observed an inverse association between carotid distensibility and BMI. Hyperglycaemic conditions can lead to increased arterial stiffness by increased collagen cross linking due to non-enzymatic glycation.[43-45] As BMI is known to be strongly related to insulin[46] this may be the mechanism underlying the association. However, no relation was found of PWV with BMI, measured concurrently, while unexpected inverse associations were found with childhood BMI. We have no explanation for the difference in findings of BMI with carotid distensibility and PWV. A possible explanation for the conflicting result is that in obese persons PWV is structurally underestimated as a proper recording of the pulse wave at the femoral artery is more difficult in obese persons. Two cross-sectional studies have reported a significant positive association between BMI and PWV in postmenopausal women.[47,48]

High systolic blood pressure measured both in childhood and in young adulthood was associated with lower carotid distensibility and higher PWV, although the latter relation was only present with systolic blood pressure measured concurrently. Thus, those who had higher blood pressure levels in childhood had stiffer arteries 20 years later, which suggests that blood pressure already in early childhood plays a role in the process of arterial stiffening. This is consistent with a recent study, showing that childhood blood pressure is predictive of arterial elasticity later in life.[8] The relation between childhood systolic blood pressure and arterial stiffness may be explained by the link between the mechanism of vascular stiffening and blood pressure. Arterial stiffening is due to degeneration of the arterial wall, probably as a consequence of repetitive cyclic stress. This repetitive strain activates vascular smooth muscle cell growth and consequently synthesis of matrix components.[49] Collagen and elastin are linked by smooth muscle whose activity modulates the contribution of each to arterial stiffness and may further increase blood pressure.

Results of studies on the associations between serum cholesterol and arterial stiffness have been controversial. In children (mean age 15 years)

with familial hypercholesterolaemia, a reduced thoraco-abdominal aortic stiffness has been observed, whereas both in adult subjects with familial hypercholesterolemia (mean age 37 years) and in adult healthy subjects (mean age 27 years), a positive association was observed between and low-density lipoprotein cholesterol and aortic stiffness. A possible explanation for these contradicting results is that the initial increase in distensibility could be due to a mechanistical effect of the presence of relatively small amounts of lipid in the wall while the later stiffening is due to sclerosis and the laying down of connective tissue in the vessel wall.[50] Therefore, a positive association between atherogenic lipoproteins and aortic stiffness can be expected after the third decade of life, when sclerotic changes appear.[51] In agreement with this view, we found a positive relation between PWV and total cholesterol, measured concurrently, but no significant association was found of PWV or carotid distensibility with total cholesterol, measured in childhood.

The current study underscores the importance of childhood cardiovascular risk factors in the development of cardiovascular disease risk. Already at very young ages BMI, systolic blood pressure and total cholesterol are related to partly irreversible vascular damage. These findings underline the importance of targeting children in delaying atherogenesis and lowering the risk of its cardiovascular sequelae with aging. At present, attention to early prevention of overweight is important.

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4.2

Alcohol intake and aortic stiffness in young men and women

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Abstract

Background

Moderate alcohol consumption has been shown to protect against cardiovascular disease. Aortic stiffness can be regarded as a marker of cardiovascular disease risk. Previously we have shown an inverse to J-shaped association between alcohol intake and aortic stiffness in middle-aged and elderly men and postmenopausal women. In the present study we examined whether such a relation is already present at a younger age.

Methods

Cross-sectional data of a cohort study in men and women aged 28 years were analysed stratified by gender (240 men and 283 women). Alcohol intake was derived from a questionnaire and aortic stiffness was assessed by pulse-wave velocity measurement.

Results

In women an alcoholic beverage intake of ≥1 glass per day was associated with a 0.36 m/s (95%CI: -0.58 to -0.14) lower pulse-wave velocity compared with non-drinkers. This effect was independent of changes in blood pressure and heart rate. In men alcohol intake was also inversely related to pulse-wave velocity but this was not significant.

Conclusion

These findings provide evidence for the view that moderate intake of alcohol may affect vascular elasticity at an early age, notably in women. These findings are compatible with a vascular protective effect of alcohol that expresses well before the occurrence of symptomatic cardiovascular disease.

Introduction

The relation between alcohol intake and the cardiovascular system seems to be U-shaped, suggesting a higher risk of cardiovascular disease in non-drinkers and heavy alcohol consumers compared to those with moderate alcohol intake.[1] Mechanisms proposed to explain a positive health effect of moderate alcohol consumption include beneficial effects of lipoprotein metabolism,[2] hemostasis[3] and inflammatory processes[4] with regard to atherogenesis. In addition, cross-sectional and large prospective studies have shown that moderate alcohol consumption reduces the risk of diabetes mellitus type 2[5] and increase insulin sensitivity.[6]
While cardiovascular protection has been demonstrated in older subjects, it is less clear whether beneficial effects of alcohol intake on the cardiovascular system express already at a younger age when subjects are still free of overt vascular disease. Early vascular evidence of cardiovascular risk may be provided by measurement of arterial characteristics, such as aortic stiffness. Aortic pulse-wave velocity (PWV) is a non-invasive measurement of the distensibility of the aorta, which is reported to be a reliable index of aortic stiffness.[7] An increased aortic stiffness at young adulthood may reflect life-long exposure to risk factors. Increased aortic stiffness has been related to unfavourable levels of risk factors, prevalent cardiovascular disease and atherosclerosis elsewhere in the arterial system. Also, increased arterial stiffness has been shown to predict cardiovascular events.[8]
Previously, we reported an inverse to J-shaped association between alcohol intake and aortic stiffness in postmenopausal women[9] and in men aged 40 to 80 years.[10] In the present study we examined whether such a relation is already present at younger age.

Methods

Subjects

The Atherosclerosis Risk in Young Adults (ARYA) Study comprises two cohorts of young adults, one performed in the city of The Hague and one in the city of Utrecht. Since vascular measurements were not performed in the Hague-cohort, this paper is restricted to the Utrecht-participants of the ARYA-study. The Utrecht cohort includes 750 young adults born between January 1, 1970 and December 31, 1973, who attended secondary school

in the city of Utrecht in the Netherlands and of whom the original medical records from the Municipal Health Care were available.[11] From October 1, 1999 to December 31, 2000, all participants visited our outdoor clinic twice within a 3-week period. PWV measurements were performed in 524 subjects (46% male). The ARYA study was approved by the Medical Ethics Committee of the UMC Utrecht. All participants gave written informed consent.

Cardiovascular risk factors

During the first visit, anthropometric measurements were performed. Height, weight, and waist-hip circumference were measured with indoor clothes without shoes. A written standardized questionnaire was completed on alcohol intake, smoking, highest education, and contraceptive pill use in women. In the questionnaire, subjects had to select 1 out of 5 options to categorize their alcohol intake (0; <1; 1-2; 3-5 and ≥6 glasses/day). Because there were only few subjects in the highest alcohol intake levels (see table 4-4), the two highest categories in men and the three highest categories in women were combined. Statistical analyses were performed with 4 alcohol intake levels for men (0, <1, 1 to 2, and ≥3 glasses/day) and 3 alcohol intake levels for women (0, <1, and ≥1 glasses/day).
During the second visit, fasting venous blood samples were drawn. The samples were stored at -20°C until all participants were enrolled into the study. Total cholesterol and high-density lipoprotein (HDL) cholesterol were determined using an automatic enzymatic procedure (Vitros950 dry-chemistry analyser (Johnson & Johnson, Rochester, New York, USA)).
At each visit, blood pressure was measured twice, after 5 minutes of rest, and at an interval of 5 to 15 minutes, in the left brachial artery in sitting position with an automated device (Dinamap, Critikon, Tampa California, USA) without replacing the cuff between the measurements. The mean of 2 consecutive measurements was used in the analyses. Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure. Mean arterial pressure was calculated as diastolic blood pressure + (1/3 * pulse pressure).

Arterial stiffness

Arterial stiffness was noninvasively assessed by measuring carotid-femoral (aortic) PWV, using an automated device (SphygmoCor device (AtCor Medical, West Ryde, Australia)). Aortic PWV was determined by sequential acquisition of pressure waves from the carotid and femoral arteries by

applanation tonometry (Millar Instruments, Houston, TX, USA). Wave transit time (t) was calculated by the system software, using the R-wave on the simultaneously recorded ECG as reference frame. The distance travelled by the pulse wave was measured in a straight line to reduce the influence of body contours. The carotid to femoral path length (D) was defined as the distance between the recording sites at the femoral artery to the suprasternal notch minus the distance from the recording site at the carotid artery to the suprasternal notch. PWV was calculated as D/t. The average of 10 successive waveforms 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 analysis.[12]

In order to evaluate the reproducibility of the technique in our research center, a subset of 25 participants had their PWV re-measured several weeks after their first visit. Absolute mean difference (standard error) in PWV of the repeated measurements between visits was 0.12 m/s (0.45). The intraclass correlation coefficient (ICC) for repeated measurements was 0.67. Since the repeated measurements were performed on 2 different occasions, the moderate ICC could partially be explained by the variability in blood pressure over time.

Statistical analysis

Data on alcohol consumption was missing in one participant, leaving 523 subjects for analysis. The alcohol intake levels (categorical) were put into a linear regression model as dummy variables with the lowest category as reference.

The association between alcohol consumption and PWV was examined, using multivariate linear regression analysis, adjusted for major determinants of PWV, namely age, mean arterial pressure and heart rate (Model A).[13, 14] In addition, Model A was extended with factors which were at least significantly ($P<0.05$) or which are biologically plausibly related to either PWV or alcohol intake (Model B). This model included waist-to-hip ratio, total cholesterol, HDL-cholesterol, highest education and current smoking.

To validate the self-reported information on alcohol intake, we evaluated the relation of HDL-cholesterol to alcohol intake using multiple linear regression analysis, adjusted for age, mean arterial pressure and heart rate.

Data were analysed using the SAS statistical software package (SAS/STAT Version 8.02, SAS Institute, Cary, NC).[15]

Results

General characteristics of the study are presented in table 4-4. Table 4-5 shows the relation of alcohol intake to PWV and HDL-cholesterol separately for men and women because the association between alcohol and PWV differed by gender (p=0.03). In both men and women HDL-cholesterol increased with increasing alcohol intake. There was an inverse association between alcohol intake and aortic stiffness in women. An alcoholic beverage intake of ≥1 glass per day decreased the PWV by 0.36 m/s (95%CI: -0.58 to -0.14) compared with non-drinkers. In men, alcohol consumption of ≥3 glasses per day lowered the PWV by 0.15 m/s (95%CI: -0.51 to 0.20). Additional adjustment for factors which were at least significantly (P<0.05) or which are biologically plausibly related to either PWV or alcohol intake (Table 4-5, Model B) did not change the magnitude of the relations.

Discussion

These findings provide evidence of an inverse association between alcohol consumption and aortic stiffness in young women. To appreciate the findings, certain aspects of the study need to be addressed. The use of self-reported information on alcohol intake may have introduced misclassification of exposure, specifically those in the heavier drinking groups.[16] However, selective misclassification of heavy drinkers as non-drinkers seems unlikely, because we observed a positive association between alcohol consumption and HDL-cholesterol, a finding that supports the rank-order validity of self-reported alcohol intake. No information was available on alcoholic beverage type consumed, changes in drinking behaviour and drinking pattern. However, type of beverage consumed is not likely to have influenced our findings, since much of the benefit is from alcohol rather than other components of each type of drink.[17, 18] In addition, changes in drinking behaviour are probably not relevant at a young age. Drinking pattern might be a factor that has influenced the results. Yet, Mukamal et al.[18] recently observed within categories of frequency of alcohol consumption similar inversely related risks of myocardial infarction, regardless of the amount of alcohol consumed per drinking day.

Table 4-4 Characteristics of the study population.

Characteristic	**Men** (n=240)	**Women** (n=283)
Age (years)	28.2 (0.9)	28.2 (0.9)
Body mass index (kg/m^2)	24.6 (3.7)	24.4 (4.5)
Waist-to-hip ratio	0.88 (0.06)	0.81 (0.06)
Alcohol (n (%)):		
0 glasses/day	31 (12.9)	70 (24.7)
<1 glasses/day	111 (46.2)	161 (56.9)
1-2 glasses/day	59 (24.6)	46 (16.3)
3-5 glasses/day	34 (14.2)	6 (2.1)
≥6 glasses/day	5 (2.1)	0 (0)
Highest education (%):		
elementary school	3.3	1.8
secondary school	19.2	21.5
lower/intermediate vocational training	41.7	38.5
higher vocational training/university	35.8	38.2
Current smokers (%)	33.3	26.5
Contraceptive pill use (%)	NA	65.4
Total cholesterol (mmol/L)	4.86 (0.94)	4.84 (0.82)
HDL-cholesterol (mmol/L)	1.30 (0.30)	1.58 (0.36)
Systolic blood pressure (mmHg)	130.4 (12.1)	120.2 (12.1)
Diastolic blood pressure (mmHg)	72.5 (7.2)	70.8 (8.4)
Mean arterial pressure (mmHg)	91.8 (8.0)	87.3 (9.0)
Pulse pressure (mmHg)	57.8 (9.3)	49.4 (7.9)
Heart rate (bpm)	63.4 (9.5)	66.4 (8.8)

Values are expressed as mean (standard deviation) in case of continuous variables, and as percentages in case of categorical variables.
HDL-cholesterol=high density lipoprotein cholesterol; bpm=beats per minute; PWV=pulse wave velocity; NA: not applicable.

Table 4-5 Pulse-wave velocity and high-density lipoprotein-cholesterol in men and women per level of alcohol intake.

		Men		
Alcohol intake level (glasses/day)	**n**	**PWV** (m/s) (Model A)	**PWV** (m/s) (Model B)	**HDL-cholesterol** (mmol/l) (Model A)
0	31	6.37 (0.13)	6.36 (0.15)	1.32 (0.05)
<1	111	6.23 (0.07)	6.20 (0.10)	1.24 (0.03)
1 to 2	59	6.30 (0.10)	6.31 (0.12)	1.36 (0.04)
≥3[a]	39	6.22 (0.12)	6.19 (0.14)	1.36 (0.05)*

		Women		
Alcohol intake level (glasses/day)	**n**	**PWV** (m/s) (Model A)	**PWV** (m/s) (Model B)	**HDL-cholesterol** (mmol/l) (Model A)
0	70	5.85 (0.07)	5.81 (0.10)	1.52 (0.04)
<1	161	5.70 (0.05)	5.63 (0.09)*	1.61 (0.03)
≥1[b]	52	5.49 (0.08)†	5.41 (0.11)‡	1.59 (0.05)

Values are expressed as mean (standard error of the mean).
**P<0.05, †P<0.01, ‡P<0.001: compared with non-drinkers.*
a 79% of the subjects had an alcoholic beverage intake of 3 to 5 glasses/day.
b 85% of the subjects had an alcoholic beverage intake of 1 to 2 glasses/day.
Model A: adjusted for age, mean arterial pressure and heart rate.
Model B: adjusted for age, mean arterial pressure, heart rate, waist-to-hip ratio, total cholesterol, high-density lipoprotein cholesterol, highest education and current smoking.
PWV=pulse-wave velocity; HDL-cholesterol= high-density lipoprotein cholesterol.

