THE DUTCH HYPERTENSION AND OFFSPRING STUDY

An epidemiological approach
to the early pathogenesis of
primary hypertension

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Hooft, Ingrid Maria Sylvia van
The Dutch Hypertension and Offspring Study:
An epidemiological approach to the early pathogenesis of primary hypertension/
Ingrid Maria Sylvia van Hooft.- [Sl.:s.n.]-Ill.
Thesis Rotterdam. - With ref. - With summary in Dutch.
ISBN 90-9007640-9
Subject headings: blood pressure / epidemiology

Cover: Alejandra van der Velden Grafische Vormgeving Oisterwijk
Printing: Spijker drukkerij, Buren (Gld)

THE DUTCH HYPERTENSION AND OFFSPRING STUDY

An epidemiological approach to the early pathogenesis of primary hypertension

EEN NEDERLANDS ONDERZOEK NAAR FAMILIAIRE HYPERTENSIE

Een epidemiologische benadering van de vroege pathogenese van primaire hypertensie

Proefschrift
ter verkrijging van graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. P.W.C. Akkermans, M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaats vinden op
woensdag 16 november 1994 om 11.45 uur

door
Ingrid Maria Sylvia van Hooft
geboren te 's-Herthogenbosch

Promotie commissie

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	Prof. J.D. Swales

Financial support by the Netherlands Heart Foundation and the Rotterdam Medical Research Foundation (ROMERES) for the publication of this thesis is gratefully acknowledged.

In remembrance of my father,

dedicated to my mother.

Acknowledgement

This thesis is based on the data collected in the Dutch Hypertension and Offspring Study. The design and conduct of the Dutch Hypertension and Offspring Study was supervised by a steering committee with the following members: prof. dr. D.E. Grobbee (chairman), prof. dr. P. van Brummelen, dr. L.J.P. van Doornen, prof. dr. A. Hofman, prof. dr. P.W. de Leeuw, dr. H.A.P Pols, prof. dr. K.H. Rahn, prof. dr. M.A.D.H. Schalekamp, prof. dr. H.A.J. Struijker-Boudier, prof. dr. Th. Thien and prof. dr. R.R.P. de Vries.

Much of the data in The Dutch Hypertension and Offspring Study presented in this thesis became collected by cooperation of the Dept. of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam, The Netherlands, with the departments of the members of the steering committee; Laboratory of Dept. of Internal Medicine I, University Hospital Dijkzigt, Rotterdam, The Netherlands (prof dr. M.A.D.H. Schalekamp, dr. F.H.M. Derkx and dr. F. Boomsma, R. de Bruin); Dept. of Pharmacology, State University Limburg, The Netherlands (prof. dr. K.H. Rahn, prof. H.A.J. Struijker-Boudier, dr. P. Schiffers, G. Jansen); Dept. of Immuno-Haematology, University Hospital Leiden, Leiden, The Netherlands (prof. dr. R.R.P. de Vries, V. Elkhuizen, H. Nieman, J. d'Amaro); Laboratory of Internal Medicine, Zuiderziekenhuis, Rotterdam, The Netherlands (dr. P.W. de Leeuw, P. Tchang).

Furthermore, data presented in this thesis became collected by cooperation with other departments in The Netherlands; Dept. of Chemical Pathology, State University Leiden (dr. M. Frölich); Laboratory for Clinical Chemistry, Sophia Hospital, Rotterdam (J. van 't Hof, J. Ambagtsheer); Dept. of Physiology, State University Limburg, Maastricht, (dr. T. van Merode); Dept. of Biochemistry, Catholic University of Nijmegen, Nijmegen (prof. dr. J.J.H.M. de Pont, E. Roose, H.P.G. Swarts) and with departments abroad; Dept. for Experimental Medicine and Endocrinology, Catholic University Leuven, Leuven, Belgium (dr. R. Bouillon); Dept. of Medicine, Glenfield General Hospital, University of Leicester, Leicester, United Kingdom (R.F. Bing).

Parts of the project were discussed with the following advisers; prof. dr. O.E. Brodde (Laboratory of Biochemistry, University Hospital Essen, Germany), dr. H.J. Waal-Manning (University of Otago Medical School, Dunedin, New Zealand), prof. J.L. Reid (Dept. of Medicine and Therapeutics, University of Glasgow, Glasgow, United Kingdom); prof. dr. F.R. Bühler (Dept. of Research, University Clinic, Kantonspital Basel, Switserland) and prof. dr. E. Ritz (Dept. of Nephrology, University Hospital Heidelberg, Germany).

The study was supported by grant 35.004 from the Netherlands Heart Foundation.

Contents

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Chapter 3 — Early prediction of primary hypertension; an epidemiological approach.
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Chapter 4.1.1 — Change in blood pressure in offspring of parents with high or low blood pressure:The Dutch Hypertension and Offspring Study.
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Chapter 4.1.3 — Haemodynamic characteristics of the early phase of primary hypertension. The Dutch Hypertension and Offspring Study.
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Chapter 4.1.4 — Characteristics of the common carotid artery in young males with and without a family history of hypertension.
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N Engl J Med 1991;324:1305-11.

Chapter 4.3.1 — Sympathetic nervous system activity and cellular-adrenoreceptors in offspring of hypertensive and normotensive parents.
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Chapter 4.4.2 — Atrial natriuretic factor and sodium intake in offspring of hypertensive and normotensive parents.
Van Hooft IMS, Grobbee DE, Hofman A, Schiffers P, de Pont JJHHM.
N Engl J Med (letter) 1989;320:867.

Chapter 4.4.3 Renal sodium handling, intra-cellular sodium, sodium-potassium ATP-ase activity, and unsaturated fatty acids in offspring of hypertensive and normotensive parents.
Van Hooft IMS, Swarts HGP, de Pont JJHHM, Hofman A, Thien Th, Grobbee DE.

Chapter 4.4.4 Alterations in calcium metabolism in young people at risk for primary hypertension.
Van Hooft IMS, Grobbee DE, Frölich M, Pols HAP, Hofman A.
Hypertension 1993;21:267-72.

When applied to hypertension, the term multifactorial serves to obscure our ignorance of the underlying mechanisms. The implication is that both genes and environment contribute to blood pressure, but 'multifactorial' avoids specifying the components or how they might interact. Nevertheless, such details are important for the development and implementation of effective preventive and treatment strategies.

(S.B. Harrap, The Lancet 1994;344:ii:169-171)

1 MECHANISMS OF HYPERTENSION

1.1 INTRODUCTION

Mechanisms to explain chronic elevation of blood pressure, hypertension, have been much debated and reviewed.[1] The development ideas can be traced back to the early 19th century, in observations by Bright of an association between high blood pressure and albuminuria.[2] In the more recent past attention has shifted to the cardiovascular consequences of elevated blood pressure. This association has repeatedly been confirmed and is well quantified by epidemiologists in large longitudinal observational studies, as summarized in several meta-analyses.[3,4] Although age standardized mortality rates from cardiovascular disease are declining in many industrialized countries, mortality and morbidity from cardiovascular disease remain a major public health problem.[5] Part of the problem might be related to early effects on the cardiovascular system of a gradually rising blood pressure starting early in life. There is therefore much interest in the various mechanisms that regulate blood pressure and might give rise to hypertension. In particular studies on the early phase of primary hypertension, before the system changes in response to sustained elevations in pressure, might indicate the mechanisms involved in the pathogenesis of primary hypertension.[6,7] This perspective forms the background of The Dutch Hypertension and Offspring Study, and of this thesis.