To our knowledge, there are only 5 studies presenting the relation of alcohol consumption on aortic PWV. A cross-sectional study in Japanese-American middle-aged and older men and women reported that the risk for high aortic PWV was lower among current drinkers and ex-drinkers than among non-drinkers.[19] In a follow-up study in middle-aged Japanese men the incidence of aortic stiffness was not related to alcohol intake,[20] whereas another longitudinal study in Japanese men suggested that alcohol is an important risk factor for development of aortic stiffness at an intake of more than 16 glasses of alcoholic beverage per week.[21] We recently reported an inverse to J-shaped association between alcohol consumption and aortic PWV in cross-sectional studies among postmenopausal women[9] and among men aged 40 to 80 years of age.[10]

In young women an alcoholic beverage intake of about 1 to 2 glasses per day decreased the PWV approximately 7% compared to non-drinkers. This is comparable with the decrease in PWV observed in postmenopausal women with an intake of 10 to 14 glasses alcoholic beverage per week (8% decrease compared to non-drinkers).[9]

In young men, the inverse relation between alcohol and PWV was less pronounced. There are several possible explanations for the sex difference in strength of association. Firstly, the smaller increase in HDL-cholesterol with increasing alcohol intake in men compared with women might suggest that men were less precise in their recall of alcohol intake. Secondly, the drinking pattern in men might be unfavourable, namely binge drinking instead of regular daily consumption. Thirdly, an alternative explanation could be that the effect of atherogenic factors, like smoking and lipids, are more pronounced in young men, in such a way that the influence of alcohol on PWV cannot be observed. Finally, it may be that the association truly does not exist in men, or that the numbers of men in our study, particularly in the non-drinking group, were too small and the variation in PWV too large for statistical detection of an inverse trend.

The mechanism by which moderate alcohol intake may reduce aortic stiffness is unknown. Alcohol consumption increases HDL-cholesterol,[2] with associated increases in paraoxonase activity[2] and cholesterol efflux.[22] These changes might decrease the amount of cholesterol within peripheral cells, and thus increase the flexibility of the vascular wall. However, the relation between alcohol intake and PWV remained when HDL-cholesterol was taken into account, suggesting that our finding could not be fully explained by an increase in HDL-cholesterol. With increasing age the arteries become

stiffer due to a decrease in elastin and an increase in collagen and connective tissues in the arterial wall.[23] Alcohol intake might delay or change this process, possibly by an effect on gene expression. Damaging of the vascular wall due to inflammation might also cause arterial stiffness. The observed decrease in the inflammation factor C-reactive protein[4] with moderate alcohol intake could decrease the risk of lesions of the vascular wall and explain the increase in vascular elasticity. Finally, epidemiological studies have shown that moderate alcohol consumption reduces the risk of diabetes mellitus type 2[5] and increase insulin sensitivity.[6] This effect of alcohol might decrease the formation and cross-linking of glycated collagen in the vascular wall, which is accelerated in hyperglycaemic milieu.[24]

Perspectives

Moderate intake of alcohol may affect vascular elasticity already at an early age, notably in women. These findings are compatible with a vascular protective effect of alcohol that expresses well before the first occurrence of symptomatic cardiovascular disease.

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CONSTITUTIONAL DETERMINANTS OF CARDIOVASCULAR DISEASE RISK

5

5.1

Birth size related changes in cholesterol from childhood to early adulthood: a 27-year follow-up study

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Abstract

Background

Current evidence on the relation between body size at birth and lipid levels in adulthood is contradictory. Our objective was to study the natural history of blood lipids from childhood to young adulthood according to birth size.

Methods

Lipid levels were annually measured from 1975 to 1993 and in 2002 in a cohort of 330 subjects from the Dutch town of Zoetermeer, initially aged 5 - 19 years. Birth data were obtained through questionnaires sent to the parents. We studied birth weight and length in relation to total cholesterol and low-density lipoprotein-, high-density lipoprotein (HDL)-, HDL_2- and HDL_3-cholesterol levels in 5-year age periods and to change of levels with increasing age.

Results

Particularly males born with low birth weight had lower adverse lipid levels before and around puberty, but had substantially higher low-density lipoprotein cholesterol when they reached young adulthood (linear regression coefficient in 30 to 37 year old males: -0.51 (-0.93 to -0.09) mmol/l /kg birth weight). Particularly females who were shorter at birth had higher adverse lipid levels from childhood to young adulthood (linear regression coefficient in 20 to 24 year old females: total cholesterol: -0.08 (-0.14 to -0.02) mmol/l /cm higher birth length).

Conclusion

Size at birth is related to the natural history of lipids from childhood to young adulthood. Children born with low birth weight or length get higher adverse lipid levels particularly when they reach young adulthood.

Introduction

There is evidence that cardiovascular disease has its origins in the fetal phase and early childhood.[1,2] Size at birth, an indicator for intra-uterine growth has an effect on atheroclerosis[3,4] and coronary artery disease,[5] but the effect is modified by adult body size. Further, birth size has been found to be inversely associated with cardiovascular risk factors such as blood pressure, insulin resistance, and obesity in adult life.[6-8] Less is known about relations between birth size and blood lipids in later life. During the first year of life babies with higher birth weight were shown to have higher apolipoprotein B and lower apolipoprotein A-I levels in the blood.[9] Studies in children showed relations between lower birth length and higher total cholesterol levels,[10] and lower birth weight with higher triglyceride levels.[11] Another study reported no relation between birth weight and lipids in early adulthood.[12] Thinness at birth as indicated by a small abdominal circumference was related to higher total and LDL-cholesterol and apolipoprotein B levels in adults.[13] Men with lower birth weight or lower weight at 1 year had higher apolipoprotein B levels in the blood in adulthood.[14] Women with lower birth weight were shown to have higher serum triglycerides and lower HDL-cholesterol levels in adulthood.[15] A recent study in adolescents and a systematic review showed a weak relation of fetal nutrition to total cholesterol.[16]
Thus, the current evidence may be indicative of a relation between birth size and blood lipid levels in later life, but particularly in the young longitudinal data on this relation is limited and to some extent inconsistent. We have therefore studied the relation between body size at birth and the natural history of total and lipoprotein cholesterol levels in a cohort of children followed up into young adulthood.

Methods

Subjects

Between 1975 and 1978 the total population aged 5 years and over of two districts of Zoetermeer, a suburban town in the western part of the Netherlands, was invited to participate in a study of chronic disease risk indicators.[17] Of 5670 eligible children aged 5-19 years, 4,649 (response 82%) took part in the study. From this group a random sample of 596 children was selected for annual follow up in a study of cardiovascular risk factors

and its determinants. The present study was based on 483 (response 81%) subjects who took part in the yearly follow-up (252 males and 231 females) until 1993. Data on parents of these 483 subjects were obtained at the baseline study (1975 - 1978). Complete data were available for 425 fathers and 454 mothers. In 2002, all subjects were invited for an examination that included measurements of atherosclerosis. In total, 362 subjects participated in this examination. The median number of visits is 15 (range 2-19). Median follow-up time for the present analyses is 23 years. Response for the majority of visits gradually declined to 83% in 1993. For the atherosclerosis measurements in 2002, the response was 61%.

Measurements

The annual measurements for each individual were performed in the same month of the year. A Hawksley random-zero sphygmomanometer was used at each examination to measure systolic and diastolic blood pressure (5th Korotkoff phase) according to a standardised protocol.[17] Body height and weight were measured without shoes and heavy clothing. At each examination a questionnaire about use of medication, alcohol intake, coffee consumption, smoking habits and use of oral contraceptives was administered. Serum blood samples were drawn by antecubital venipuncture for measurement of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and its subfractions, HDL_2 cholesterol and HDL_3 cholesterol. In all children who were 5 - 14 years of age initially, skeletal age was determined at each follow-up examination, when they had not yet reached skeletal maturity. Skeletal age was determined using a radiograph of the hand and wrist according to the procedures and the rating system of Tanner et al.[18]

Laboratory analysis

The laboratory analysis of lipoprotein-cholesterol concentrations for this cohort study is described in detail elsewhere.[19] Briefly, serum total cholesterol at baseline was measured with an automated enzymatic method[20] and from 1983, with a modified reagent (CHOD/PAP High Performance, Boehringer Mannheim, FRG). The standard deviation of duplicate serum cholesterol measurements stored at -20°C for up to 4 years did not exceed 3.0% and did not show a significant drift. Measurements of HDL-cholesterol (from 1979) and LDL-cholesterol (from 1984) were performed by the same method after precipitation. For HDL-cholesterol we used a phosphotungstate

method[21] with a minor modification[22] and polyvinylsulphate (Boehringer Mannheim, FRG) for LDL-cholesterol. All measurements were carried out at the Department of Epidemiology & Biostatistics at Erasmus University Rotterdam, the Netherlands, which from 1978 participated in the lipid standardization program of the World Health Organization (WHO) Regional Lipid Reference Centre in Prague, Czechoslovakia, and from 1977 in the Dutch National Cholesterol standardization program (KCA foundation). During the baseline period quality control was indirectly checked on the CDC protocol by monthly comparison with cholesterol determination using the Abell-Kendall method.[23] Accuracy and precision of total cholesterol and HDL-cholesterol measurements were within acceptable limits (CDC/WHO) over the entire period. Automated analyses were initially carried out on a Technicon Auto Analyzer-II system (Technicon Instruments, Tarrytown, New York) and from 1989 on a Kone Specific Analyzer (Kone Instruments, Espoo, Finland) using frozen (-20°C) serum samples. From 1987, HDL_2-cholesterol and HDL_3-cholesterol subfractions in serum were assayed as described by Gidez et al[24] with slight modifications and separated using stepwise precipitation of apolipoprotein B containing lipoproteins with heparin/Mn^{2+} in two steps and HDL_2 with dextran-sulphate. In 2002, serum total cholesterol was determined by an automated enzymatic procedure using Roche CHOD-PAP reagent kit. HDL-cholesterol was measured with the Roche direct HDL-cholesterol assay using PEG-modified enzymes and dextran sulphate.

Collecting birth data

To obtain birth data, a questionnaire was sent to the parents of the children at baseline. Questions were asked about birth weight (gram), birth length (cm), placental weight (gram), gestational age, complications during pregnancy and whether or not the mother had smoked during pregnancy. The addresses of 33 parents could not be found and 2 children had died during follow-up. Of the 448 questionnaires sent, 353 (response 78.8%) were completed and returned to the investigators. Of the 353 returned questionnaires, 330 had usable data on birth weight. All 330 subjects had sufficient follow-up data on lipoprotein-cholesterol and possible confounders. In 59% of the returned questionnaires, the parents reported the birth weight as they remembered it (59% of these reported birth weights to the nearest 100g). In 19% parents obtained the birth weight from a maternity center (55% reported to the nearest 100g). In 22%,

parents took the birth weight from birth announcements (43% reported to the nearest 100 grams). In 2002, birth weight was asked again from the participants themselves via a questionnaire. Forty-one subjects without information on birth size at baseline reported their birth weight. In total, birth weight was known in 371 subjects.

Data analysis

Aim was to analyse body size at birth as a determinant of offspring (lipoprotein) cholesterol over time. To that end, an unbalanced repeated measures model was used with repeated measurements of offspring (lipoprotein) cholesterol as dependent variable and birth weight respectively birth length as independent variables. As the influence of birth weight on total cholesterol changed with increasing age (highly significant interaction term between birth weight and age (p=0.002)), this was first done within 5-year age-categories starting 5 to 9 to 25 to 29 and then 30 to 37 years. The same model was used to adjust for offspring current body weight, height, use of cigarettes, alcohol consumption and gestational age. Furthermore, both a variable indicating the change in skeletal age per calendar year and an interaction term between birth weight and the repeated measurements of the BMI were added to the regression models. The influence of gestational age was also evaluated by both restricting the analyses to children born at term and by adding the variable gestational age to the model. In each model a random intercept and slope were used.
The relation between change of lipoprotein cholesterol with age and birth weight and length was studied using a linear regression model with repeated measures of lipoprotein cholesterol as the dependent variable and age, birth weight, birth length and the interaction terms birth weight*age or birth length*age as independent variables. In analyses of change the covariance structure was assumed to be random. Each subject's responses (lipids) were assumed to have a linear relation with age and to have its own random intercept and slope.
The basic model for the influence of birth weight can be written as:

$$\text{Cholesterol} = \beta_0 + \beta_1 * \text{age} + \beta_2 * \text{birth weight} * \text{age} + \beta_3 * \text{birth weight}.$$

Written as a function of age this makes:

$$\text{Cholesterol} = (\beta_0 + \beta_3 * \text{birth weight}) + (\beta_1 + \beta_2 * \text{birth weight}) * \text{age},$$

whereby (β_0 + β_3*birth weight) is the intercept and (β_1 + β_2*birth weight) is the slope of cholesterol change with age.

All statistical analyses were performed using the Statistical Analysis System (SAS), with the Proc Mixed module for unbalanced repeated measurement analysis.[25]

Results

Table 5-1 shows characteristics of the subjects on entry into the cohort and birth data. Subjects were on average 13 years old, with 72% under 16 years of age. Males had slightly higher birth weights and lengths than females. Males who dropped out of the study before 1992 (21%) had on average a 213 grams lower birth weight and 0.1 mmol/l lower baseline cholesterol levels than those who were completely followed up. In those subjects of whom we did not obtain birth data, the baseline mean lipid levels in males (n=76); total cholesterol: 4.7 mmol/l, LDL-cholesterol: 3.4 mmol/l and HDL-cholesterol: 1.4 mmol/l, and in females (n=77); total cholesterol: 4.8 mmol/l, LDL-cholesterol: 3.2 mmol/l and HDL-cholesterol: 1.4 mmol/l, were comparable to levels in the studied group (table 5-1).

Birth weight and lipids

Males showed a tendency towards a negative association for total cholesterol levels with birth weight in childhood. For both total cholesterol and LDL-cholesterol levels progressively inverse associations were found at young adulthood (table 5-2). Among females, total cholesterol and LDL-cholesterol levels were positively associated with birth weight in childhood. In adulthood, females also showed inverse associations. In young adult males a positive association was found of birth weight with HDL-cholesterol and particularly with HDL_2-cholesterol, but not with HDL_3-cholesterol. In females, associations with HDL-cholesterol and subfractions were not statistically significant except for the positive association between birth weight and HDL_3-cholesterol at the ages 20-24 years. Adjustment for current weight and height, use of cigarettes and alcohol consumption did not materially change the results, nor did adjustment for total cholesterol level, systolic blood pressure and body mass index in both fathers and mothers. Inclusion in the model of a variable indicating whether subjects were breast-fed as

Table 5-1 Baseline characteristics at entry into the study cohort and birth data.

Variable	**Males** (n = 178)	**Females** (n = 152)
Age (years)	13.1 (5.5-21.1)	12.7 (5.6-21.1)
Body height (cm)	157.0 (109-195)	157.0 (114-181)
Body weight (kg)	44.5 (18-92)	45.0 (17-81)
Body Mass Index (kg/m^2)	17.8 (13.2-28.4)	18.5 (12.0-28.5)
Systolic blood pressure (mmHg)	116.0 (77-156)	112.0 (71-156)
Diastolic blood pressure (mmHg)	69.0 (34-89)	67.0 (38-96)
Total serum cholesterol (mmol/l)	4.5 (2.7-6.7)	4.7 (3.1-6.3)
LDL-cholesterol (mmol/l)	3.0 (0.8-8.0)	3.1 (0.8-6.8)
HDL-cholesterol (mmol/l)	1.3 (0.6-2.2)	1.4 (0.7-2.2)
HDL_2-cholesterol (mmol/l)	0.3 (0.05-1.1)	0.4 (0.05-1.2)
HDL_3-cholesterol (mmol/l)	0.9 (0.5-1.5)	0.9 (0.5-1.4)
Birth weight (kg)	3.5 (0.6)	3.3 (0.5)
Birth length (cm)	51.6 (2.8)	50.4 (2.1)
Gestational age (weeks)	39.1 (1.6)	39.1 (1.5)
Complications during pregnancy* (%)	28.2	29.6
Mother smoked during pregnancy (%)	19.1	19.7

Values are median (range), birth data are mean (SD). LDL = low density lipoprotein, HDL = high density lipoprotein. including hypertension and diabetes mellitus.*

babies still yielded an inverse relation in males for LDL-cholesterol in young adulthood (-0.24 mmol/l/kg, -0.45 to -0.04) and no changes compared to unadjusted analyses in females. Further, to examine the mediating role of maturation, we added a variable indicating the change in skeletal age per calendar year to the regression models. For both males and females this yielded decreased and non-significant relations for total cholesterol and LDL-cholesterol.