To study the early phase of primary hypertension, animal and human observational and experimental study designs have been used, with the aim of finding determinants of primary hypertension. Each design has its own advantages and disadvantages for the mechanism studied. From experimental models, especially in various species of genetically hypertensive rats, it follows that many different mechanisms may cause hypertension, alone or in combination. Changes in cardiovascular physiology, in renal physiology, neuro-endocrinology and recently, characteristics at the cellular and DNA level have been put forward. Concepts thought to be of relevance at the time The Dutch Hypertension and Offspring Study was designed, are reviewed in chapter 1.2.

In studies of the early pathogenesis of primary hypertension, three major problems appear. First the multifactorial nature makes it impossible to define one initiating or necessary cause for development of primary hypertension. The only early marker of future hypertension we are certain about is elevated blood pressure itself.[6] This issue will be discussed in chapter 2.1, in view of the Platt-Pickering debate on monogenetic versus multifactorial origin of primary hypertension.[8]

Second, the multifactorial origin and presence of blood pressure increases early in life make it difficult to separate genetic and environmental influences in the development of primary hypertension, as discussed in chapter 2.2.

Third, the physiology of blood pressure regulation may obscure mechanisms involved in the early phase of primary hypertension; a rise in blood pressure evokes secondary changes that cannot easily be distinguished from putative primary mechanisms. This problem will be discussed for family studies in chapter 2.3.

Studies comparing subjects selected on the basis of parental blood pressure profiles, may be best suited to study human primary hypertension in its early phase.[9] The Dutch Hypertension and Offspring Study, a family based study of early predictors of primary hypertension, forms the core of this thesis. In chapter one hypotheses explored in The Dutch Hypertension and Offspring Study are discussed (see above). In chapter two methodological problems encountered in studies on the early phase of human primary hypertension are dealt with (see above). A general description of the design, protocol and methods will be given in chapter three, including results of the selection method used. In chapter four, grouped according to the hypotheses described in chapter one, the results of The Dutch Hypertension and Offspring Study will be presented. Finally in chapter five, conclusions from these findings will be drawn and in light of the vast body of knowledge already available suggestions for further research will be given.

1.2 THE EARLY PHASE OF HYPERTENSION

Many studies of the pathophysiology of primary hypertension and its association with aging,[10,11] studies of subjects with so called "borderline" hypertension[12,13,14,15,16,17] and observational studies on determinants of blood pressure and blood pressure rise in young normotensive subjects,[18,19,20,21] have explored the mechanisms that regulate blood pressure. Although hypertension is of multifactorial origin, and separation of mechanisms is somewhat artificial, hypotheses have focussed on:

- cardiovascular mechanisms that balance blood flow through cardiac function and vascular tone and contraction (section 1.2.1)
- the role of the kidney in body fluid homeostasis and vasoactive peptides (section 1.2.2)
- the autonomous nervous system at rest and during stress and the sensitivity of the cardiovascular system to its activity (section 1.2.3)
- mechanisms that regulate the cellular and general homeostasis of electrolytes, such as sodium and calcium, and their correlates with blood pressure (section 1.2.4)
- certain immunogenetic factors that may affect blood pressure (section 1.2.5).

According to these five mechanistic areas, major studies addressing these topics both in the early phase of primary hypertension and in established primary hypertension will be summarized. At the beginning of each section a lay out of the subdivisions is given.

1.2.1 HAEMODYNAMIC CHARACTERISTICS

- *Introduction*
- *Primary increase of peripheral resistance*
- *Primary increase of cardiac output*
- *Summary*

Introduction

The nature of the haemodynamic characteristics of the initial phase of primary hypertension is controversial. Two major hypotheses compete; the development of high blood pressure may begin either with an increased peripheral resistance or with an increased cardiac output.[22] Once hypertension is established an elevated peripheral resistance and a low to normal cardiac output prevails.[22] It is assumed that in hypertension peripheral resistance either gradually rises with age or that hypertension develops after an initial period of a high cardiac output, a so called hyperkinetic circulatory phase, followed secondarily by an increase in peripheral resistance.[22] In 'early', 'mild', 'borderline' or 'labile' young hypertensives an increased cardiac output, measured by invasive methods, has been reported.[12,14,15,23,24,25] Others, however, using noninvasive methods, could not confirm this,[13] or even reported a decrease in cardiac output and increase of peripheral resistance in young borderline hypertensive subjects compared to normotensive subjects.[26,27] More recently, results were presented suggesting an elevated cardiac output in a proportion of borderline hypertensive subjects.[28] The total group of borderline hypertensive subjects compared to normotensive subjects, however, did not show an increased cardiac output. It was argued that the hyperkinetic circulatory phase is a clinical entity, applicable to a sizable proportion of up to 37% of the borderline hypertensive subjects.[28] It is not known whether borderline hypertensive subjects with a hyperkinetic circulation are at an increased risk to develop primary hypertension compared to those with a normokinetic circulation. Follow-up for up to 17 years of borderline hypertensive subjects with an initially high cardiac output, has shown a decrease in cardiac output and an increase in peripheral resistance, without a clear rise in blood pressure after 10 years and a mild increase in blood pressure after 17 years.[29] Borderline hypertensive subjects initially showing a normal cardiac output and an increased peripheral resistance had a more substantial rise in blood pressure that occurred earlier during the 17 years follow-up.[29] Blood pressure of either young hypertensive or young normotensive subjects did not show a relation with cardiac output.[30,31] In subjects,

participating in the Muscatine Study, cardiac output was only weakly related to blood pressure, and the level of cardiac output did not predict future blood pressure during follow-up.[20] In this study, future blood pressure was best predicted by left ventricular mass, resting and exercise systolic blood pressure.[20]

In addition to the persisting discussion on the initial haemodynamic change in the early phase of primary hypertension, hypotheses have been proposed to define the mechanisms responsible and to describe the transition of these haemodynamic changes to primary hypertension.

Primary increase of peripheral resistance

With respect to the increased peripheral resistance, Folkow proposed that structural vascular changes are responsible for the early rise in peripheral resistance.[32] The theory of an increased arterial wall to lumen ratio was based on findings of a diminished maximal vasodilatation after arterial occlusion during isometric exercise and local heating in hypertensive compared to normotensive subjects.[22,32] This was associated with a steeper pressor response curve and lower pressor threshold dose in hypertensive subjects.[33] Recently, however, Distler's group proposed that at least part of this phenomenon may arise from a functional component in the elevated resistance in hypertensive subjects.[34] They showed that the difference between hypertensive and normotensive subjects in maximal vasodilatation and vascular resistance seen during either infusion of nifedipine or after arterial occlusion during isometric exercise and local heating, disappeared when both manoeuvres were applied simultaneously.[34] Still, findings in experimental[35] and human hypertension both in isolated resistance vessels[36] and in large arteries in vivo[37] support the presence of an increased arterial wall to lumen ratio in hypertension, which may precede the development of hypertension.[38] Aalkjær et al. did not find a difference in media thickness compared to lumen diameter in isolated resistance vessels of offspring of hypertensive and normotensive parents.[39] Functional changes in the vascular smooth muscle cell might also increase vascular tone and peripheral resistance.[22,34] Myogenic activity may be increased either intrinsically, due to an increased sensitivity to stimuli or a decreased sensitivity to inhibition, or extrinsically due to an increased activity of the sympathetic nervous system and other humeral pressor factors. The latter possibilities are discussed in section 1.2.3. Resistance vessels of offspring of hypertensive and offspring of