Birth length and lipids

In males and females, total cholesterol and LDL-cholesterol levels were persistently inversely associated with birth length (table 5-3). An inverse association was found in males with HDL-cholesterol and subfractions. In females, mostly a positive relation with HDL-cholesterol and subfractions was found. Adjustment for current weight and height, use of cigarettes and alcohol, for total cholesterol level, systolic blood pressure and BMI of both fathers and mothers, and for breast-feeding did not change the results. Adjustment for change in skeletal age per calendar year still showed associations in young adult males (LDL-cholesterol (n=87): -0.05 mmol/l/cm, -0.10 to -0.003) and in young adult females (LDL-cholesterol (n=59): -0.09 mmol/l/cm, -0.16 to -0.008). In males, there was a negative interaction between birth length and the repeated measurements of the BMI with total cholesterol (p=0.052) and LDL-cholesterol (p=0.03). In females, a positive interaction was found for HDL-cholesterol (p=0.03).

The effects of birth weight and birth length, particularly on total cholesterol and LDL-cholesterol, were similar in direction in males and females, but differed somewhat in magnitude. In figure 5-1, the mean values of total cholesterol by birth weight are given for males. The mean values of LDL-cholesterol by birth length are given for females (figure 5-2).
Based on the data in table 5-2 and in figure 5-1 the analysis of the relation between birth weight and lipid change was performed from the age of 20 years as from this age lipid change was linear and indicative of differences in change by birth weight. Statistically significant associations were found between birth weight and age in males for models with total cholesterol and LDL-cholesterol as dependent variable and similarly in females for LDL-cholesterol. Table 5-4 shows intercepts and slopes that were calculated for subjects with a birth weight of 2 kg and of 4 kg. Both in males and in females with a birth weight of 2 kg the intercepts of total cholesterol and

Table 5-2 Age specific regression coefficients for total and lipoprotein-cholesterol levels depending on birth weight.

		Males		Females	
	Age (yrs)	***n***	**b** (95% CI)	***n***	**b** (95% CI)
Total cholesterol	5-9	*51*	0.03 (-0.23 to 0.29)	*62*	-0.08 (-0.49 to 0.32)
	10-14	*126*	-0.06 (-0.25 to 0.13)	*125*	0.002 (-0.18 to 0.19)
	15-19	*199*	-0.02 (-0.21 to 0.18)	*172*	-0.02 (-0.19 to 0.15)
	20-24	*190*	-0.08 (-0.28 to 0.13)	*149*	0.01 (-0.19 to 0.22)
	25-29	*146*	-0.16 (-0.42 to 0.10)	*110*	-0.17 (-0.43 to 0.08)
	30-37	*151*	**-0.26** (-0.55 to -0.03)	*141*	-0.02 (-0.30 to 0.25)
LDL-cholesterol	15-19	*56*	0.04 (-0.25 to 0.33)	*47*	**0.61** (0.09 to 1.13)
	20-24	*103*	-0.08 (-0.37 to 0.21)	*85*	0.08 (-0.20 to 0.36)
	25-29	*115*	-0.26 (-0.57 to 0.05)	*90*	-0.12 (-0.40 to 0.15)
	30-37	*65*	**-0.51** (-0.93 to -0.09)	*46*	-0.08 (-0.51 to 0.35)
HDL-cholesterol	7-14	*127*	0.02 (-0.05 to 0.08)	*132*	0.02 (-0.09 to 0.014)
	15-19	*199*	-0.01 (-0.07 to 0.04)	*172*	0.05 (-0.02 to 0.12)
	20-24	*190*	0.02 (-0.03 to 0.07)	*149*	0.04 (-0.04 to 0.12)
	25-29	*146*	0.02 (-0.04 to 0.08)	*110*	0.07 (-0.02 to 0.17)
	30-37	*151*	0.03 (-0.05 to 0.10)	*141*	0.02 (-0.09 to 0.13)
HDL_2-cholesterol	15-19	*56*	-0.04 (-0.08 to 0.01)	*47*	-0.09 (-0.21 to 0.04)
	20-24	*102*	-0.01 (-0.06 to 0.03)	*83*	-0.0001 (-0.07 to 0.07)
	25-29	*113*	0.04 (-0.02 to 0.09)	*89*	0.03 (-0.04 to 0.10)
	30-37	*65*	**0.08** (0.01 to 0.15)	*46*	0.07 (-0.06 to 0.21)
HDL_3-cholesterol	15-19	*56*	0.0008 (-0.06 to 0.06)	*47*	0.04 (-0.05 to 0.13)
	20-24	*102*	-0.002 (-0.04 to 0.04)	*83*	**0.06** (0.01 to 0.12)
	25-29	*113*	-0.02 (-0.05 to 0.02)	*89*	0.03 (-0.03 to 0.08)
	30-37	*65*	0.02 (-0.04 to 0.08)	*46*	0.006 (-0.09 to 0.10)

Values based on repeated measurement regression models, stratified by age and gender, with birth weight as independent variable.
n = number of subjects; b = linear regression coefficients (repeated measurement analysis) (mmol/l/kg); 95% CI = 95 percent confidence interval; Bold =p-value < 0.05.

LDL-cholesterol are consistently lower, but there is a more marked rise in lipid levels than in those with a birth weight of 4 kg. For HDL-cholesterol the opposite is found in males.
In males, there was a negative interaction between birth weight and the repeated measurements of the BMI in models with total cholesterol ($p<0.0001$) and LDL-cholesterol ($p<0.0001$), indicating that particularly low birth weight subjects with currently higher relative body weight had increased levels of total cholesterol and LDL-cholesterol. In females, positive interactions were found for HDL-cholesterol ($p<0.0001$), HDL_2-cholesterol ($p=0.06$) and HDL_3-cholesterol ($p=0.02$).

Gestational age

Analyses restricted to subjects born at term ($\geq$ 37 weeks of gestation) still showed inverse associations in males for birth weight with LDL-cholesterol and in females for birth length with total cholesterol and LDL-cholesterol. Inclusion of gestational age in models with either birth weight or birth length did not materially change the results.

Discussion

In this study we found that low birth weight or low birth length are related to higher lipid levels in the blood of children and adolescents particularly when they reach young adulthood.
Associations of birth weight with total cholesterol changed from positive in childhood to inverse at young adulthood. Both in males and females, birth length was inversely associated with lipoprotein-cholesterol levels from childhood to adulthood. These findings were consistent with analyses of birth size in relation to individual change of lipids over time.
Before we can interpret these findings some methodological aspects of the study should be discussed. Birth data were obtained through the parents and without reference to the annual lipid measurements. At the time of birth of the subjects in the present study, there was no national registry of birth data, nor are these available from vital statistics. Furthermore, a large proportion of children in the Netherlands, was, and still is, born at home. Accurate birth weight data may be obtained from maternity center cards and from personal birth announcements. Although the recall from memory of particularly, birth weights by mothers, as opposed to other prenatal and

Table 5-3 Age specific regression coefficients for total and lipoprotein-cholesterol levels depending on birth length.

		Males		Females	
	Age (yrs)	***n***	**b** (95% CI)	***n***	**b** (95% CI)
Total cholesterol	5-9	*39*	-0.03 (-0.10 to 0.04)	*39*	-0.02 (-0.12 to 0.07)
	10-14	*89*	-0.007 (-0.05 to 0.04)	*82*	**-0.07** (-0.13 to -0.01)
	15-19	*129*	-0.003 (-0.05 to 0.04)	*113*	**-0.07** (-0.12 to -0.03)
	20-24	*124*	-0.02 (-0.07 to 0.03)	*102*	**-0.08** (-0.14 to -0.02)
	25-29	*86*	-0.007 (-0.07 to 0.06)	*76*	**-0.08** (-0.16 to -0.001)
	30-37	*90*	-0.04 (-0.12 to 0.03)	*82*	-0.08 (-0.16 to 0.005)
LDL-cholesterol	15-19	*50*	-0.05 (-0.12 to -0.02)	*38*	-0.06 (-0.18 to 0.05)
	20-24	*85*	-0.02 (-0.08 to 0.05)	*71*	-0.08 (-0.16 to 0.007)
	25-29	*76*	-0.04 (-0.12 to 0.03)	*68*	**-0.08** (-0.16 to -0.003)
	30-37	*36*	-0.08 (-0.20 to 0.05)	*31*	-0.02 (-0.14 to 0.09)
HDL-cholesterol	7-14	*89*	-0.010 (-0.03 to 0.009)	*82*	-0.002 (-0.04 to 0.03)
	15-19	*129*	-0.002 (-0.02 to 0.01)	*113*	0.007 (-0.01 to 0.03)
	20-24	*124*	-0.003 (-0.02 to 0.01)	*102*	0.01 (-0.01 to 0.03)
	25-29	*86*	-0.0001 (-0.02 to 0.02)	*76*	0.02 (-0.008 to 0.05)
	30-37	*90*	0.008 (-0.01 to 0.03)	*82*	0.02 (-0.01 to 0.05)
HDL_2-cholesterol	15-19	*49*	-0.007 (-0.02 to 0.006)	*38*	-0.008 (-0.04 to 0.02)
	20-24	*85*	-0.002 (-0.01 to 0.009)	*70*	0.005 (-0.02 to 0.03)
	25-29	*75*	-0.0001 (-0.01 to 0.01)	*68*	0.02 (-0.003 to 0.04)
	30-37	*36*	0.006 (-0.01 to 0.03)	*31*	0.006 (-0.03 to 0.04)
HDL_3-cholesterol	15-19	*49*	-0.006 (-0.02 to 0.01)	*38*	-0.001 (-0.02 to 0.02)
	20-24	*85*	-0.006 (-0.02 to 0.004)	*70*	0.006 (-0.01 to 0.02)
	25-29	*75*	-0.004 (-0.01 to 0.005)	*68*	-0.0008 (-0.02 to 0.02)
	30-37	*36*	-0.006 (-0.02 to 0.01)	*31*	0.002 (-0.02 to 0.02)

Values based on repeated measurement regression models, stratified by age and gender, with birth length as independent variable
n = number of subjects; b = linear regression coefficients (repeated measurement analysis) (mmol/l/cm); 95% CI = 95 percent confidence interval; Bold = p-value < 0.05.

Table 5-4 Lipid change over the age period of 20 to 37 years

Lipid	Gender	Birth weight (kg)	Mean cholesterol level at age 20 (mmol/l)	Slope (mmol/l/ year)	Effect of birth weight*age (β_2)	p-value
Total cholesterol	Males	2	4.94	0.047	-0.0056	0.04
		4	4.78	0.036		
	Females	2	4.99	0.019	-0.00003	0.99
		4	4.95	0.019		
LDL-cholesterol	Males	2	3.19	0.094	-0.025	0.02
		4	3.06	0.045		
	Females	2	2.97	0.064	-0.035	0.02
		4	3.22	-0.010		
HDL-cholesterol	Males	2	1.18	-0.007	-0.00047	0.65
		4	1.60	-0.008		
	Females	2	1.33	-0.0002	0.0026	0.12
		4	1.41	0.005		

*Values are based on the following repeated measurement model: lipidsrepeated = $\beta_0 + \beta_1$*age + β_2*birth weight*age + β_3*birth weight:*

*Written as a function of age the model is: lipidsrepeated = ($\beta_0 + \beta_3$*birth weight)+ ($\beta_1 + \beta_2$*birth weight)*age.*

*Slope of lipid change with age = $\beta_1 + \beta_2$*birth weight*

β_2: regression coefficient indicating interaction effect of birth weight and age on lipid development

LDL = low density lipoprotein; HDL = high density lipoprotein

Figure 5-1 Mean levels of low density lipoprotein (LDL)-cholesterol by birth weight in males.

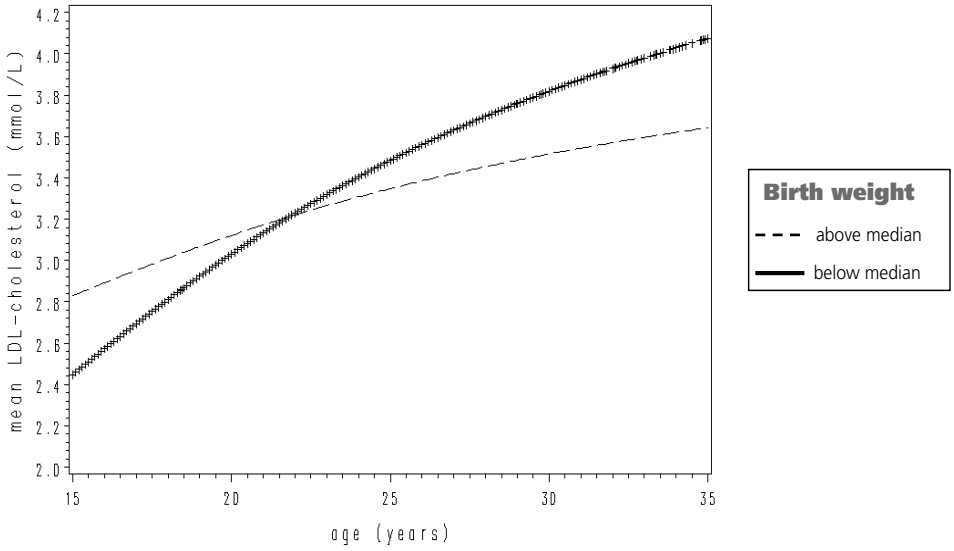
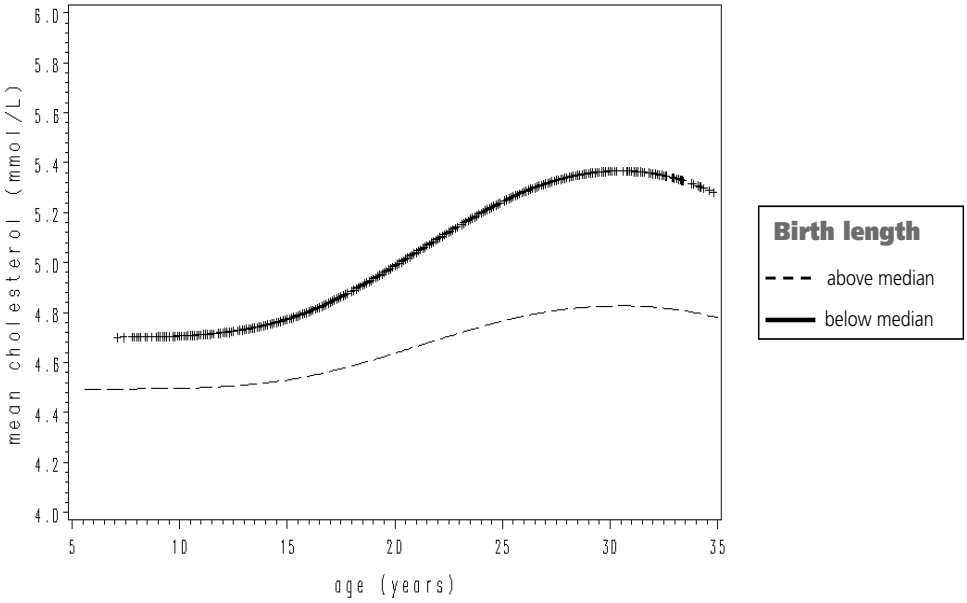


Figure 5-2 Mean levels of total cholesterol by birth length in females.



postnatal characteristics, has been shown to be reliable[26] it may be expected to affect precision. Some imprecision in the self-reported birth weights occurred as there were many rounded-off numbers, in particular when birth weight was reported from memory. A sex adjusted analysis of the pooled data of males and females restricted to subjects whose parents reported birth weights (41% of total group) from maternity center cards of birth announcements showed associations similar to those reported, although a gender specific analysis included too small numbers of subjects to be meaningful. Therefore, misclassification with respect to birth data is most likely to be random and, if anything, may have underestimated the real association for birth weight and lipids. Baseline values of lipids in responders and non-responders to the birth data questionnaire were similar for both males and females. Differences in birth weight and cholesterol levels in those who dropped out of the study before 1992 may have affected the relation. These differences, however, were small as compared to the mean birth weight and baseline total cholesterol levels of the total group. Furthermore, all data provided by the dropouts are included in the analysis. In a previous report, we have shown that secular trends in lipid levels in this study group, if any, are small[19] and not likely to explain the results with respect to birth data.

One study showed an association between low birth weight and increased triglyceride levels in white male and female children born at term, but not with variables examined in the present study such as total-, LDL- and HDL-cholesterol.[11] It was further shown that differences in cardiovascular risk factors across groups of British children from areas with high and low cardiovascular mortality were unaffected by standardisation for birth weight.[27] Another study showed that in prepubertal children total cholesterol was inversely related only to birth length.[10] Thus, in these studies an association between birth weight and lipids could, except for triglycerides, not be shown. Recently, a systematic review of the effect of birth weight on total cholesterol showed a significant decrease in total cholesterol of 0.05 mmol/l per kg higher birth weight.[16] We found an inverse relation with birth weight in adulthood, particularly in males, which is in line with previous reports.[13] The inverse relation between lipids and birth length in our study was somewhat stronger in females than in males, which is to the authors' knowledge not described earlier for these age groups.

Like in other studies,[13] the relations found in the present study were independent of gestational age and of life-style factors including use of

oral contraceptives, although we did not measure current dietary habits. Walker et al. reported that the relation between birth weight and adult blood pressure level attenuated when adjusted for parental blood pressure level.[28] Adjustment for parental cardiovascular risk by total cholesterol and blood pressure levels and BMI in our analysis did not change the findings. Therefore, the relations found seem to be independent of familial aggregation of cardiovascular risk factors at least as measured in parents and offspring at baseline. It has been postulated that lower social class in early life is associated with risk of ischaemic heart disease,[29] while socio-economic status is also associated with fetal growth.[30] It was shown that for the inverse relation between birth weight and blood pressure in 50 year olds, confounding by social-economic status is not a very likely explanation.[31] There is evidence to suggest that prolonged breast feeding is associated with increased low density lipoprotein cholesterol levels and higher death rates in adulthood.[12,27] Adjustment for breast-feeding, however, did not change our results.

The mechanism by which birth size could be related to cholesterol levels is largely unknown. It is postulated that intra-uterine growth retardation, particularly during late gestation, results in a disproportionate effect on liver growth, which may lead to altered lipoprotein cholesterol metabolism.[13] Earlier findings indicated a relation between an adverse intra-uterine environment during the first two trimesters of pregnancy and adult obesity.[32] The fact that obesity has been repeatedly reported to modify relations between birth size and cardiovascular risk factors, diabetes mellitus and incident coronary heart disease points at the importance of later life exposures as well,[2,6,7] although others reported such modification to be absent.[8] Previously, we have shown in this study group that the inverse relation between birth weight and blood pressure is mediated by the BMI later in life.[33] The interaction between BMI and birth size in males suggests that total cholesterol and LDL-cholesterol are particularly raised in those with low birth weight or length and with high current body mass. Data in females suggests that HDL-cholesterol is lower in those with low birth weight and high current body mass. A mediating effect of current body size in the relation between infant body mass and adult cholesterol levels was also found in one other study.[12]

Relations in children between birth weight and blood pressure have been reported to amplify with increasing age.[34] This could in principle be due to tracking of blood pressure together with dispersion of the distribution with

increasing age, or to detracking of blood pressure such that children with low birth weight change to higher ranks in the blood pressure distribution with increasing age. Our findings with regard to lipid levels, but particularly lipid change with age, indicate detracking (excessive change) of lipids by birth weight to the extent that the relation even reverses. This reversal appears to express after puberty. In another study, restriction of analyses to prepubertal children yielded no relation between birth weight and lipids.[11] Our data suggest that the rate of maturation is involved and may be a confounding factor in the birth weight and lipid relation. Birth length showed a consistent inverse relation with lipids. Like with birth weight the associations between birth length and lipids appeared to get slightly stronger in adulthood. Adjustment for rate of maturation left the relation between birth length and lipids unchanged. This supports our observation that low birth length is related to less favourable lipid levels early in life, remaining throughout adulthood regardless of growth related factors.

Our data support the view that differences in levels of lipids may have their origins in early life. Differences amount to almost 0.5 mmol/l per kilogram difference in birth weight for LDL-cholesterol in adulthood and are as such important. These differences are graded and particularly pertain to children born at term. Further, as the relations between birth size and lipids in this study were obtained without any adjustment, unlike relations with blood pressure in these age groups,[33] these findings may have implications from a public health point of view.

We conclude that size at birth is related to the natural history of lipids from childhood to young adulthood. Children born with low birth weight or length have higher adverse lipid levels particularly when they reach young adulthood.

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5.2

Variation in the IGF-1 gene, birth size and cardiovascular risk in the young

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Abstract

Background

The potential role of insulin-like growth factor (IGF)-1 in cardiovascular pathophysiology has raised considerable interest over the past years. A common functional polymorphism in the promoter region of IGF-1 may play an important role in the development of type 2 diabetes and myocardial infarction. Our aim was to study the relation of this polymorphism with birthsize and cardiovascular disease risk in young adulthood.

Methods

Data of 2 prospective population based studies in men and women aged at baseline between 5 and 21 years were pooled. Birth characteristics were collected using school health records and questionnaires. Repeated measurements of cardiovascular risk factors were extended with carotid intima-media thickness and aortic pulse-wave velocity measurements during the last visit. A functional polymorphism in the promoter region of the IGF-1 gene was determined in 1079 subjects.

Results

70% were homozygous carriers of either the 192 base pairs (bp) or the 194-bp allele or heterozygous carriers of both the 192-bp allele and the 194-bp allele (wild type group), 30% was non-carrier or heterozygous carrier of either the 192-bp allele or the 194-bp allele (variant carriers). At the age of 30 years, the wild type group was on average 1.7 cm taller compared to variant carriers (p=0.006), while they were born with a 0.3 cm larger birth length (p=0.13). No associations were observed between IGF-1 genotype and body mass index, blood pressure, serum cholesterol and carotid intima-media thickness, aortic pulse-wave velocity.

Conclusion

Birth length and subsequent height growth from childhood into young adulthood are affected by IGF-1 genotype. In our age group of young adults, no relation could be found between the IGF-1 genotype and cardiovascular risk factors and measures of vascular damage.

Introduction

Insulin-like growth factor (IGF-1) is an essential regulator of developmental growth as IGF-1 levels are strongly associated with both fetal and postnatal growth.[1-4] Over the last years, IGF-1 has been suggested to be involved in the pathophysiology of type 2 diabetes and cardiovascular disease.[5-8] Subjects with low circulating IGF-1 levels have a significantly increased risk of myocardial infarction.[9, 10] There is also strong evidence that IGF-1 is a critical determinant of vascular growth responses in vitro and in vivo.[11-13] A direct interaction between the activity of the growth hormone (GH) - IGF-1 axis and the endothelium may play a role. It is possible that the modulating effect of IGF-1 on nitric oxide synthesis is a mediating factor,[14] together with an anabolic effect on the myocardiocytes.[15] Recently, a polymorphism in the promoter region of IGF-1 on chromosome 12q21 has been identified, which has been found to be related to IGF-1 levels.[16, 17] In elderly individuals, Vaessen et al. showed that non-carriers of the wild-type allele of this polymorphism had an increased risk of type 2 diabetes mellitus and myocardial infarction and also low circulating IGF-1 levels, reduced body height and low birth weight.[18, 19] However, a second study in young adults showed no relation between the IGF-1 promoter polymorphism and either type 2 diabetes, birth size and growth and showed an increase in IGF-1 levels in non-carriers.[20] To expand upon these contradicting results, we examined the relation between this functional polymorphism in the promoter region of the IGF-1 gene and birth size, childhood growth and cardiovascular risk in young adulthood.

Methods

Subjects

Data of the Atherosclerosis Risk in Young Adults (ARYA) study[9] and the Epidemiological Preventive Study Zoetermeer (EPOZ)[10] were pooled to increase statistical power for analysis. The ARYA study was approved by the Medical Ethics Committee of the UMC Utrecht and the EPOZ study was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam. All participants gave written informed consent.

ARYA-STUDY

From the Municipal Health Service medical records were obtained from all young adults born between 1970-1973 who attended secondary school in the city of Utrecht in the Netherlands. The available charts (n=15,592) were checked for the presence of adequately registered birth weight and at least one blood pressure measurement during adolescence. The median number of childhood measurements was 1 (range 1-4). All young adults with a complete chart (n=4,208; 27%) were invited by mail to participate. Eventually, 750 subjects completed both visits to the research center within a 3-week period.[21]

EPOZ-STUDY

Families with children aged 5-19 years old who were living in 2 districts in the Dutch town of Zoetermeer were invited to participate in a cross-sectional population based study on risk-indicators for chronic disease. All participants were included between 1975 and 1978.[22] Zoetermeer is a suburban residential community of in that time about 55,000 inhabitants which is situated near The Hague in the Netherlands. Of all persons aged 5-19 years, 4,649 (82 %) took part in the study. From this group, a random sample of 596 children was selected for annual follow-up in a study on the natural history of cardiovascular risk factors and their determinants. The median number of visits was 15 (range 2-19). The median follow-up time for the present analyses was 23 years. The response for the majority of visits gradually declined to 83% in 1992. For the atherosclerosis measurements in 2002, the response was 61%. The present study involved 362 persons that participated in the last visit.

IGF-1 GENOTYPE

For both studies, DNA was isolated at the last visit for IGF-1 genotyping in the same laboratory using the same techniques. Polymerase chain reaction was performed using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine (CA) repeat 1 kb upstream of the human IGF-1 gene.[23] The reaction was carried out in a final volume of 10 ml 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-GCTAGCCAGCTGGTGTTATT-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).

Measurements

Clinical data were obtained without knowledge of IGF-1 genotype. At each visit, anthropometric measurements were performed with indoor clothes without shoes and a written questionnaire was completed on alcohol intake, smoking and highest education. In ARYA, information on birth characteristics was obtained from filed records of child health facilities. In EPOZ, information about birth weight was collected both from the mothers at the baseline visit and from the participants themselves at the last visit. Total cholesterol and HDL-cholesterol were measured using an automatic enzymatic procedure (ARYA: Vitros950 dry-chemistry analyser (Johnson & Johnson, Rochester, New York, USA); EPOZ: Roche Diagnostics (Mannheim, Germany)). At each visit, blood pressure was measured twice in the left brachial artery in sitting position (ARYA: semi automated device (Dinamap, Critikon, Tampa California, USA); EPOZ: Hawksley random-zero sphygmomanometer (Hawksley and Sons, Ltd)). The mean of 2 consecutive measurements was used in the analyses. Mean arterial blood pressure (MAP) was calculated as [diastolic blood pressure + (1/3 * (systolic blood pressure - diastolic blood pressure))]. Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure. The indicators of atherosclerosis and arterial stiffness used in these analyses were measured once at the last examination phase. Intima-media thickness (IMT) was measured by recording of ultrasonographic images of both the left and right carotid artery, using a 7.5 MHz linear array transducer (ARYA: Acuson Aspen; EPOZ: ATL Ultramark IV (Advanced Technology Laboratories, Bethel Washington, USA)). The lumen-intima interface and the media-adventitia interface of the near and far wall of the distal common carotid artery were measured off-line. The protocol has been described in detail elsewhere.[24] In short, the common carotid intima-media thickness was determined as the average of maximal near and far wall measurements of both left and right side. In ARYA reproducibility was assessed by repeating the common carotid IMT

of 21 participants at the same visit by another sonographer. Absolute mean difference of IMT was 0.012 mm (standard error 0.004). The intraclass correlation coefficient for repeated measurements was 0.84. The PWV was determined using an automated device (ARYA: SphygmoCor device (PWV Medical, Sydney, Australia); EPOZ: Complior (Colson, Garges-lès-Gonesse Cx, France)), which allowed an online pulse wave recording and automatic calculation of PWV with 2 transducers, 1 positioned at the base or the neck for the common carotid artery and the other over the femoral artery. The average of 10 successive waveforms 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 analysis. Reproducibility of the PWV measurement was only evaluated in the ARYA study. A subset of 25 participants had their PWV re-measured several weeks after their first visit. Absolute mean difference (standard error) in PWV of the repeated measurements between visits was 0.12 m/s (0.45). The intraclass correlation coefficient (ICC) for repeated measurements was 0.67. Since the repeated measurements were performed on 2 different occasions, the moderate ICC could be explained in part by the variability in blood pressure over time.

Data analysis

The present analyses are based on 1,079 subjects in whom IGF-1 genotype was determined. The wild-type allele in the promoter region of the IGF-1 gene had a length of 192 base pairs, which is equivalent to 19 cytosine-adenine (CA) repeats. As the levels of IGF-1 are similar in homozygous carriers of the wild-type allele as well as homozygous carriers of the 194-bp allele,[25] we grouped on one hand all homozygous carriers of either the wild-type allele or the 194-bp allele and all heterozygous carriers of both a 192-bp allele and a 194-bp allele (wild type group). The second group comprised the non-carriers and all heterozygous carriers of either the 192-bp allele or the 194-bp allele (variant carriers).

First, a multivariate linear regression model was used to study the relation between genotypes and birth size. Due to differences between men and women in birth size, gender was added as a possible confounder. Secondly, repeatedly measured cardiovascular risk factors blood pressure, weight, height, total- and HDL-cholesterol were studied as outcome. As these repeated measurements within children were mutually dependent observations we used unbalanced repeated measures analysis (Proc Mixed, SAS).[26] In the analysis each subject's response was assumed to have a linear

relation with age and to have its own random intercept and slope and therefore a random covariance structure was chosen. This model was used to obtain mean cardiovascular risk factor by genotype adjusted for gender and age. An interaction term genotype*age was added. Finally, a multivariate linear regression model was used to study the relation between genotype and the measures of vascular damage as continuous variables (Proc GLM, SAS). Because of the differences between men and women in the measures of vascular damage, gender was always included. As PWV is related to wall elasticity and thus depends on blood pressure, analyses using PWV were adjusted for mean arterial pressure.[27]
Because the association between IGF-1 genotype and both cardiovascular risk factors and measures of vascular damage did not differ by study population, pooled results are presented only. Still, in order to correct for possible differences between the studies, all analyses were adjusted for study. Data were analyzed using the SAS statistical software package (SAS/STAT Version 8.02, SAS Institute, Cary, NC).[26]

Results

Table 5-5 shows the general characteristics of the two cohorts according to gender. Men (49.0%) were born significantly heavier and taller. At young adulthood, blood pressure, weight, height, levels of total cholesterol and measures of vascular damage were higher in men, and HDL-cholesterol was lower in women.

IGF-1 genotype

The IGF-1 genotype was determined in 728 (97.2% of the 750) participants of the ARYA study (47% male) and in 351 (96.7% of the 362) participants of the EPOZ study (54% male). Table 5-6 shows that 9 alleles were identified. Genotype and allele distribution were in Hardy-Weinberg equilibrium (for the ARYA study p=0.44 and for the EPOZ-study p=0.56).