normotensive parents were compared in vitro and, in the absence of morphological differences between the offspring groups, similar responses to various stimuli were obtained.[39] This does not favour differences in intrinsic vascular smooth muscle activity between offspring of hypertensive and normotensive parents. In vivo studies comparing blood flow and arterial resistance during maximal vasodilatation between offspring of hypertensive and offspring of normotensive parents have not provided consistent results. Maximal forearm blood flow after arterial occlusion and ischaemic exercise was observed to be decreased in offspring of hypertensive parents compared to offspring of normotensive parents in two studies,[40,41] but others have reported a similar maximal forearm blood flow.[42] Moreover, maximal calf muscle blood flow did not show a difference between groups.[42,43] The finding of an enhanced responsiveness of blood pressure to intravenous noradrenaline infusion with similar responses to angiotensin-II infusion in offspring of hypertensive parents and offspring of normotensive parents, suggests differences in adrenergic sensitivity.[44] Another approach to study vascular properties is to measure noninvasively the change in arterial diameter or cross sectional area relative to pressure changes during the cardiac cycle using ultrasound techniques, to estimate the distensibility and cross sectional compliance of the artery respectively. With this method, both young and old borderline hypertensive subjects have shown a diminished distensibility and cross sectional compliance of the common carotid artery compared to normotensive subjects.[45] In the Bogalusa Heart Study, children in the upper race-, sex- and age-specific tertile for cholesterol and blood pressure showed an increased stiffness of the common carotid artery (i.e. a decreased distensibility) compared to children in the lowest tertile.[46] When grouped according to family history of hypertension, no clear differences in distensibility were seen. However, a positive parental history of myocardial infarction was associated with an increased arterial stiffness.[46] A decrease in venous vascular compliance that was observed in offspring of hypertensive parents compared to offspring of normotensive parents during saline infusion using an invasive technique,[42] will be discussed below as it is more relevant to the theory of a hyperkinetic circulation than to peripheral resistance.

In summary, the increased peripheral resistance in established hypertension appears to depend largely on an increased arterial wall to lumen ratio.[36,37] In the early phase of primary hypertension intrinsic or extrinsic factors affecting vascular smooth muscle tonic contraction might increase peripheral resistance. Intrinsic contractile factors like guanosine

triphosphate binding protein and phospholipase C have trophic influences on cardiovascular muscle growth.[47] Moreover, vascular smooth muscle cells could even have an intrinsic, "genetic" tendency for hypertrophy.[48] Therefore, in the early phase of primary hypertension structural cardiovascular changes may be present together with an increase in vascular tone, both leading to an increase in total peripheral resistance.

Primary increase of cardiac output

With regard to the hyperkinetic circulatory phase, both an increased chrono-inotropic cardiac stimulation and an increased venous return have been put forward as responsible for an increase in cardiac output.[22] An increased cardiac stimulation may arise from an increased sympathetic stimulation (see section 1.2.3), or decreased parasympathetic inhibition resulting in an increased heart rate and/or stroke volume.[49] A rise in venous return may result from an increased volume load or a decreased vascular capacity.[22] In offspring of hypertensive parents an exaggerated rise in central venous pressure was found compared to offspring of normotensive parents during a saline infusion of 1000 ml.[42] As a consequence the estimated total vascular capacity, calculated from the change in blood volume divided by the change in central venous pressure, was diminished in the offspring of hypertensive parents. Unfortunately, in this study the change in cardiac output was not registered. Yet, it was argued that cardiac output must have risen in excess in the offspring of hypertensive parents compared to the offspring of normotensive parents, because an exaggerated response in systolic blood pressure and pulse pressure was observed in the offspring of hypertensive parents.[42] Under physiologic circumstances, changes in volume load may arise from changes in water and sodium retaining mechanisms (see section 1.2.2, 1.2.4). A combination of an increased water and sodium retention and a decreased total vascular capacity could lead to an increased venous return and subsequently an increased cardiac output. However, an increased venous return with shifts from the systemic to the cardiopulmonary circulation would, in theory, only lead to a short-term increase in cardiac output, lasting until the central blood volume has disposed of excess volume.[50]

Regardless of the origin of an increased cardiac output, the hyperkinetic circulatory phase is followed by the haemodynamic state of fully developed primary hypertension with a relative increase in peripheral resistance and decrease in cardiac output.[16] This might occur from autoregulation of the perfusion; i.e. excess flow (so called luxury perfusion) is

counteracted by an increase in resistance, followed by a decrease in venous return and cardiac output.[51] The increase in resistance could occur from either a "myogenic" response to nonmetabolic flow, or from a presumably greater than normal washout of vasodilator substances.[22] Korner argued against the autoregulation theory as an explanation for the secondary rise in peripheral resistance and decrease in cardiac output. He postulated that pressor stimuli have a more pronounced effect on the resistance vessels compared to the heart that eventually leads to a predominance of an increased peripheral resistance.[52] Lund-Johansen argued against the whole-body auto regulation theory to explain the transition from high cardiac output to high peripheral resistance, because in his 20-year follow-up study of borderline hypertensive subjects no sign of a luxury perfusion with an abnormally low arterio-venous oxygen difference was found.[29] He postulated that through a decrease in left ventricular compliance, due to left ventricular hypertrophy, the stroke index declines, and finally cardiac output.[29] Julius et al. reasoned that, at the end of the supposed hyperkinetic circulatory phase, the elevation of cardiac output becomes less through a decrease in cardiac compliance and a decrease of cardiac-ß-adrenergic receptor responsiveness.[28] By then, however, responsiveness of the resistance vessels could become accentuated through structural hypertrophic vascular changes resulting from the hyperkinetic circulatory phase. This would imply that a relatively increased cardiac output and peripheral resistance combine in the initial phase of primary hypertension. Support for this view is found in the observation that some borderline hypertensive subjects have a peripheral resistance that is raised in relative excess of an increased cardiac output.[25,50,53] Moreover, normokinetic borderline hypertensive subjects had an increased minimal forearm vascular resistance compared to hyperkinetic borderline hypertensive subjects, and in the former a significant correlation existed between the minimal forearm vascular resistance and left ventricular mass.[54] This could indicate that structural cardiac and vascular changes develop in parallel and relatively early in the course of blood pressure elevation.

At variance with a hyperkinetic circulation in the early phase of primary hypertension are not only the above discussed results using noninvasive methods in borderline hypertensive subjects that did not show an increased cardiac output for the group as a whole,[13,26,27,28] but also the findings in normotensive youngsters with a contrasting family history of hypertension. The haemodynamic state of young normotensive offspring with a positive family history of hypertension, compared to those without, is characterized

by a similar cardiac output,[55,56,57,58,59,60,61,62] a similar[55,63,64,65,66,67] to increased[41,56,57,59,68,69] left ventricular mass, an altered ventricular diastolic filling[55,56] and a similar[56] or slightly increased[55,58] calculated total peripheral resistance (table 1).

In conclusion, it is debatable whether an increased cardiac output is a separate mechanism in the initial phase of primary hypertension and whether it leads to an increased peripheral resistance.