Birth size and growth

The wild type group had a mean birth length of 51.1 cm (50.9-51.4) and the variant carriers had a mean birth length of 50.8 cm (50.5-51.2). This 0.3 cm difference was not statistically significant (p=0.13). The wild type group had a mean birth weight of 3.41 kg (95% confidence interval 3.37-

3.48), which was not significantly different from the mean birth weight of the variant carriers (3.40 kg (3.33-3.46); p=0.68). The associations between IGF-1 genotype and birth height and weight were similar in men and women (p=0.99 respectively 0.92).
The increase in height from childhood into adulthood differed per genotype group (p=0.006). Thirty year-old men from the wild type group had a mean height of 183.3 (182.6-184.0) cm compared to a mean height of 181.6 cm (180.6-182.6) cm in male variant carriers of either a 192-bp allele or a 194-bp allele. In women, the differences were similar (170.0 (169.3-170.6) respectively 168.4 (167.5-169.3)).

Cardiovascular risk factors and vascular damage

No differences were seen in cardiovascular risk factors between genotype groups (table 5-7). Common carotid intima-media thickness and pulse wave velocity did not differ between the genotypes either (table 5-8).

Table 5-5 General characteristics of the study populations.

Characteristic	Men			Women		
	ARYA (n=340)	EPOZ (n=189)	ARYA+EPOZ (n=529)	ARYA (n=388)	EPOZ (n=162)	ARYA+EPOZ (n=550)
Birth characteristics						
Birth weight (kg)	3.48 (0.5)	3.50 (0.6)	3.49 (0.6)	3.36 (0.5)	3.33 (0.6)	3.35 (0.6)
Birth length (cm)	51.3 (2.4)	51.7 (3.0)	51.4 (2.6)	50.4 (2.5)	50.5 (2.1)	50.4 (2.4)
Last visit						
Age (years)	28.2 (0.9)	38.1 (4.4)	32.6 (5.7)	28.2 (0.9)	36.9 (4.5)	31.4 (5.1)
Height (m)	1.84 (0.07)	1.82 (0.07)	1.83 (0.07)	1.70 (0.06)	1.69 (0.08)	1.70 (0.07)
Weight (kg)	83.5 (13.7)	85.2 (13.6)	84.1 (13.7)	71.1 (14.6)	72.7 (14.9)	71.6 (14.7)
Body mass index (kg/m^2)	24.6 (3.7)	25.6 (3.4)	25.0 (3.6)	24.4 (4.5)	25.4 (4.7)	24.8 (4.6)
Waist-to-hip ratio	0.88 (0.06)	0.90 (0.06)	0.89 (0.06)	0.81 (0.06)	0.80 (0.08)	0.81 (0.07)
Alcohol (n (%)): 0 glasses/day	31 (12.9)	27 (14.2)	58 (13.5)	70 (24.7)	47 (28.7)	117 (26.2)
≤1	111 (46.2)	35 (18.4)	146 (33.9)	161 (56.9)	63 (38.4)	224 (50.1)
1-2	59 (24.6)	86 (45.3)	145 (33.7)	46 (16.3)	44 (26.8)	90 (20.1)
3-5	34 (14.2)	30 (15.8)	64 (14.9)	6 (2.1)	3 (1.8)	9 (2.0)
≥6	5 (2.1)	12 (6.3)	17 (4.0)	0 (0)	7 (4.3)	7 (1.6)
Highest education (%):						
elementary school	3.3	0	1.9	1.8	0.6	1.4
secondary school	19.2	23.5	21.1	21.5	31.2	25.1
lower/intermediate vocational training	41.7	50.8	45.6	38.5	49.4	42.4
higher vocational training/university	35.8	25.7	31.4	38.2	18.8	31.1
Current smokers (%)	33.3	40.0	36.3	26.5	28.7	27.3
Contraceptive pill use (%)	NA	NA	NA	65.4	41.9	56.9
Total cholesterol (mmol/l)	4.86 (0.94)	5.22 (1.05)	5.02 (1.01)	4.84 (0.82)	4.91 (0.87)	4.87 (0.84)
HDL-cholesterol, (mmol/l)	1.30 (0.30)	1.12 (0.29)	1.22 (0.31)	1.58 (0.36)	1.37 (0.32)	1.50 (0.36)
Systolic blood pressure (mmHg)	130.4(12.1)	124.1 (13.7)	127.6 (13.2)	120.2 (12.1)	115.5 (11.7)	118.5 (12.2)
Diastolic blood pressure (mmHg)	72.5 (7.2)	82.4 (9.2)	76.9 (9.5)	70.8 (8.4)	76.4 (8.9)	72.8 (9.0)
Mean Arterial Pressure (mmHg)	91.8 (8.0)	96.3 (9.8)	93.8 (9.1)	87.3 (9.0)	89.4 (9.2)	88.1 (9.2)
Pulse Pressure (mmHg)	57.8 (9.3)	41.6 (10.1)	50.7 (12.6)	49.4 (7.9)	39.1 (7.9)	45.6 (9.3)
Heart rate (beats per minute)	63.4 (9.5)	65.6 (11.2)	64.4 (10.3)	66.4 (8.8)	69.2 (10.9)	67.4 (9.7)
Mean common carotid maximum IMT(mm)	0.67 (0.09)	0.72 (0.08)	0.69 (0.09)	0.69 (0.08)	0.69 (0.07)	0.69 (0.08)
Pulse Wave Velocity (m/s)	6.26 (0.76)	9.98 (1.50)	7.91 (2.18)	5.70 (0.69)	8.63 (1.15)	6.77 (1.67)

Values are expressed as mean (standard deviation) in case of continuous variables, and as percentages in case of categorical variables. For definitions see text.
n: number; NA: not applicable; HDL: high-density lipoprotein; IMT: intima-media thickness

Table 5-6 IGF-I promoter polymorphism- and 192-bp/ 194-bp genotype frequency distributions and of the study populations.

Polymorphism		Men		Women	
Allele (bp)	**$(CA)_n$**	**ARYA** (n=340)	**EPOZ** (n=189)	**ARYA** (n=388)	**EPOZ** (n=162)
176	11	0 (0)	1 (0.3)	1 (0.1)	0 (0)
186	16	2 (0.3)	0 (0)	0 (0)	0 (0)
188	17	6 (0.9)	8 (2.1)	12 (1.5)	5 (1.5)
190	18	29 (4.3)	23 (6.1)	46 (5.9)	19 (5.9)
192 (wild-type)	19	439 (64.6)	241 (63.8)	493 (63.5)	206 (63.6)
194	20	142 (20.9)	73 (19.3)	145 (18.7)	63 (19.4)
196	21	49 (7.2)	25 (6.6)	58 (7.5)	28 (8.6)
198	22	13 (1.9)	6 (1.6)	21 (2.7)	3 (0.9)
200	23	0 (0)	1 (0.3)	0 (0)	0 (0)
Genotypes					
Homozygous *194-bp*		17 (5.0)	6 (3.2)	15 (3.9)	4 (2.5)
Homozygous *192-bp*		145 (42.6)	78 (41.3)	158 (40.7)	62 (38.3)
Heterozygous *194-bp/192-bp*		89 (26.2)	50 (26.5)	90 (23.2)	47 (29.0)
Non-carrier		10 (2.9)	9 (4.8)	13 (3.4)	6 (3.7)

Data are n (%). The allele distribution is based on 2 alleles per participant.
bp: length of PCR product in base pairs; $(CA)_n$: number of cytosine-adenine repeats;
wild-type allele: most frequent allele in this population;
IGF-1: insulin-like growth factor-1

Table 5-7 IGF-1 promoter polymorphism and cardiovascular risk factors in young adulthood.

Cardiovascular risk factor	Genotype		
	wild type group	variant carriers	p-value
Systolic blood pressure (mmHg)	125.1 (124.2-126.1)	124.6 (123.3-125.8)	0.43
Diastolic blood pressure (mmHg)	73.8 (73.3-74.4)	73.8 (73.0-74.6)	0.98
Weight (kg)	79.8 (78.7-80.9)	79.0 (77.6-80.3)	0.22
Body mass index (kg/m²)	25.0 (24.7-25.3)	25.0 (24.6-25.4)	0.91
Total cholesterol (mmol/l)	5.0 (4.9-5.1)	5.0 (4.9-5.1)	0.62
LDL-cholesterol (mmol/l)	3.07 (2.99-3.14)	3.13 (3.02-3.23)	0.31
HDL-cholesterol (mmol/l)	1.35 (1.32-1.37)	1.35 (1.31-1.39)	0.90

Values are means (standard error of the mean) adjusted for age and gender.
wild type group: homozygous 192, homozygous 194, heterozygous 192/194 or 194/192
variant carriers: non-carriers 192 and 194, heterozygous 192 without 194 and 194 without 192
IGF-1: insulin-like growth factor-1; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Table 5-8 IGF-1 promoter polymorphism and vascular damage in young adulthood.

Parameter for vascular damage	Genotype		
	wild type group	variant carriers	p-value
Maximal common carotid intima-media thickness (mm) *	0.669 (0.661-0.678)	0.670 (0.659-0.681)	0.90
Pulse Wave Velocity (m/s) †	6.72 (6.61-6.68)	6.72 (6.61-6.83)	0.52

Values are means for men and women combined at age 30 (standard error of the mean).
** : adjusted for age, gender.*
† : adjusted for age, gender, mean arterial pressure, height.
IGF-1: insulin-like growth factor-1.

Discussion

Our findings show a clear association between a polymorphism in the promoter region of the IGF-1 gene and changes in body height growth from childhood into adulthood. Already at birth, carriers of this polymorphism were taller, although the difference was not statistically significant. However, no association was seen between this polymorphism and birth weight. Also, no relation was found between this polymorphism and cardiovascular risk factors measured from childhood into young adulthood and vascular damage at young adulthood.

Before interpreting our results, some methodological issues need to be discussed. Circulating levels of IGF-1 do not necessarily reflect the local production of IGF-1 in pancreatic beta cells, myocardium or bone cells. Over 90% is bound to specific IGF binding proteins. For this reason we examined a genetic polymorphism in the promoter region of IGF-1 on chromosome 12q, that appears to be functional as studies showed that it is related to the IGF-1 level and body height.[16-18] Two longitudinal studies were pooled in order to increase statistical power. Both cohorts consisted of subjects who were included in childhood or young adolescence and their last visit took place when they were young adults. Although the EPOZ participants were older than the ARYA participants, the associations between IGF-1 genotype and the different outcomes did not differ by study. Nevertheless, a dichotomous variable for study was added in all analyses in order to take possible measurement and study differences between the studies into account. In the ARYA study, measurement error is minimized by using birth- and adolescent characteristics obtained from filed medical health records, instead of parental recall and self-report. Although only 750 of the 4,208 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 relations under study did not occur. Data at birth and adolescence did not differ between the responders and the non-responders, and the obtained data between the participants with and without a pulse wave velocity measurement were similar. Furthermore, the invited adults in the ARYA study were unaware of their IGF-1 genotype, 87% of our participants had a 19-CA repeat in the promoter region of the IGF-1 gene similar to the EPOZ study and similar to other population based studies.[16-18, 20, 28, 29] During the 27-year follow-up period in the EPOZ study, a single research assistant performed the vast majority of the average of 15

measurements per participant, reducing measurement variation. The large numbers of measurements that were performed within the EPOZ study in each individual further enhanced more accurate estimation of subject's true underlying levels at every age. The population selected for follow-up was a random sample from the youngsters who participated in the baseline study. As BMI, blood pressure and total cholesterol values at previous EPOZ visits were similar among those who were and those who were not lost to follow-up, we do not think that selective loss-to-follow-up has affected our results. Accuracy of maternally reported birth size has been shown to be higher for the eldest child and in younger mothers.[30] As most participants in the EPOZ study were firstborns and mean maternal age at baseline was 41 years, we think that this added to the validity of reported birth size.

Similar to our study, the Rotterdam Study[18] and a U.K. study[20] found that homozygous carriers of the 192-bp allele were the tallest subjects. The Nurses Health Study described a non significant test for trend for body height in women (p=0.47) but did not mention body height per genotype.[17] Our data further showed that already at birth the wild type group was 0.3 cm taller compared to the variant carriers. However, like Frayling et al.[20] we could neither confirm the results of Vaessen et al.[19] who found that non-carriers of the 192-bp allele had a low birth weight nor the results of Arends et al. The latter study showed an association between birth length, birth weight, head circumference and an intronic repeat in the promoter region of the IGF-1 gene in a cohort of 124 short children with a mean age of 7 years and born small for gestational age.

Our data showed no relation between the IGF-1 genotype and cardiovascular risk factors at a young age. The Rotterdam Study has previously shown that homozygous carriers of the 192-bp allele had a lower risk to develop type 2 diabetes mellitus and myocardial infarction.[18] Within our study, we did not find an association between genetic variation in the IGF-1 gene and arterial wall thickness and arterial stiffness at young adulthood. Ultrasonographically measured common carotid intima-media thickness is a recognized measure of atherosclerosis24, [31-34] which correlates well with pathological measurements of human carotid arteries[35] and is an established risk indicator of cardiovascular disease. Finally, pulse wave velocity has repetitively been related to type 2 diabetes mellitus, myocardial infarction and mortality in adults and elderly subjects.[36, 37]

Given the results of the Rotterdam Study,[18] we would have expected a relation between IGF-1 genotype and cardiovascular risk factors and vascular

damage. It is possible that another pathogenic mechanism is responsible for the association between IGF-1 and CVD than convential cardiovascular risk factors. IGF-1 is a potent survival factor preventing apoptosis of vascular smooth muscle cells[12] as well as stimulating the proliferation[38] and migration of vascular smooth muscle cells.[39] IGF-1 may contribute to the balance between apoptosis and survival in atherosclerotic lesions.[40] Circulating IGF-1 levels stimulate nitric oxide, which is essential for the integrity of the vessel wall. Decreased NO production by vascular endothelium, due to low IGF-1 levels, might be another pathway linking IGF-1 levels and CVD. In addition, IGF-1 can promote vasodilatation through activation of potassium channels,[41, 42] and aortic angiogenesis through stimulation of tube-forming activity and migration of vascular endothelial cells.[43] Also, IGF-1 has been shown to inhibit the adherence of human peripheral blood monocytes to an endothelial cell line.[44]

In conclusion, our results indicate that a functional polymorphism in the promoter region of the IGF-1 gene is associated with childhood growth, whereas no significant association is seen with birth size. Our results do not support the view that this polymorphism is associated with increased cardiovascular risk or vascular damage at young adulthood.

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GENERAL DISCUSSION

6

Over the past century, research into the aetiology of cardiovascular diseases (CVD) has focussed on adult risk factors, such as blood pressure, cholesterol, diabetes, diet, physical exercise and smoking. Advances in the diagnosis and treatment of coronary heart disease have increased the lifespan and quality of life for many patients. It is estimated that about half of the dramatic decline in CVD between 1980 and 1990 is attributed to reductions in risk factors, while the other half has been attributed to improved treatment of clinically apparent disease.[1] However, CVD is still the major cause of mortality in most western societies and an increasing health threat in developing countries. CVD still claim large parts of health budgets and are responsible for many quality adjusted life years lost.[1-3] Since the 1980s, there has been a revived interest in the early origins of adult disease. It has been shown that atherosclerosis is present and progresses for many decades before the onset of clinical manifestations.[4] Furthermore, the dramatic increase in the prevalence of childhood obesity in the past three decades[5-9] may have implications for children's current and future cardiovascular health. Finally, family dynamics may play an important role in the development of childhood risk factors and subsequent vascular changes in adulthood.

The work presented in this thesis aims at exploring early determinants of vascular damage in healthy adults and gaining insight into the aetiology and the development of later cardiovascular disease.

This chapter will focus on the main findings, considered in the light of current knowledge and ongoing research in the field of the early origins of adult disease. Subsequently, some methodological issues concerning the EPOZ-study are discussed. Finally, I will elaborate on the importance of cardiovascular risk assessment in the young and consider the implications for public health and future research.

Main findings

Prospective studies have shown that cardiovascular disease aggregates in families.[10, 11] This is probably partly due to familial aggregation of important cardiovascular risk factors such as blood pressure and plasma cholesterol.[12, 13, 14-17] and was shown to be detectable in children at a very young age.[18]

In longitudinal studies, children with a family history of hypertension were shown to have persistently higher blood pressure levels than children without

such a history over follow-up periods of up to 10 years.[19-21] Still the usefulness of recording a positive family history in the prediction of hypertension in the individual is considered to be limited.[22, 23] It is of particular interest to know whether parental blood pressure levels are predictors of their offspring's subsequent blood pressure development into young adulthood and whether this holds for the whole distribution of parental blood pressure. In our 27-year follow-up study, actual parental blood pressure showed to be an important predictor of blood pressure development from childhood into young adulthood. Our findings add to the current knowledge in several ways. The impact of parental blood pressure was present over the whole distribution of parental blood pressure levels, meaning the lower the parental blood pressure the lower the blood pressure levels of the offspring. Furthermore, our data suggest that the impact of parental blood pressure starts at an early age and is strong and long lasting. A genetic basis for the differences found in our study is supported by the fact that familial differences are not only present at a young age when environments are shared, but persist into young adulthood when environments become more different.

Already at young ages, family dynamics also play an important role in the development of obesity.[24] In a retrospective cohort study it was shown that parental obesity doubles the risk of adult obesity among both obese and nonobese children under 10 years of age.[25] The impact of parental body mass index (BMI) on the manifestation of overweight in offspring has been shown in cross-sectional studies as well.[6, 26-28] In our study, we used actual measurements of parental BMI and repeatedly measured BMI in offspring from childhood into adulthood. We showed that the impact of parental BMI was present over the whole range of parental BMI levels, meaning that the lower the parental BMI the lower the child's BMI. Furthermore, parental BMI was also associated with increased cardiovascular risk in the offspring, already at young ages. Parental and children's BMI did not only affect the current health status but were also predictors of future vascular damage and thereby the risk of manifest cardiovascular disease in later life. These results underline the importance of early prevention of excessive weight gain and the necessity of involving the parents in fighting the obesity epidemic.

Thus, parental risk status is important for one's cardiovascular risk profile. But which childhood risk factor determines cardiovascular risk at later age? The study of the relation between cardiovascular risk factors in children and clinically manifest CVD requires a follow-up of many decades as in most

cases manifest CVD occurs after the fifth decade. As an alternative, we can estimate the relation between cardiovascular risk factors in childhood and early vascular damage, measured non-invasively. Knowledge about the predictive value of childhood cardiovascular risk factors may result in identification of young subjects at high risk of CVD. Early detection of those at highest risk can subsequently lead to early treatment or preventive measures. We found that BMI, systolic blood pressure and total cholesterol measured during childhood were strongly related to both carotid intima-media thickness (IMT) and presence of plaques in the carotid arteries. For example, IMT increased with 0.025 mm per kg/m² increase in childhood BMI, measured 20 years before assessment of IMT. The risk of having plaques in the carotid arteries increased with 60% per mmol/l increase in childhood total cholesterol level. Four other longitudinal studies showed that BMI, systolic blood pressure and low-density-lipoprotein cholesterol measured both in childhood and in adolescence predicted carotid IMT in adulthood.[29-32] However, the associations were not found in the Thousand Families cohort Study.[33] In our study, we also found relations between childhood risk factor levels and arterial stiffness, measured by carotid-femoral pulse-wave velocity (PWV) and carotid distensibility. Those who had higher blood pressure levels in childhood had stiffer arteries 20 years later, suggesting that blood pressure even in early childhood plays a role in the process of arterial stiffening. This is consistent with recent findings of the Bogalusa Heart Study.[34] Notably, we found an association between childhood systolic blood pressure and both PWV and distensibility in adult offspring indicating an increase in arterial stiffness with increasing blood pressure. Similar results were found in our study for BMI and common carotid distensibility as marker for arterial stiffness. Hyperglycaemic conditions can lead to increased arterial stiffness by increased collagen cross linking due to non-enzymatic glycation.[35-37] As BMI is known to be strongly related to insulin[38] this may be the mechanism underlying our observation of an association between BMI and carotid distensibility. However, no relation was found of PWV with BMI, measured concurrently, while unexpected inverse associations were found with childhood BMI. We have no explanation for the difference in findings of BMI with carotid distensibility and PWV. A possible explanation for the conflicting result is that in obese persons PWV is structurally underestimated as a proper recording of the pulse wave at the femoral artery is more difficult in obese persons. Finally, we found a positive relation between PWV and total cholesterol, measured concurrently, but no significant association was

found of PWV or carotid distensibility with total cholesterol, measured in childhood.

There is evidence that cardiovascular disease may partly have its origins in the fetal phase.[39-42] Birth size has been found to be inversely associated with cardiovascular risk factors such as blood pressure, insulin resistance and obesity in adult life.[43-45] Longitudinal data on the relation between birth size and blood lipid levels in later life are limited in the young and to some extent inconsistent.[46-52] Within the EPOZ study, size at birth was related to the natural history of lipids from childhood to young adulthood. Children born with low birth weight or length obtained higher levels of adverse lipids particularly when they reached young adulthood. Associations of birth weight with total cholesterol changed from positive in childhood to inverse in young adulthood. Both in males and females, birth length was inversely associated with total cholesterol levels from childhood to adulthood. The mechanism by which birth size could be related to cholesterol levels is largely unknown. Intra-uterine growth retardation, particularly when occurring during late gestation, results in a disproportionate effect on liver growth, which may lead to altered lipoprotein cholesterol metabolism.[50]

Another factor that influences one's cardiovascular risk is alcohol consumption. The relation between alcohol intake and risk of CVD has been shown to be U-shaped, suggesting a higher risk of cardiovascular disease in non-drinkers and heavy alcohol consumers compared to those with moderate alcohol intake.[53] Mechanisms proposed to explain a positive health effect of moderate alcohol consumption include beneficial effects of alcohol consumption on lipoprotein metabolism,[54] hemostasis[55] and inflammatory processes[56] and insulin sensitivity.[57] While cardiovascular protection has been demonstrated in middle-aged and older subjects, it is less clear whether beneficial effects of alcohol intake on the cardiovascular system express well before the occurrence of symptomatic cardiovascular disease. We found that moderate intake of alcohol affected vascular elasticity at an early age, notably in women. In young women, an alcohol intake of 1 to 2 glasses per day decreased arterial stiffness, measured by the PWV by approximately 7% compared to non-drinkers. In young men, the relation between alcohol and PWV was less pronounced. The mechanism by which moderate alcohol intake may reduce arterial stiffness is unknown. Alcohol consumption increases HDL-cholesterol,[54] with associated increases in paraoxonase activity[54] and cholesterol efflux.[58] These changes might decrease the amount of cholesterol within peripheral vascular cells and thus increase the flexibility

of the vascular wall. However, the relation between alcohol intake and PWV remained when HDL-cholesterol was taken into account, suggesting that our finding could not be fully explained by an increase in HDL-cholesterol. With increasing age, the arteries become stiffer due to a decrease in elastin and an increase in collagen and connective tissues in the arterial wall.[59] Alcohol intake might delay or change this process, possibly by an effect on gene expression. Furthermore, epidemiological studies have shown that moderate alcohol consumption reduces the risk of diabetes mellitus type 2 [60] and increase insulin sensitivity.[57] Therefore, alcohol consumption might decrease the formation and cross-linking of glycated collagen in the vascular wall, which is accelerated in hyperglycaemic milieu,[61] and thereby increase arterial elasticity.

The potential role of insulin-like growth factor (IGF)-1 in cardiovascular pathophysiology has raised considerable interest over the past years. IGF-1 is an essential regulator of developmental growth as IGF-1 levels are strongly associated with both fetal and postnatal growth.[62-65] Recently, a polymorphism in the promoter region of IGF-1 on chromosome 12q21 has been identified, which may influence IGF-1 production and body height.[66-68] Our findings show a clear association between the IGF-1 gene polymorphism and body height growth from childhood into adulthood. Already at birth the carriers of the wild-type allele of this polymorphism were 0.3 cm taller compared to the variant carriers. Like Frayling et al.,[69-71] we could neither confirm the results of Vaessen et al.,[72] who found that non-carriers of the 192-bp allele had a low birth weight, nor the results of Arends et al.,[73] who showed an association between birth size and an intronic repeat in the promoter region of the IGF-1 gene in a cohort of 124 short children with a mean age of 7 years and born small for gestational age. Our data showed no relation between the IGF-1 gene polymorphism and cardiovascular risk profile at a young age nor between the polymorphism and parameters of vascular damage.

Methodological considerations

The EPOZ study is a population-based cohort study in healthy children. At baseline between 1975 and 1978, 596 children, aged 5-19 years, were randomly included in the study. The long annual follow-up creates on the one hand the advantage of using repeated measurements but on

the other hand causes problems concerning loss-to-follow-up while the correlation between the repeated measures need to be dealt with. Another methodological issue that will be discussed is the use of surrogate endpoints in the EPOZ study.

Repeated measurements

Within the EPOZ study, many measurements of growth and of cardiovascular risk factors are repeated in each person during the 27-year follow-up period. These measurements have within-subject variability and measurement error. One of the advantages of the large number of measurements that was performed in each individual is the enhancement of a more accurate estimation of the person's true underlying levels. Moreover, as a single research assistant performed the vast majority of the average of 15 measurements per participant, measurement variation was reduced. One complication in the analysis of the data is that the repeated measures for the same subject are correlated. When this correlation is ignored and the responses in the same subject are treated as independent observations the effect estimates are usually still valid but the standard errors are usually incorrect (usually too small for between-subjects effects and usually too large for within-subject effects). Sometimes it is satisfactory to do separate analyses for each time point, but problems of multiple testing arise because many tests of the association are conducted within one person. Moreover, one may loose statistical power, as not all information is exhaustively used. Another difficulty is the interpretation of the results: how to combine all time points? To overcome these problems, all measurements should be included in one model and the correlation between the measurements must be taken into account. This is possible with unbalanced repeated measurement analysis. We used the MIXED procedure within SAS (PROC MIXED). A mixed linear model is a generalization of the standard linear model, the generalization being that the data are permitted to exhibit correlation and nonconstant variability. In most repeated measurements analyses we used a model with intercept and age as random effects. This means that, besides an estimated fixed intercept and fixed age-effect for each subject a personal deviation from this mean intercept and mean age-effect is estimated. In summary, with the Proc Mixed model data can be used more exhaustively. Secondly, this model takes into account the correlation between the repeated measurements within each participant. Thirdly, the model is very flexible as not only the means but also the variances and covariances

are modelled. Finally and very importantly, each subject with at least 1 measurement can be used in the analysis.

Non-response and loss-to-follow-up

Selection bias can lead to either an under- or an overestimation of the studied relations, depending on the selection process and cannot be corrected for in the analysis. Non-response can cause selective losses leading to selection bias. The population selected for follow-up in the EPOZ study was a random sample from the youngsters who participated in the baseline study. The only selection criterion was an age between 5 and 19 years. Theoretically, selection bias occurs only when the relation between the determinant and the outcome is different for those who participated and those who were eligible but did not participate. We do not consider selection bias by non-response to have occurred, as it is unlikely that the children or the parents based their participation in EPOZ on awareness of relations between early determinants and later disease.
Another potential problem in our 27-year follow-up study is selective loss-to follow-up that may have biased our results in the sense that determinant-outcome relations were different among those who pursued and those who terminated study participation. However, our data do not indicate such bias. BMI, blood pressure and total cholesterol values at previous visits were similar among those who were and those who were not lost to follow-up at a later stage. Moreover, loss-to-follow up only biases an association, for example between blood pressure and atherosclerosis, if particularly persons with high blood pressure and extensive atherosclerosis were lost. We think that this is unlikely, as the EPOZ cohort is a young population with a mean age of 37 years at the last visit. At this age, persons are usually not aware of the their extent of atherosclerosis, as this is usually non-symptomatic and seldom measured in practice. Thus, even though high risk factor levels, as measured during the EPOZ visits, were reported to the general practitioners and were known to the participants, possible loss to follow-up based solely on this information would not have affected our risk estimates. Finally, as the EPOZ study comprises a young population, not many persons are lost due to death or illnesses, causing selective loss-to-follow-up.

Surrogate endpoints

The mean age of the participants of the EPOZ 2002 study was 37 years. Therefore, we could not use clinically manifest coronary heart disease or

death as endpoints in our studies. Therefore, we chose surrogate endpoints that reflect the risk for such clinical endpoints or end-organ damage properly and were positioned on the time axis in between the causal pathway.[74-76] Some main advantages of using these biomarkers are that sufficient power can be attained, as they can be measured in all participants, and that the participants can be studied at a young age. To use a measure as surrogate endpoint, the measure must first be valid and well reproducible. Secondly, the measure must be significantly related to the disease of interest. A change in the surrogate measure should be reflected in a change in risk of the disease of interest Thirdly, a biologically plausible mechanism should underlie the pathway between the measure and the disease of interest. We used the measurements of IMT of the carotid arteries, which is a validated surrogate marker for atherosclerosis and cardiovascular disease risk.[77] Ultrasonographic measurement of carotid IMT correlates well with pathological measurements of atherosclerosis.[78, 79] Prospective epidemiological studies have shown that an increase in IMT is associated with an increase in relative risk for myocardial infarction and stroke, and that a decrease in IMT due to drug treatment is associated with a decrease in the incidence of vascular events.[80-84] The presence of carotid plaques is associated with cardiovascular and cerebrovascular disease as well, irrespective of the side and location of the plaques.[83, 85, 86] Other measures that we used in our study were femoral-carotid PWV as a measure of aortic stiffness and carotid distensibility. Aortic stiffness, measured through PWV is increased in the presence of a myocardial infarction with a strength of association comparable to that of atherosclerosis and myocardial infarction.[87-89] Common carotid arterial distensibility is strongly associated with previous stroke and also, but less strongly with previous myocardial infarction.[90]

Cardiovascular risk prediction in the young

Early origins of adult disease

Children's health could influence their well being for the rest of their life. The public health interest in early life determinants of adult disease already emerged a long time ago.[91] Studying adult mortality in Britain before 1920, Derrick had shown that each succeeding generation displayed a lower mortality at all ages from childhood to old age.[92] He concluded that 'each generation is endowed with a vitality peculiarly of its own, which persistently

manifests itself through the succeeding stages of its existence'. At the beginning of the twentieth century, the idea that early life conditions and experience affect adult health was an important component of the prevailing public health model. This idea went hand in hand with the expanding knowledge on nutrition in the 1930s with its emphasis on the importance of nutrition in childhood. One of the first studies to suggest that adverse conditions in early life affect health in the adult was performed by Kermack and co-workers and includes data going back to as early as 1751.[93, 94] The authors conclude that their data on relative adult mortality for various years of birth 'behave as if expectation of life was determined by conditions, which existed during the child's earlier years' and that maternal health during and around the time of pregnancy may be important in this respect. In 1964, Geoffrey Rose had reported that siblings of patients with coronary heart disease had stillbirth and infant mortality rates that were twice as high as those of controls.[95] He concluded that 'ischaemic heart disease tends to occur in individuals who come from a constitutionally weaker stock'. During this period, McCance and Widdowson showed in animals that undernutrition before or shortly after birth profoundly and permanently modifies the morphology and physiology of the body.[96] McCance already in 1976 pointed at the critical periods of growth.[97] In 1973, Anders Forsdahl found a geographical link between past infant mortality and subsequent adult mortality from heart disease.[98] He hypothesized that poor living conditions in early life, which are known to increase infant death rates, make babies that survive more vulnerable to heart disease.