Summary

The collective findings in hypertensive subjects, and in youngsters at risk for hypertension in view of their family history of hypertension (table 1), do not support the presence of a hyperkinetic circulation as the initial haemodynamic derangement in primary hypertension. In none of the studies comparing offspring of hypertensive parents to offspring of normotensive parents, a difference in cardiac output was observed. In the borderline hypertensive youngsters, this may only be present in a minority of subjects studied. Normokinetic borderline hypertensive subjects tend to have a relatively increased peripheral resistance, that is related to left ventricular mass. In 50% of studies, using a family history approach, in which left ventricular mass was measured, a relative increase was seen in the offspring of hypertensive parents. It seems much more likely that early structural cardiovascular changes occur parallel with an early rise of blood pressure and total peripheral resistance.

It remains difficult to assess the extent to which the youngsters at risk for hypertension selected in the family history studies will actually develop hypertension. Not many of the family history studies actually measured the blood pressure of the parents at the start of the selection.[70] In some studies parental blood pressure was given, however, the primary selection was based on questionnaires. It cannot be excluded that the overall picture of the results is influenced by inadequate methodology to select the youngsters at risk for hypertension in a number of studies. Also, one cannot exclude that the smaller group of hyperkinetic borderline hypertensive youngsters may represent a different entity of a pre-hypertensive state of primary hypertension, compared to the prevailing group of normokinetic borderline hypertensive youngsters. If a hyperkinetic circulation does occur in the pre-hypertensive phase in a subset of the population that develops primary hypertension, the transition to primary hypertension is unlikely to result from autoregulation, but from

structural cardiovascular changes by which cardiac output declines and peripheral resistance rises.

Table 1 Haemodynamic characteristics of the prehypertensive state; review of family history studies.*

Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
- Pressure response							
to noradrenaline	questionnaire	unknown	24.0	48	SBP 0, DBP 0	yes,+	Bianchetti[44]
to angiotensin-II	questionnaire	unknown	24.0	48	SBP 0, DBP 0	no	Bianchetti[44]
- Wall to lumen ratio							
(resistance vessels)	history of R_x	unknown	24.1	43	SBP 5 (ns), DBP 8 (s)	no	Aalkjær[39]
- Forearm vascular resistance							
at rest	history	unknown	23.7	40	SBP 8(ns), DBP 10 (s)	no	Takeshita[40]
at maximal vasodilatation	history	unknown	23.7	40	SBP 8 (ns), DBP 10 (s)	yes,+	Takeshita[40]
	BP	>160/95 vs <140/90	22.4	30	SBP 12 (s), DBP 5 (s)	yes,+	Parati[41]
	BP	>175/115 vs <130/90	36	30	SBP 3 (ns), DBP 2 (ns)	no	Widgren[42]
	history	unknown	19-22	24	SBP 4 (ns), DBP 1 (ns)	no	Iwase[61]
- Calf muscle vascular resistance							
at rest	BP father	>175/115 vs <130/90	31.1	32	MAP 5 mmHg (ns)	yes,+	Gudmundsson[43]
at maximal vasodilatation	BP father	>175/115 vs <130/90	31.1	32	MAP 5 mmHg (ns)	no	Gudmundsson[43]
	BP	>175/115 vs <130/90	36	30	SBP 3 (ns), DBP 2 (ns)	no	Widgren[42]
- Carotid Artery Wall stiffness	questionnaire	unknown	13	99	SBP 10 (s)°	no	Riley[46]
- Venous vascular compliance	BP	>175/115 vs <130/90	36	30	SBP 3 (ns), DBP 2 (ns)	yes,-	Widgren[42]
- Total peripheral resistance	history	unknown	37	29	SBP 15 (s), DBP 7 (s)	no (ns +)	Ohlsson[58]
	history	unknown	26	38	SBP 1(ns), DBP 4(ns)	no (ns +)	Graettinger[55]
	BP	>175/115 vs <130/90	36	30	SBP 6 (ns), DBP 2 (ns)	no	Widgren[56]
	history	unknown	19-22	24	SBP 4 (ns), DBP 1 (ns)	no	Iwase[61]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Cardiac output	history	unknown	26	38	SBP 1(ns), DBP 4(ns)	no	Graettinger[55]
	BP	>175/115 vs <130/90	36	30	SBP 6 (ns), DBP 2 (ns)	no	Widgren[56]
	questionnaire, BP	>95 or R_x vs <95	14-19	106	SBP 2 (ns), DBP 1 (ns)	no	Radice[57]
	history	unknown	37	29	SBP 15 (s), DBP 7 (s)	no	Ohlsson[58]
	BP	DBP >95 vs < 95	22	100	SBP 2 (ns), DBP 1 (ns)	no	Celentano[59]
	questionnaire, BP	>95 or R_x vs <95	14-19	96	SBP 4 (ns), DBP 1 (ns)	no	Alli[60]
	history	unknown	19-22	24	SBP 4 (ns), DBP 1 (ns)	no	Iwase[61]
Left ventricular mass	BP	>160/95 vs <140/90	22.4	30	SBP 12 (s), DBP 5 (s)	yes,+	Parati[41]
	history	unknown	26	38	SBP 1(ns), DBP 4(ns)	no	Graettinger[55]
	BP	>175/115 vs <130/90	36	30	SBP 6 (ns), DBP 2 (ns)	yes,+	Widgren[56]
	questionnaire, BP	>95 or R_x vs <95	14-19	106	SBP 2 (ns), DBP 1 (ns)	yes,+	Radice[57]
	BP	DBP >95 vs <95	22	100	SBP 2 (ns), DBP 1 (ns)	yes,+	Celentano[59]
	questionnaire, BP	>95 or R_x vs <95	14-19	96	SBP 4 (ns), DBP 1 (ns)	yes,+	Alli[60]
	history	unknown	26	29	SBP 12 (s), DBP 6 (s)	no	Casati[63]
	questionnaire, BP	148/92 vs 130/80	8 - 10	107	SBP 5 (s), DBP 3 (s)	no	Hansen[64]
	questionnaire	unknown	26	61	SBP 5(s), DBP 2(ns)	no	Allemann[65]
	history, BP	>160/90 or R_x vs <160/90	14	29	SBP 1 (ns), DBP 0	no	DeLeonardis[67]
	BP	>160/95 or R_x vs <140/90	22	30	SBP 13 (s), DBP 5 (ns)	yes,+	Ravogli[68]
	history	unknown	28	43	SBP -7 (ns), DBP -8 (ns)	yes,+	RokkedahlN[69]

* If three groups were studied, the data on groups at highest contrast are presented.
FH+=offspring with positive, FH-= offspring with negative family history of hypertension.
HT= hypertension, NT=Normotension, BP=blood pressure, P_{xx}=xx^{th} percentile for blood pressure position of the parents.
R_x=treatment for hypertension.
Difference FH+ vs FH-: +=increased in FH+, -=decreased in FH+.
s=significant difference, ns=nonsignificant difference between FH+,FH-.
°=selection based on BP offspring.

1.2.2 THE KIDNEY

- *Introduction*
- *Renal perfusion*
- *The renin-angiotensin-aldosterone system*
- *Sodium excretion, renin-angiotensin and renal perfusion*
- *Summary*

Introduction

In theory the role of the kidney as a long-term regulator of blood volume and blood pressure level is well established.[71] In work by Mizell et al. the long-term control of renal perfusion pressure on renal electrolyte and water excretion was convincingly demonstrated.[72] The physiology of the kidney in regulating body-fluid homoeostasis determines that any increase in blood pressure is compensated for by an increase in renal excretion of water and salt. Therefore, initially blood pressure elevation may be viewed as a homoeostatic response to a deficient renal sodium output.[51] Yet, once sustained hypertension has developed, changes in renal function must have occurred to prevent excessive diuresis. The pathogenetic mechanism that sets, during the development of hypertension, the pressure-natriuresis relationship at a higher level of arterial pressure is unknown.[73] However, from the above reasoning, it is likely that the kidney is either primarily or secondarily involved in the pathogenesis of primary hypertension, or even both.