Fetal origins of cardiovascular disease

In 1986, David Barker found that ischaemic heart disease was strongly correlated with both neonatal and postneonatal mortality and suggested that poor nutrition in early life increases susceptibility to the effects of an affluent diet.[99] Subsequently, in 1989, Barker first showed that the strong correlation between ischaemic heart disease and past infant mortality originated in poor conditions in utero, rather than poor conditions in childhood, although the latter also contributed. He hypothesized that a baby's nourishment before birth and during infancy 'programmes' the development of risk factors for CVD.[100] Undernutrition of the fetus during critical periods of development would lead to adaptations in the structure and physiology of the fetal body, and these adaptations would increase the risk of coronary heart disease, hypertension, hypercholesterolaemia

and diabetes in later life. Barker's fetal origins of adult disease hypothesis is supported by findings from animal experiments that show that undernutrition during pregnancy has permanent adverse effects on the cardiovascular health of the offspring.[101-103] Also, the effects of undernutrition during gestation on adult health are studied in humans, mostly using data from persons born during famines.[104-112] One of the outcomes considered in Barker's hypothesis is blood pressure. There are a number of possible mechanisms by which restricted intrauterine growth could either initiate or amplify raised blood pressure and lead to cardiovascular disease.[113] First, there is evidence that the fetal renin-angiotensin system is activated in intrauterine growth retardation.[114] Fetal hypoxaemia stimulates the release of renin and the subsequent generation of angiotensin II induces vasoconstriction. Secondly, Brenner hypothesized that retarded fetal growth leads to reduced numbers of nephrons, which in turn leads to increased pressure in the glomerular capillaries and the development of glomerular sclerosis.[115, 116] It has been empirically shown that the number of nephrons is reduced in hypertensives.[117] Thirdly, fetal undernutrition could lead to lifelong changes in the fetal hypothalamic-pituitary-adrenal axis, which in turn resets homeostatic mechanisms controlling blood pressure. Treatment of pregnant rats with low dose dexamethasone led to persistently raised blood pressure in the offspring.[118] Finally, fetal undernutrition may lead to a higher risk for childhood excess weight gain, which in turn may induce hypertension as weight is among the most important determinants of blood pressure.

In the past decade, this fetal origins of adult disease hypothesis has been subject to heated debate in the literature.[119-128] An alternative explanation of the association of low birth weight with diabetes and vascular disease is the fetal insulin hypothesis.[129] Hattersley and Tooke proposed that genetically determined insulin resistance results in impaired insulin-mediated growth in the fetus as well as insulin resistance in adult life. Low birth weight, measures of insulin resistance in life, and ultimately glucose intolerance, diabetes, and hypertension could all be phenotypes of the same insulin-resistant genotype. Concerning Barker's hypothesis, the critics point at problems of selection bias and confounding by socio-economic status. Furthermore, within and across studies exploring the fetal origins of adult disease hypothesis, a range of different obstetric and perinatal measures as marker for intra-uterine growth have been used as explanatory variables and this may have led to inconsistencies. Another issue comprises the rationale of additional

adjustment for attained body size in the analysis between birth weight and cardiovascular endpoints. On the one hand, adjustment for adult body size can distort the results since one assigns a higher rate of growth to smaller infants and a slower rate of growth to larger infants. On the other hand, at any point in time, body size is correlated with both earlier and later size. Therefore, even when birth size is correlated directly with CVD endpoints, the postnatal growth must be taken into account by adjusting for adult body size. If this adjustment attenuates the effect of early size, later size is likely to be more relevant than early size in the aetiology of CVD. Amplification of the effect due to adjustment for adult body size indicates a dominant impact of catch-up growth on future CVD in the infants with lower birth weight. Finally, some facts remain conflicting with the fetal origins of adult disease hypothesis. CVD-mortality is higher in northern Europe than in southern Europe[130] despite higher birth weights up north. But then again, adults also grow taller in the northern countries compared to the south. The independent contribution to the high CVD-mortality of restricted fetal growth has been shown to be very small. Also, a 1-kg difference in birth weight is huge and not achievable as public health target.
In this thesis, we examined the relation between birth size and blood lipids. Our data support the view that differences in levels of lipids may have their origins in early life. Low birth weight and low birth length were related to higher blood lipid levels of children and adolescents particularly when they reached young adulthood. Differences amounted to almost 0.5 mmol/l per kilogram difference in birth weight for LDL-cholesterol in adulthood and are as such important. These differences are graded and particularly pertain to children born at term. Further, as the relations between birth size and lipids in this study were obtained without any adjustment, unlike relations with blood pressure in these age groups,[131] these findings may have implications from a public health point of view.

The obesity epidemic

The recent increase in the global prevalence of obesity qualifies as an epidemic.[5, 132] An increasing number of deaths worldwide is due to medical complications of obesity.[133] Moreover, obesity claims 1.6% of the total costs in health care in the Netherlands annually and even 9.8% of the total health budget in the United States, amounting to 99 billion dollar.[134] The obesity epidemic is recognized by the World Health Organisation (WHO) as one of the top 10 global health problems. According to the WHO it is

essential to develop new preventive public health strategies which affect the entire society and not only children and adults who have a high BMI.[135] It has been proposed that early life prevention is the only effective solution to the problem of adult obesity.[136] Children are a vulnerable population, because they may not be prepared to make informed health-related choices on their own. Childhood obesity seems to be increasing at a disturbing rate and thereby obese children tend to become obese adults. Of children who were overweight at around 12 years of age some 80% were still obese at around 30 years.[137] The results of the EPOZ study about familial clustering of body weight and its impact on the cardiovascular risk profile underline the importance of parents in solving the problem. Having both parents in the highest tertile of BMI was associated with an average 3 kg/m² higher BMI in comparison with having both parents in the lowest tertile. Moreover, children with paternal BMI in the highest tertile had a significantly higher IMT compared to offspring of fathers with a BMI in the lowest tertile (chapter 3.2). The impact of parental BMI was present over the whole range of parental BMI levels, meaning the lower the parental BMI the lower the child's BMI. Unfortunately, we could not discriminate between effects of environment and biology in our study as we lack information on main environmental determinants of obesity, physical activity and nutrition. The rise in overweight, also in children, occurred very rapidly over the last 3 decades. This suggests strong environmental determinants of childhood obesity, which may well explain our finding of familial aggregation. We must inspire parents to make behavioural changes that are sufficient to resist the pressures of current environmental factors towards excessive weight gain of their offspring. Parents' attention should be drawn to this huge medical problem and to their responsibility regarding the future health of their children. Apart from the impact of parents, demonstrated with our data, also other parts of the society should be more willing to carefully manage the food and physical activity environments of our children at home, in school, and in other places frequented by children.

Starting earlier to prevent heart disease

Research investigating the early origins of adult cardiovascular disease provides new insight into the aetiology of cardiovascular disease and the early phases of life may be an interesting starting-point in the prevention of cardiovascular disease. The EPOZ study stressed the importance of risk assessment in younger individuals by showing (1) evident relations between

childhood levels of cardiovascular risk factors blood pressure, body mass index and total cholesterol and vascular damage in adulthood, (2) evident relation between childhood lifestyle and vascular damage in adulthood and (3) the important parental impact in developing overweight and vascular damage. The importance of the study of cardiovascular risk assessment in the young has been underlined by several other observations as well. First, a considerable number of school-age children have risk factors which in adults are predictive of coronary heart disease.[138] Thereby, clustering of risk factor variables occurs as early as childhood and adolescence and is associated with atherosclerosis in young adulthood and thus risk of later CVD.[139, 140] Secondly, the process of atherosclerosis may start at a very early age[4] and there is evidence that the relation between arterial wall changes and cardiovascular risk factors already exist in children and adolescents.[140, 141] For example, the Bogalusa Heart Study showed that as the number of cardiovascular risk factors increased in young people, so does the severity of asymptomatic coronary and aortic atherosclerosis.[140] Thirdly, longitudinal studies of children followed into adulthood showed that adverse life style, for example physical inactivity due to television watching, and cardiovascular risk factors tend to track throughout life.[32, 142-151] In older children and adolescents, habitual tobacco use has been shown to contribute to raised blood pressure and the development of other risk factors in early life, most of which track into adulthood.[152]

Thus, the existing evidence indicates that in order to stop the atherosclerotic process one may have to start intervening early in life but the key questions are how early and in whom? The feasibility of the proposed guidelines as described in the American Heart Association Guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood[153] is questionable. In this report, Kavey et al. recommend that from the age of 3 years, blood pressure should be measured at each doctor's visit, for whatever reason the child's examined. Like growth, blood pressure should be entered on a chart for age/sex/height. Furthermore, targeted screening of fasting lipids is recommended in children >2 years of age with a family history of dyslipidemia or premature CVD. For children in whom the family history is unknown and other risk factors are present, lipids and lipoproteins should be assessed. If averaged results of 3 fasting lipid profiles are above cut points, first additional therapeutic lifestyle changes should be initiated including diet. However, these guidelines are not yet evidence based.

When starting pharmacological treatment in young children according

to the proposed guidelines, it is particularly relevant to know what is the potentially longstanding preventive effect of several intervention strategies. Also, issues of medicalization of children, the cost-effectiveness of shifting the clinical implementation towards the young age group, and the possible stigmatization should be considered. To know whether treatment or a preventive measure succeeds in improving the cardiovascular risk profile and delaying the development of vascular damage, a randomized controlled trial that prospectively evaluates the amount of atherosclerosis at different time-points is needed. The effects of intervention strategies could be monitored using new promising tools as surrogate endpoints. The newest generation multidetector CT (MDCT) has the ability to non-invasively quantify the amount of coronary-, carotid- and aortic calcification very accurately as well as plaque composition.[154, 155] A major drawback using MDCT in the young is radiation. MRI is another promising tool for measuring atherosclerotic plaque composition in extracoronary arteries.[156] In view of primary prevention, MRI has the big advantage of absence of radiation.

These considerations will put medical treatment of blood pressure and cholesterol or even dietary advises on a longer track. An exception should be made here for obesity. As far as overweight is concerned, primary prevention is non-pharmacological, involving mainly lifestyle changes both in parents and children. Moreover, as discussed in the previous paragraph, obesity is a highly frequent, increasing and therefore urgent problem. Therefore, considering the current obesity epidemic, action to overcome the problem of overweight must and could start early in life. In the previous paragraph, different strategies to attack the obesity epidemic are discussed.

Summarizing, our results emphasized the importance of childhood factors and parental influence on developing overweight and vascular damage in adulthood. The proper strategy for tackling the obesity epidemic and the worldwide number-one killer, CVD, should probably target children and young adults. The time for serious considerations about primary prevention beginning in childhood has come, although the consequences in clinical practice can only be realized when early treatment and prevention have proven effective and when the issues of costs, medicalization, stigmatisation have been considered.

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SUMMARY / SAMENVATTING

7

Summary

Despite recent advances in treatment, leading to a considerable reduction in cardiovascular mortality, cardiovascular morbidity is still a huge health problem in the industrialized countries and rapidly increasing in the rest of the world. Aging of the population and improved survival after non-fatal cardiovascular disease (CVD) events bear heavily on medical costs. For stopping the cardiovascular epidemic it is essential to improve primary prevention of CVD in order to delay the development of atherosclerosis and hence the incidence of CVD. Already in the seventies, much attention was paid to risk-indicators of CVD. The Framingham Heart Study showed strong associations of blood pressure, levels of total cholesterol and smoking cigarettes with the occurrence of CVD. Overweight appears to influence the cardiovascular risk profile already at young ages. This thesis aims at exploring the early determinants of overweight and cardiovascular disease risk. Most studies presented in this thesis are based on data from the Epidemiological Preventive Study of Zoetermeer (EPOZ study), a prospective population-based cohort study in the Netherlands that was started in 1975 among 5 to 19 years old children. **Chapter 2** describes the rationale and the design of this longitudinal study.

Chapter 3 focuses on the impact of parents' risk factor status on the cardiovascular risk in the offspring. Prospective studies have shown that cardiovascular disease aggregates in families. This is probably partly due to familial aggregation of cardiovascular risk factors such as blood pressure and plasma cholesterol, which was shown to be detectable in children at a very young age. In longitudinal studies, children with a family history of hypertension were shown to have persistently higher blood pressure levels than children without such a history over follow-up periods of up to 10 years. Still the usefulness of recording a positive family history in the prediction of hypertension in the individual is considered to be limited. It is of particular interest to know whether parental blood pressure levels are predictors of their offspring's subsequent blood pressure development into young adulthood and whether this holds for the whole distribution of parental blood pressure. In our 27-year follow-up study, actual parental blood pressure showed to be an important predictor of blood pressure development from childhood into young adulthood (**chapter 3.1**). Our findings add to the current knowledge in several ways. The impact of

parental blood pressure was present over the whole distribution of parental blood pressure levels, meaning the lower the parental blood pressure the lower the blood pressure levels of the offspring. Furthermore, our data suggest that the impact of parental blood pressure starts at an early age and is strong and long lasting.
Already at young ages, family dynamics also play an important role in the development of obesity. Contrary to earlier studies, we used actual measurements of parental body mass index (BMI) and repeatedly measured BMI in offspring from childhood into adulthood. We showed that the impact of parental BMI was present over the whole range of parental BMI levels, meaning the lower the parental BMI the lower the child's BMI (**CHAPTER 3.2**). Furthermore, our data suggest that the impact of parental BMI starts at an early age, is strong and long lasting and is also associated with increased cardiovascular risk. Our results showed that parental and children's BMI do not only affect the current health status but are also predictors of future vascular damage and thereby the risk of manifest cardiovascular disease in later life. These results underline the importance of early prevention of excessive weight gain and the necessity of involving the parents in fighting the obesity epidemic.

Thus, parental risk status is important for one's cardiovascular risk profile. In **CHAPTER 4**, the associations between several childhood risk factors and vascular damage at young adulthood are presented. When knowing more about the predictability of early levels of cardiovascular risk factors, such as BMI, systolic blood pressure and total cholesterol, for atherosclerosis and arterial stiffness in young adulthood, identification of those at highest risk would be possible at a younger age. And early detection can lead to early treatment. We found that BMI, systolic blood pressure and total cholesterol measured during childhood were strongly related to both carotid intima-media thickness (IMT) and presence of plaques in the carotid arteries (**CHAPTER 4.1**). For example, IMT increased with 0.025 mm per 1 kg/m² increase in BMI during childhood, 20 years before assessment of IMT. The risk of having plaques in the carotid arteries increased with 60% per 1 mmol/l increase in total cholesterol level measured in childhood. Notably, we found an association between childhood systolic blood pressure and both pulse wave velocity (PWV) and carotid distensibility in adult offspring indicating an increase in arterial stiffness with increasing blood pressure. No relation was found between childhood total cholesterol and vascular

stiffness.
The relation between alcohol intake and the occurrence of CVD has found to be U-shaped, suggesting a higher risk of CVD in non-drinkers and heavy alcohol consumers compared to those with moderate alcohol intake. Mechanisms proposed to explain a positive health effect of moderate alcohol consumption include beneficial effects of alcohol on lipoprotein metabolism, hemostasis and inflammatory processes and insulin sensitivity. While cardiovascular protection has been demonstrated in middle-aged and older subjects, it is less clear whether beneficial effects of alcohol intake on the cardiovascular system express well before the occurrence of symptomatic cardiovascular disease. We found that moderate intake of alcohol affected vascular elasticity at an early age, notably in women (**CHAPTER 4.2**). In young women, an alcohol intake of 1 to 2 glasses per day decreased the PWV by approximately 7% compared to non-drinkers. In young men, the relation between alcohol and vascular elasticity was less pronounced.

There is evidence that cardiovascular disease has its origins in the fetal phase and early childhood. Birth size has been found to be inversely associated with cardiovascular risk factors such as blood pressure, insulin resistance and obesity in adult life. Longitudinal data on the relation between birth size and blood lipid levels in later life are limited in the young and to some extent inconsistent. Within our 27-year follow-up study, size at birth was related to the natural history of lipids from childhood to young adulthood (**CHAPTER 5.1**). Children born with low birth weight or length obtain higher lipid levels particularly when they reach young adulthood. Associations of birth weight with total cholesterol changed from positive in childhood to inverse at young adulthood. Both in males and females, birth length was inversely associated with cholesterol levels from childhood to adulthood. These findings were consistent with results of analyses of birth size in relation to individual change of lipids over time. The mechanism by which birth size could be related to cholesterol levels is largely unknown. Possibly, intra-uterine growth retardation, particularly when occurring during late gestation, results in a disproportionate effect on liver growth, which may lead to altered lipoprotein cholesterol metabolism.
Next to intra-uterine growth, also genes could play a role in the early origins of CVD. The potential role of insulin-like growth factor (IGF)-1 in cardiovascular pathophysiology has raised considerable interest over the past years. IGF-1 is an essential regulator of developmental growth as IGF-1 levels

are strongly associated with both fetal and postnatal growth. Recently, a polymorphism in the promoter region of IGF-1 on chromosome 12q21 has been identified, which may influence IGF-1 production and body height. The fetal insulin hypothesis by Hattersley suggests that genes involved in insulin resistance cause low birth size, type 2 diabetes mellitus and in later life CVD through adverse vascular development and body-fat distribution. This hypothesis provides an alternative explanation of the association of low birth weight with vascular disease as proposed by Barker's fetal programming hypothesis. Our findings show a clear association between the IGF-1 polymorphism and body height growth from childhood into adulthood (**CHAPTER 5.2**). Already at birth, carriers of this polymorphism were 0.3 cm taller compared to the variant carriers. Like Frayling et al., we could confirm neither the results of Vaessen et al., who found that non-carriers of the 192-bp allele had a low birth weight, nor the results of Arends et al., who showed an association between birth size and an intronic repeat in the promoter region of the IGF-1 gene in a cohort of 124 short children with a mean age of 7 years and born small for gestational age. Our data showed no relation between the IGF-1 gene polymorphism and cardiovascular risk profile at a young age nor between the polymorphism and parameters of vascular damage.

In the general discussion in **CHAPTER 6**, the main findings of this thesis are considered in the context of current scientific knowledge and ongoing research in the field of early origins of adult disease. In addition, relevant methodological aspects are discussed together with implications for public health and future research.

Samenvatting

Ondanks verbeterde behandelmethoden van hart- en vaatziekten, die de laatste jaren geleid hebben tot een daling van de cardiovasculaire mortaliteit, blijven hart- en vaatziekten het grootste gezondheidsprobleem in de geïndustrialiseerde landen. Daarnaast is er in de rest van de wereld een alarmerende toename van hart- en vaatziekten. De vergrijzing van de bevolking en de verbeterde overleving na hart- en vaatziekten eisen een groot deel van de totale medische kosten op. Het is daarom essentieel primaire preventie van hart- en vaatziekten te verbeteren waardoor de ontwikkeling van atherosclerose wordt vertraagd en daarmee de incidentie van hart- en vaatziekten. Al in de jaren zeventig is veel aandacht besteed aan de risicofactoren voor hart- en vaatziekten. De Framingham Heart Study liet sterke associaties zien tussen bloeddruk, cholesterol, het roken van sigaretten en het voorkomen van hart- en vaatziekten. Overgewicht lijkt het cardiovasculaire risicoprofiel al op jonge leeftijd te beïnvloeden. Dit proefschrift heeft als doel de vroege voorspellers van overgewicht en risico op hart- en vaatziekten te onderzoeken. Veel van de studies die in dit proefschrift beschreven zijn, zijn gebaseerd op gegevens van het Epidemiologisch Preventief Onderzoek Zoetermeer (EPOZ), een prospectieve vervolgstudie in Nederland die in 1975 van start is gegaan in een groep van 5 tot 19 jaar oude kinderen. **Hoofdstuk 2** beschrijft de achtergronden en het ontwerp van deze longitudinale studie.

Hoofdstuk 3 richt zich op de invloed van de ouders op het cardiovasculaire risico van hun kinderen. Prospectieve studies hebben laten zien dat hart- en vaatziekten clusteren in families. Dit komt waarschijnlijk door clustering van cardiovasculaire risicofactoren als bloeddruk en cholesterol, zoals al op heel jonge leeftijd bij kinderen is aangetoond. Kinderen met een familiegeschiedenis van hoge bloeddruk hadden een bij herhaling hogere bloeddruk dan kinderen zonder zo'n familiegeschiedenis, zoals bleek uit een aantal longitudinale studies met een follow-up oplopend tot 10 jaar. Voor het voorspellen van de kans op hoge bloeddruk bij een individu blijft de familiegeschiedenis van beperkte waarde. Het is met name interessant te weten of ouderlijke bloeddrukwaarden de ontwikkeling van de bloeddruk bij hun kinderen voorspellen en of dit geldt voor de hele verdeling van ouderlijke bloeddruk, van hoog tot laag. In onze 27-jarige vervolgstudie hebben we laten zien dat ouderlijke bloeddruk een belangrijke voorspeller is

van de bloeddruk ontwikkeling vanaf de kindertijd tot in jong volwassenheid (**HOOFDSTUK 3.1**). Wat is nieuw aan onze bevindingen? De invloed van ouderlijke bloeddruk was aanwezig over de hele verdeling van bloeddruk. Dit betekent dat hoe lager de ouderlijke bloeddruk hoe lager de bloeddruk bij hun kinderen. Daarnaast lieten onze data zien dat de invloed van ouderlijke bloeddruk sterk is, al op jonge leeftijd begint en voortduurt tot in volwassenheid.
Al op jonge leeftijd, speelt de familie ook een belangrijke rol bij de ontwikkeling van obesitas. In tegenstelling tot eerdere studies, hebben wij metingen van ouderlijke body mass index (BMI) gebruikt en BMI metingen van de kinderen vanaf de kindertijd tot in volwassenheid. We hebben laten zien dat de invloed van de ouderlijke BMI aanwezig was over de hele verdeling van ouderlijke BMI: hoe lager de ouderlijke BMI, hoe lager de BMI van het kind (**HOOFDSTUK 3.2**). Daarnaast blijkt de sterke invloed van ouderlijke BMI al vanaf jonge leeftijd aanwezig te zijn en voort te duren tot in volwassenheid. Ook is de ouderlijke BMI geassocieerd met een toegenomen risico op hart- en vaatziekten. De BMI van zowel de ouders als de kinderen beïnvloeden niet alleen de huidige gezondheidsstatus maar zijn ook voorspellers voor toekomstige vaatschade en daarmee voor het optreden van hart- en vaatziekten. Deze resultaten onderstrepen het belang van vroege preventie van excessieve gewichtstoename en de noodzaak de ouders hierin te betrekken teneinde de obesitas epidemie te bestrijden.

Het risicoprofiel van ouders blijkt dus belangrijk voor het cardiovasculaire risicoprofiel van hun kinderen. In **HOOFDSTUK 4** worden de associaties tussen verschillende risicofactoren gemeten in de kindertijd en vasculaire schade, gemeten in jong volwassenheid, gepresenteerd. Vroege identificatie van personen met een hoog risico op hart- en vaatziekten is alleen mogelijk wanneer meer bekend is over de mate waarin cardiovasculaire risicofactoren, die al op jonge leeftijd gemeten zijn, atherosclerose en vaatwandstijfheid bepalen. Vroege detectie kan leiden tot vroege behandeling. In onze studie bleken BMI, systolische bloeddruk en totaal cholesterol, gemeten tijdens de kindertijd, sterk gerelateerd te zijn aan zowel intima-media dikte van de halsslagaders als aanwezigheid van plaques in de halsslagaders (**HOOFDSTUK 4.1**). Bijvoorbeeld, de intima-media dikte nam toe met 0.025 mm per 1 kg/m² toename van de BMI, gemeten tijdens de kindertijd. De kans op plaques in de halsslagaderen nam toe met 60% per 1 mmol/l toename in totaal cholesterol gemeten in de kindertijd. Een duidelijke relatie vonden we ook tussen

systolische bloeddruk op de kinderleeftijd en zowel de polsgolfsnelheid (PWV) als de distensibiliteit van de halsslagaderen op de volwassen leeftijd: de vaatwandstijfheid neemt toe met toename van bloeddruk. Geen relatie is gevonden tussen totaal cholesterol en vasculaire stijfheid.
De relatie tussen alcoholinname en het optreden van hart-en vaatziekten is U-vormig. Dit betekent dat er een hoger risico op hart- en vaatziekten bestaat bij geheelonthouders en zware alcoholgebruikers vergeleken met hen die matig alcohol gebruiken. Een aantal mechanismen is geopperd om het gunstige effect van matig alcoholgebruik te verklaren, te weten gunstige effecten van alcohol op het lipoproteine metabolisme, hemostasis en ontstekingsprocessen en insuline gevoeligheid. Aangezien cardiovasculaire bescherming aangetoond is bij mensen van middelbare- en bejaarde leeftijd, is het minder duidelijk of de gunstige effecten van alcoholgebruik op het cardiovasculaire systeem al ver voor het optreden van symptomatische hart- en vaatziekten zichtbaar zijn. Onze studies lieten zien dat matig alcoholgebruik al een gunstig effect had op de vaatwandelasticiteit op jonge leeftijd, met name in vrouwen (**HOOFDSTUK 4.2**). Jonge vrouwen die per week 1-2 glazen alcohol nuttigden, hadden een ongeveer 7% lagere PWV vergeleken met geheelonthoudende vrouwen. De relatie tussen alcohol en vaatwandelasticiteit was minder duidelijk bij jonge mannen.

Er zijn aanwijzingen dat hart- en vaatziekten hun oorsprong hebben tijdens de foetale fase en vroege kindertijd. Eerdere studies lieten een inverse relatie zien tussen geboortegewicht en geboortelengte en cardiovasculaire risicofactoren, zoals bloeddruk, insuline resistentie en obesitas in het volwassen leven. Longitudinale data met betrekking tot de relatie tussen geboortegewicht/-lengte en lipidenconcentraties in het bloed op latere leeftijd zijn beperkt en ook inconsistent. Binnen onze 27-jarige vervolgstudie was geboortegewicht/-lengte gerelateerd aan de cholesterolwaarden vanaf de kindertijd tot in jong volwassenheid (**HOOFDSTUK 5.1**). Kinderen die geboren worden met een laag geboortegewicht of klein zijn bij de geboorte hebben hogere cholesterolwaarden, met name wanneer ze volwassen zijn. De associaties tussen geboortegewicht en totaal cholesterol veranderden van positief tijdens de kindertijd naar invers tijdens jong volwassenheid. Zowel bij mannen als bij vrouwen was geboortelengte invers geassocieerd met cholesterolwaarden van de kindertijd tot in volwassenheid. Het mechanisme die deze relatie tussen geboorteproporties en cholesterol zou kunnen verklaren is onbekend. Mogelijk resulteert intra-uteriene groeivertraging,

met name wanneer deze optreedt tijdens het laatste trimester van de zwangerschap, in een disproportioneel effect op de groei van de lever, waardoor een veranderd cholesterol metabolisme ontstaat.
Naast intra-uteriene groei, zouden ook de genen een rol kunnen spelen bij de vroege oorsprong van hart- en vaatziekten. De laatste jaren is er toenemende interesse voor een potentiële rol van de insuline-achtige groeifactor-1 (IGF-1) in de cardiovasculaire pathosfysiologie. IGF-1 is een essentiële regulator van ontwikkelingsgroei aangezien IGF-1 concentraties sterk geassocieerd zijn met zowel foetale als postnatale groei. Recent is een polymorfisme in de promotor regio van IGF-1 op chromosoom 12q21 geïdentificeerd, welke mogelijk de IGF-1 productie en lichaamslengte beïnvloedt. De foetale insuline hypothese van Hattersley suggereert dat genen die betrokken zijn bij insuline resistentie een laag geboortegewicht, type 2 diabetes mellitus en op latere leeftijd hart- en vaatziekten veroorzaken door ongunstige vasculaire ontwikkeling en lichaamsvetdistributie. Deze hypothese verschaft een alternatieve verklaring voor de associatie tussen laag geboortegewicht en vasculaire ziekte zoals voorgesteld door Barker's foetale programmeringshypothese. Onze bevindingen laten een duidelijke associatie zien tussen het IGF-1 polymorfisme en lichaamslengte van de kindertijd naar volwassenheid (**HOOFDSTUK 5.2**). Al bij de geboorte waren de dragers van dit polymorfisme 0.3 cm groter vergeleken met de variabele dragers. Zoals Frayling et al., konden we noch de gegevens van Vaessen bevestigen, die vond dat de niet dragers van het 192-bp allel een lager geboortegewicht hebben, noch de gegevens van Arends et al., die een associatie aantoonde tussen geboortegewicht en een intron repeat in de promotor regio van het IGF-1 gen in een cohort van 124 kinderen van gemiddeld 7 jaar die bij geboorte te klein waren voor de zwangerschapsduur. Onze data lieten geen relatie zien tussen het IGF-1 gen polymorfisme en het cardiovasculaire risicoprofiel op jonge leeftijd en ook geen relatie tussen het polymorfisme en parameters van vasculaire schade.

In de algemene discussie in **HOOFDSTUK 6** worden de belangrijkste bevindingen van dit proefschrift geplaatst in een breder kader van huidige wetenschappelijke kennis en lopend onderzoek naar de vroege oorsprong van volwassen ziekte. Daarnaast worden relevante methodologische aspecten van de studie besproken en ook de gevolgen voor de maatschappelijke gezondheidszorg en voor toekomstig onderzoek.

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Tot het volgende weekend!

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About the author

Annette van den Elzen was born on August 19th, 1973 in Geldrop, The Netherlands. In 1991 she passed secondary school at the 'Varendonck College' (previously 'College Asten-Someren') in Asten (atheneum). In 1991 she started Medical School at the Radboud University Nijmegen (previously 'Katholieke Universiteit Nijmegen'). In 1995, she participated in research on malaria at l'Hôpital Central in Yaoundé, Cameroun (dr. J.P. Verhave, department of Medical Microbiology, UMC St Radboud, Nijmegen, the Netherlands). In 1998, she did research on the Pierre Robin Sequence (dr. B.A. Semmekrot and dr. H.A.M Marres, departments of pediatrics and ENT, UMC St Radboud, Nijmegen, the Netherlands). Following this, she spent 3 months working at Sumve Hospital in Tanzania and participated in research on chloroquine resistence in malaria in children.

She graduated cum laude from Medical School in 1999. Subsequently she worked as a resident at the department of Pediatric Surgery in the Wilhelmina Children's Hospital in Utrecht (prof.dr. N.M.A. Bax) and at the department of Pediatrics in Meander Medical Center in Amersfoort. In September 1999, she started working at Erasmus MC in Rotterdam, first as a resident in the Sophia Children's Hospital, thereafter as junior researcher at the department of Epidemiology & Biostatistics (prof.dr. A.Hofman), resulting in the work described in this thesis. In 2003, she obtained a Master of Science degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences (n i h e s) in Rotterdam. She starts her residency in Pediatrics in the Sophia Children's Hospital, Erasmus MC, Rotterdam (prof.dr. A.J. van der Heijden), January 2005.

She is living together with Paul Bremer in Woerden. They have 2 beautiful boys: Jens and Ties.