From elegant kidney transplant experiments in animals and humans it appears possible to transplant hypertension with the kidney. In animals "normotensive rats" (NR) receiving a kidney from a young, normotensive "spontaneously hypertensive rat" (SHR) developed hypertension while transplantation of kidneys from NR to an already hypertensive SHR decreased blood pressure.[74] In humans, essential hypertensive patients with nephrosclerosis who received a kidney from a normotensive donor became normotensive.[75] Kidney transplant recipients with a negative family history of hypertension receiving a kidney from a normotensive donor with a positive family history of hypertension needed more anti-hypertensive treatment after transplantation compared to recipients of a kidney from a normotensive donor with a negative family history of hypertension.[76] These findings underline the possibility that the kidney is primarily involved in the pathogenesis of primary hypertension. However, in the donor kidney during life, changes might have occurred that influence blood pressure after transplantation in the

recipient. To exclude renal changes secondary to an increased perfusion pressure in the kidney grafts of genetic HR, kidney transplant experiments were done with kidneys of genetic HR that had normal blood pressures during life until transplantation through treatment with either ramipril or hydralazine. It was shown that recipients of a kidney from a normotensive but genetic HR developed posttransplantation hypertension and that recipients of a kidney from a genetic normotensive rat (NR) did not.[77] These results point to a primary role for the kidney in primary hypertension. Further experiments with transition from low to high salt diet in kidney graft recipients, showed an exaggerated sodium retention in recipients from grafts of genetic HR compared to recipients from grafts of genetic NR. This suggests that renal sodium retention is increased in graft kidneys from genetic HR, compared to graft kidneys from genetic NR, and points to a role of sodium retention in the pathogenetic mechanism involved in hypertension that can be transplanted by the kidney.[77]

Before these experiments, it was postulated that a primary defect residing in the kidney decreases water and sodium excretion.[78] Many mechanisms in the kidney contribute to its sodium excretion capacity,[79] both intrinsic mechanisms such as a redistribution of blood flow between cortical and juxta-medullary nephrons[80], the glomerulo-tubular balance, the tubulo-glomerular feedback, and the renin-angiotensin system may be important. Major extrarenal mechanisms include renal perfusion pressure and flow. Of these systems both renal perfusion and the renin angiotensin system have been studied in the early phase of primary hypertension.

Renal perfusion

Renal function tests in young and borderline hypertensive subjects[13,81,82,83,84,85] have not shown a consistent derangement of renal perfusion in the early phase of hypertension. Relative to the systemic circulation, renal blood flow in these subjects was reported to be either in a normal range[13,15,81] or decreased.[82] Compared to normotensive subjects, renal blood flow was reported to be either relatively increased,[13,15,81] similar[82] or decreased.[83] Moreover, studies comparing offspring of hypertensive parents and offspring of normotensive parents showed a similar renal blood flow in the groups studied (see table 2).[85,86,87,88,89] In two of five studies the offspring groups were observed both on a low and a high sodium diet.[87,89] With regard to renal blood flow, no difference was seen between the

groups of offspring whether on the high or the low sodium diet.[87,89] However, on the low sodium diet, in the offspring of hypertensive parents compared to the offspring of normotensive parents, an exaggerated increase in renal blood flow was seen in response to diltiazem,[87] suggestive of an increased renal vascular tone in the offspring of hypertensive parents on a low sodium diet. This is in accordance with the observation of an increased renal vascular resistance on a low sodium diet in offspring of hypertensive parents compared to normotensive parents.[89] However, by Bianchi and co-workers an increase in renal blood flow in the offspring of hypertensive parents has been reported.[90,91] It is important to note that in the studies of Bianchi et al.[90,91] cardiac output was similar in the offspring groups, suggesting that in the offspring of hypertensive parents a relative increase in renal perfusion was established by selective renal vasodilatation. This was interpreted as an increased pressure diuresis secondary to a possible increased tubular reabsorption, due to a genetic membrane defect (see section 1.2.4). In the long run this could lead to hypertension, either by a further increase of tubular reabsorption or by an acceleration of the age-related process of renal arteriosclerosis.[91] However, other findings do not support an increase in renal vasodilatation; during stress renal perfusion decreases in offspring of hypertensive parents while it increases in offspring of normotensive parents.[85] Under angiotensin converting enzyme inhibition[86] or calcium entry blockade[87,88] renal perfusion increased in the offspring of hypertensive parents and not in the offspring of normotensive parents, suggesting an increase of renal vasoconstriction in the offspring of hypertensive parents. Moreover, in offspring of hypertensive parents on a high sodium diet, acute sodium loading resulted in a significant increase in renal vascular resistance, while it decreased in offspring of normotensive parents.[89] From these findings it might be concluded that renal perfusion and renal vascular resistance are differently maintained and regulated in offspring of hypertensive parents compared to offspring of normotensive parents. Results might have been influenced by small differences in blood pressure that were found between the groups of offspring, either nonsignificant,[85,86,87,88] or significant for diastolic blood pressure,[89,90,91] systolic blood pressure.[90,91]

The renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system could also be involved in a change of renal sodium excretory capacity or renal perfusion in the early phase of primary hypertension. An

increase in renin and angiotensin-II may raise renal vascular resistance and decrease sodium excretion. In Japanese schoolchildren with a family history of hypertension a higher plasma renin activity was found compared to schoolchildren without a family history of hypertension.[92] Similar differences were observed by Grim et al. in US adults.[93] However, in the above mentioned studies comparing offspring of hypertensive parents to offspring of normotensive parents, plasma renin activity was not significantly different between the groups in five of six studies[85,86,87,88,90] with a tendency to a lower plasma renin in the offspring of hypertensive parents in three studies[85,87,90] and a clearly lower plasma renin in one study[91] (see table 2). A decreased renin activity in the early phase of primary hypertension might result from an increased body sodium and body volume state. This is, however not supported by two studies addressing this directly, which could not reveal a difference in total exchangeable sodium, plasma or blood volume at either normal[94,95] or high sodium intake[94] between offspring of hypertensive and normotensive parents. A decrease in plasma renin activity might also occur secondary to an increased renal vasoconstriction, as was suggested by the findings mentioned above.[85,86,87,88,89]

Sodium excretion, renin-angiotensin, renal perfusion

Changes in renal haemodynamics and/or the renin-angiotensin system in the early phase of primary hypertension could be reflected in a difference in rate of excretion of an acute sodium-volume load in offspring of hypertensive parents in comparison with offspring of normotensive parents. Again, from several studies no consistent pattern has emerged. After sodium loading, offspring of hypertensive parents compared to offspring of normotensive parents showed an increased sodium elimination rate with a similar renin concentration before loading that was more suppressed during sodium loading.[96] In the sons of normotensive parents, but not those of hypertensive parents, a correlation has been observed between sodium excretion per hundred ml of glomerular filtration and blood pressure.[96] Another study described the opposite, i.e. a decreased sodium elimination rate in the offspring of hypertensive parents, together with an elevated basal renin concentration that remained relatively increased during sodium loading compared to the offspring of normotensive parents.[93] A similar sodium elimination rate in both groups of offspring at either normal or high sodium intake[94] has also been reported.

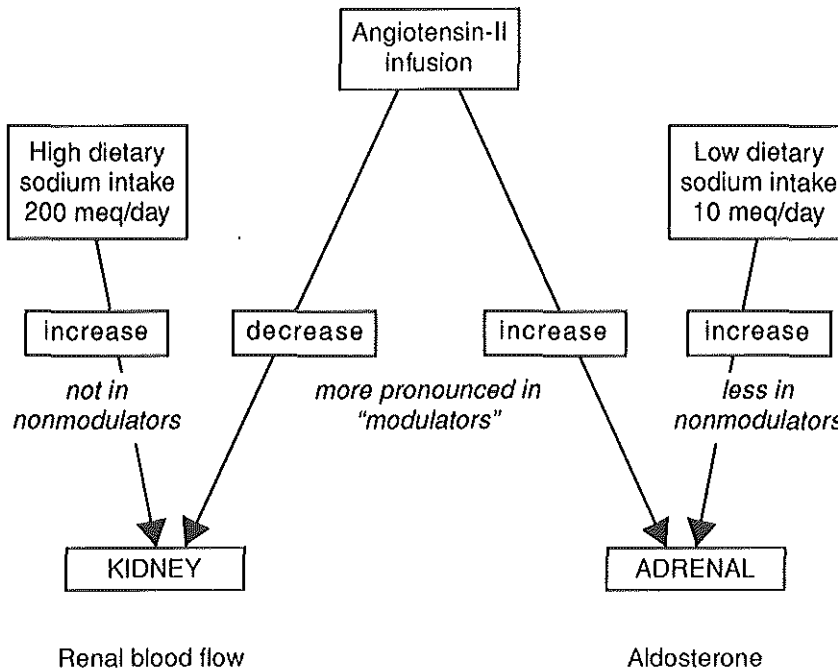
Other characteristics besides sodium excretion capacity have been postulated to be

involved in the alteration of renal function in the early phase of primary hypertension; an increased sympathetic nervous system activity[97] (see section 1.2.3), a change in adrenergic sensitivity of the kidney due to changes in adrenoreceptors[98] (see section 1.2.3), a membrane abnormality for electrolyte transport[22,79,99] (see section 1.2.4), or a state of the kidney in which there is a relative insensitivity to modulate renal perfusion and aldosterone secretion in response to angiotensin-II infusion, associated with changes in sodium intake.[100] The presence of the first three abnormalities in the early phase of primary hypertension, i.e. in subjects at risk for hypertension, will be discussed in the sections hereafter. The latter will be discussed here.

The concept of "nonmodulation" opposed to "modulation", has emerged from studies in essential hypertensive subjects with normal to high renin levels.[100] Nonmodulators show no clear decrease in renal blood flow and increase in aldosterone in response to angiotensin-II infusion on respectively a high or low sodium diet (figure 1).[100] This abnormality is normalized during converting enzyme inhibition[101,102] without a change in the blood pressure response to angiotensin-II infusion[101] and without a change in the plasma angiotensin-II level.[102] This may suggest that a local tissue-defect in the renin-angiotensin system is present in nonmodulators. Special interest in this concept as a possible primary mechanism in the pathogenesis of hypertension arose from the observation of a higher prevalence of a positive family history of hypertension among nonmodulators (85%) compared to modulators (27%).[103] A study in hypertensive sibship showed a familial aggregation of the nonmodulation trait. The sibship studied however, had a mean age of 45 years and were almost all hypertensive themselves so that nonmodulation as a secondary phenomenon could not be excluded.[104] Interestingly, from observations in pre-hypertensive subjects, nonmodulating characteristics can be deduced; normotensive offspring of hypertensive parents showed a lower plasma aldosterone level on sodium restriction and an increased response of renal blood flow to diltiazem, but not to acetylcholine, compared to normotensive offspring of normotensive parents.[87] It was postulated that this finding represents an increased angiotensin-II mediated renal vasoconstriction in the offspring of hypertensive parents.[87] This possibility is supported by other findings showing an increased rise in renal blood flow in offspring of hypertensive parents, compared to offspring of normotensive parents, to either a converting enzyme inhibitor,[86] or a calcium antagonist.[88] Moreover, similar to nonmodulating hypertensive adults, a reduced adrenal response to

angiotensin-II at low sodium intake was shown in offspring of hypertensive compared to offspring of normotensive parents.[105]

Figure 1 Schematic representation of the nonmodulation theory.[100]



The nonmodulation-modulation concept stresses one other important feature of the kidney. Shifts in sodium intake are able to modify renal and adrenal responses in normotensive youngsters and a balanced high or low sodium intake is needed to demonstrate differences between nonmodulators and modulators. A liberal sodium intake may therefore in part explain the inconsistent findings in the above described studies of renal function and the renin-angiotensin system in youngsters at different risk for hypertension.

In conclusion, from the above reviewed studies both on renal physiology in relation to blood pressure regulation, and renal transplantation in relation to hypertension, it follows that the kidney is primarily involved in the pathogenesis of hypertension. Studies in borderline hypertensive subjects and in offspring of hypertensive parents, although not conclusive, suggest that the renal derangement might either be an increased renal vasoconstriction or a derangement in sodium excretion capacity. An increased renal vasoconstriction might be related to either a local tissue defect in the renin-angiotensin system, or an increased sensitivity to pressor substances. The possible derangement in sodium excretion might be related to abnormalities in membrane transport of electrolytes or renal haemodynamic changes.

Table 2 Renal function, renin-angiotensin-aldosterone and sodium excretion in the prehypertensive state; review of family history studies.*

Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Renal blood flow							
- at rest	history	unknown	39 (22-66)	24	MAP 7 (ns)	no	Hollenberg[85]
- change during stress test	history	unknown	39 (22-66)	24	MAP 2 (ns)	>in FH-, <in FH+(ns)	Hollenberg[85]
- at rest	history, BP check	unknown	21.4	108	SBP 4 (ns), DBP 1 (ns)	no	Uneda[86]
- change after captopril	history, BP check	unknown	21.4	41	SBP 1 (ns), DBP 0	=in FH-, >in FH+(s)	Uneda[86]
- at rest on high sodium diet	history of R_x	unknown	38.5	27	SBP 3 (ns), DBP 3 (ns)	no	Blackshear[87]
- at rest on low sodium diet	history of R_x	unknown	40	25	SBP 1 (ns), DBP 0	no	Blackshear[87]
- change after diltiazem	history of R_x	unknown	40	25	not given	> FH-, >>> FH+ (s)	Blackshear[87]
- at rest	history of R_x, BP	R_x vs <140/90	24	18	SBP 2 (ns), DBP -1 (ns)	no	Montanari[88]
- change after Nifedipine	history of R_x, BP	R_x vs <140/90	24	18	SBP 0, DBP 1 (ns)	= FH-, >> HF+ (s)	Montanari[88]
- at rest on low sodium diet	BP	>169/95 vs <140/90	41 vs 47	22	SBP 6 (s), DBP 14 (s)	no	Textor[89]
- at rest on high sodium diet	BP	>169/95 vs <140/90	41 vs 47	22	SBP 10 (s), DBP 10 (s)	no	Textor[89]
- at rest	BP, 2 parents	>150/95 vs <140/90	23	91	SBP 5 (s), DBP 5 (s)	yes, + (s)	Bianchi[90]
- at rest	BP, 2 parents	>150/95 vs <140/90	23	108	SBP 8 (s), DBP 5 (s)	yes, + (s)	Bianchi[91]
Renal vascular resistance							
- at rest	history, BP check	unknown	21.4	108	SBP 4 (ns), DBP 1 (ns)	yes, + (s)	Uneda[86]
- change after captopril	history, BP check	unknown	21.4	41	SBP 1 (ns), DBP 0	=in FH-, <in FH+(s)	Uneda[86]
- at rest on low sodium diet	BP	>169/95 vs <140/90	41 vs 47	22	SBP 6 (s), DBP 14 (s)	yes, + (s)	Textor[89]
- at rest on high sodium diet	BP	>169/95 vs <140/90	41 vs 47	22	SBP 10 (s), DBP 10 (s)	yes, + (ns)	Textor[89]
Glomerular filtration							
- at rest	history, BP check	unknown	21.4	108	SBP 4 (ns), DBP 1 (ns)	no	Uneda[86]
- at rest	history of R_x, BP	R_x vs <140/90	24	18	SBP 2 (ns), DBP -1 (ns)	no	Montanari[88]
- at rest on low sodium diet	BP	>169/95 vs <140/90	41 vs 47	22	SBP 6 (s), DBP 14 (s)	no	Textor[89]
- at rest on high sodium diet	BP	>169/95 vs <140/90	41 vs 47	22	SBP 10 (s), DBP 10 (s)	no	Textor[89]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Glomerular filtration							
- at rest	BP, 2 parents	>150/95 vs <140/90	23 (14-30)	91	SBP 5 (s), DBP 5 (s)	yes, + (ns)	Bianchi[90]
- at rest	BP, 2 parents	>150/95 vs <140/90	23	108	SBP 8 (s), DBP 5 (s)	yes, + (s)	Bianchi[91]
Plasma renin activity	history	unknown	39 (22-66)	24	MAP 7 (ns)	no	Hollenberg[85]
Plasma renin activity	history	unknown	21.4	108	SBP 4 (ns), DBP 1 (ns)	no	Uneda[86]
PRA on low sodium diet	history of R_x	unknown	40	20	SBP 1 (ns), DBP 0	no	Blackshear[87]
Plasma renin activity	history of R_x, BP	R_x vs <140/90	24	18	SBP 2 (ns), DBP -1 (ns)	no	Montanari[88]
Plasma renin activity§	BP	>160/95 vs <140/90	41 vs 47	22	SBP 6 (s), DBP 14 (s)	no	Textor[89]
Plasma renin activity	BP, 2 parents	>150/95 vs <140/90	23 (14-30)	91	SBP 5 (s), DBP 5 (s)	yes, - (ns)	Bianchi[90]
Plasma renin activity	BP, 2 parents	>150/95 vs <140/90	23	108	SBP 8 (s), DBP 5 (s)	yes, - (s)	Bianchi[91]
Plasma renin activity	BP,	>160/95,R_x vs <160/95	10-14	567	SBP 3 (ns), DBP 1 (ns)	yes,+(s)in boys	Shibutani[92]
Plasma renin activity	questionnaire+BP	141/84,R_x vs 126/78	similar	62	SBP 8 (s), DBP 4 (ns)	yes,- (ns)	Beretta-Pic[95]
Plasma renin activity	BP	DBP>85 vs <140/80	18 - 26	34	MAP 1(ns)	no	Wiggins[96]
Plasma renin activity	BP, History	not given	28 (16-60)	86	SBP 6 (s), DBP 8 (s)	yes, + (s)	Grim[93]
Plasma renin activity§	BP	>160/95 vs <140/90	25 (20-32)	24	SBP 3 (ns)/ DBP 5 (ns)	no	Beretta-Pic[105]
Aldosterone	history	unknown	39 (22-66)	24	MAP 7 (ns)	no	Hollenberg[85]
Aldosterone(low sodium diet)	history of R_x	unknown	40	17	SBP 1 (ns), DBP 0	yes, - (s)	Blackshear[87]
Aldosterone	history of R_x, BP	R_x vs <140/90	24	18	SBP 2 (ns), DBP -1 (ns)	no	Montanari[88]
Aldosterone	BP	>160/95 vs <140/90	41 vs 47	22	SBP 6 (s), DBP 14 (s)	no	Textor[89]
Aldosterone (urine)	BP, 2 parents	>150/95 vs <140/90	23	108	SBP 5 (s), DBP 5 (s)	no	Bianchi[90]
Aldosterone (urine)	BP, 2 parents	>150/95 vs <140/90	23	108	SBP 8 (s), DBP 5 (s)	no	Bianchi[91]
Aldosterone	questionnaire+BP	141/84,R_x vs 126/78	similar	62	SBP 8 (s), DBP 4 (ns)	no	Beretta-Pic[95]
Aldosterone	BP, History	not given	28 (16-60)	86	SBP 6 (s), DBP 8 (s)	no	Grim[93]
Aldosterone§	BP	>160/95 vs <140/90	25 (20-32)	24	SBP 3 (ns), DBP 5 (ns)	no	Beretta-Pic[105]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Angiotensin II	history	unknown	39 (22-66)	24	MAP 7 (ns)	no	Hollenberg[85]
Ang II on low sodium diet	history of R_x	unknown	40	21	SBP 1 (ns), DBP 0	no	Blackshear[87]
Angiotensin II§	BP	>160/95 vs <140/90	25 (20-32)	24	SBP 3 (ns), DBP 5 (ns)	no	Beretta-Pic[105]
Exchangeable sodium							
- at normal sodium intake	BP father, History	R_x vs NT	31	32	MAP 5 (ns)	no	Gudmundsson[94]
- at high sodium intake	BP father, History	R_x vs NT	31	32	MAP 3 (ns)	no	Gudmundsson[94]
- at normal sodium intake	questionnaire+BP	141/84,R_x vs 126/78	similar	62	SBP 8 (s), DBP 4 (ns)	no	Beretta-Pic[95]
Total blood volume							
- at normal sodium intake	questionnaire+BP	141/84,R_x vs 126/78	similar	62	SBP 8 (s), DBP 4 (ns)	no	Beretta-Pic[95]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Acute sodium load and							
1) sodium elimination rate							
- at normal sodium intake	BP father, History	R_x vs NT	31	32	MAP 5 (ns)	no	Gudmundsson[94]
- at high sodium intake	BP father, History	R_x vs NT	31	32	MAP 3 (ns)	no	Gudmundsson[94]
- at normal sodium intake	BP	DBP>85 vs <140/80	18 - 26	34	MAP 1(ns)	yes, +	Wiggins[96]
- at normal sodium intake	BP, History	not given	28 (16-60)	86	SBP 8 (s), DBP 6 (s)	yes, -	Grim[93]
2) suppression of renin							
- at normal sodium intake	BP	DBP>85 vs <140/80	18 - 26	34	MAP 1(ns)	yes, +	Wiggins[96]
- at normal sodium intake	BP, History	not given	28 (16-60)	86	SBP 6 (s), DBP 8 (s)	no	Grim[93]
Angiotensin II infusion and aldosterone increase							
- at low sodium intake	BP	>160/95 vs <140/90	25 (20-32)	24	SBP 3 (ns), DBP 5 (ns)	yes, -	Beretta-Pic[105]
- at high sodium intake	BP	>160/95 vs <140/90	25 (20-32)	24	SBP 12 (s), DBP 12 (ns)	yes, --	Beretta-Pic[105]

* If three groups were studied, the data on normotensive groups at greatest contrast are presented.
FH+=offspring with positive, FH-= offspring with negative, family history of hypertension.
HT= hypertension, NT=Normotension, BP=blood pressure.
R_x=treatment for hypertension.
Difference FH+ vs FH-: += increased in FH+ versus FH-, -=decreased in FH+ versus FH-, during manoeuvre: = means unaltered, > means increase, < means decrease, >>> means greater increase (relative to the other group) of the parameter during the manoeuvre.
s=significant difference, ns=nonsignificant difference between FH+,FH-.
§ studied on a high and a low sodium intake (not presented separately as similar results for parameter X were found).

1.2.3 THE SYMPATHETIC NERVOUS SYSTEM

- *Introduction*
- *Activity of the sympathetic nervous system in hypertension*
- *Stress reactivity and sympathetic nervous system activity in hypertension*
- *Sympathetic nervous system activity and blood pressure regulation*
- *Summary*

Introduction

A role of the sympathetic nervous system in the pathophysiology of primary hypertension has long been suspected and has been extensively studied. Much controversy, however, remains, resulting in part from many serious methodological problems involved in the measurement of sympathetic nervous system activity and reactivity.[22] Grossly, five approaches can be distinguished. Measurement of catecholamine levels and metabolites in venous or arterial blood or urine samples has been used repeatedly, in particular in blood sampled from the antecubital vein.[106,107] Discussion arose whether peripheral venous plasma noradrenaline represents systemic sympathetic nervous activity.[107,108,109,110] Recently Chang et al. reasoned that this might be appropriate only during conditions when change in muscle sympathetic nerve activity parallels changes in general sympathetic nerve activity, i.e. not during rest, but during physical activity.[111] As a more sophisticated measure, total or regional spillover rate and total clearance of noradrenaline and adrenaline may be estimated.[112] Similarly, adrenergic end-organ effects of various stimuli and changes produced by pharmacological autonomic blockade,[49,113,114] and microneurographic electrophysiological registrations of muscle sympathetic nerve activity,[115,116] have been used. An indirect approach measures the balance between neural sympathetic and parasympathetic activity by way of spectral analysis of heart rate, blood pressure and respiration variability.[117,118] Regardless the complexities involved in each method, not one is "perfect" in assessing sympathetic nervous system activity at the effector site.[22,112] Not withstanding these problems, from various studies, it appears that the sympathetic nervous system activity might be involved, especially in the early phase of primary hypertension.

Activity of the sympathetic nervous system in hypertension

Venous plasma noradrenaline levels at rest were frequently found to be elevated in young

established hypertensive subjects compared to young normotensive subjects, as reviewed by Goldstein.[106,107] However, in general in borderline hypertensive subjects compared to normotensive subjects[14,116,119] and in offspring with a positive family history of hypertension compared to offspring with a negative family history of hypertension[44,90,91,120,121,122] no clear differences in blood levels of noradrenaline were found. In contrast, recently, Ferrier et al. reported elevated plasma noradrenaline levels in normotensive offspring of hypertensive parents compared to offspring of normotensive parents using arterial plasma samples.[123] Plasma adrenaline appears to be somewhat increased in established hypertensive subjects of all ages,[106] but no clear elevation of plasma adrenaline at rest has been detected in borderline hypertensive subjects[14,119] and offspring of hypertensive parents[44,90,91,120,121,122,123] compared to their respective control groups.

After mental stress, increased plasma levels of noradrenaline and/or adrenaline in borderline hypertension[14,119,124] and in subjects with a positive family history of hypertension[124] have been described. During light, submaximal physical exercise an increased plasma noradrenaline was seen in both young normotensive and borderline hypertensive offspring of hypertensive parents compared to normotensive offspring of normotensive parents.[125] These findings may point at a relative increase in systemic sympathetic nervous system activity during physical stress in youngsters at risk for hypertension.[111,125]

Changes in reactivity at the level of the adrenoreceptor may also be implicated, but the role and presence of changes in adrenoreceptor characteristics in established hypertension is controversial. Studies comparing platelet α_2- and lymphocytic β_2-adrenoreceptor densities in hypertensive and normotensive subjects have yielded conflicting results.[126,127,128,129,130,131] Recently, Blankesteijn et al. concluded that blood-cell adrenoreceptor characteristics are unchanged in primary hypertension, based on their negative results in studies of differences in cellular adrenoreceptors across relatively large groups of hypertensive subjects and normotensive subjects, and a review of the literature.[132] Not included in the review was the one study in hypertensive subjects that took into account the family history of hypertension.[131] In that study a higher density of α_2-adrenoreceptors in the group of hypertensive subjects with a positive family history compared to hypertensives without a family history for hypertension was observed.[131] Moreover, Michel et al. reported an increased density of platelet α_2-adrenoreceptors in

normotensive offspring of one hypertensive parent compared to offspring of normotensive parents, in the absence of differences in circulating catecholamine levels.[133] However, Skrabal et al. could not detect any difference in platelet α_2-adrenoreceptor density between normotensive youngsters with or without a family history of hypertension, but did observe an increased platelet α_2-adrenoreceptor density in salt-sensitive normotensive youngsters compared to salt-resistant normotensive youngsters.[134] In genetically hypertensive rat strains (SHR) renal α_2-adrenoreceptor density appears to be increased before hypertension develops.[135] Therefore, although the adrenoreceptor characteristics might not be altered once hypertension has developed, one cannot exclude changes in the pre-hypertensive phase.

An increased response to noradrenaline infusion in offspring of hypertensive parents compared to offspring of normotensive parents has been reported.[44,120,136,137] This was postulated not to result from structural vascular changes, because of the pre-hypertensive state of the youngsters,[44,136] and because noradrenaline and angiotensin evoked different pressor responses.[44] These findings suggest an increased sensitivity to noradrenaline in offspring of hypertensive parents, that may be related to alterations in adrenoreceptors or receptor density.

Stress reactivity and sympathetic nervous system activity in hypertension

With regard to the effect of sympathetic nervous system activity on blood pressure and heart rate, many studies have been done to estimate the effect of various stress forms on these cardiovascular parameters.[138,139] Interpretation of the responses to stress are difficult as functional and structural cardiovascular alterations, besides the sympathetic nervous system reactivity, may contribute to the response.[22] However, these studies might be informative about the effect of "stress" on daily and longterm blood pressure levels, as high responders to an active laboratory stress task appear to be high responders during everyday stress too.[140] A positive association between responses to active laboratory stress-tasks and future blood pressure rise has been demonstrated.[17,141] Recently, a quantitative review of studies on stress reactivity in subjects with parental hypertension, showed hyperresponsiveness of blood pressure in offspring of hypertensive parents in both mental and physical stress tasks.[70] Moreover, an association between the response to real life stressors and parental hypertension is known.[142] Falkner suggested that, although it has not

been proven that stress causes hypertension, a continued hyper-response to daily stressors, producing repeated burst of elevated blood pressure that occur more often in those at risk, may, when extended over time, play a role in the development of hypertension.[143]

Several other hypotheses have been developed to clarify how relatively small differences in sympathetic nervous system activity might lead to hypertension. The adrenaline hypothesis fits the stress related hypothesis for hypertension, by suggesting that a long term effect on blood pressure may result from repeated adrenaline excretion. Even though these would give only small and short term increases of plasma adrenaline, after re-uptake in sympathetic nerve endings adrenaline could facilitate noradrenaline release during subsequent sympathetic stimulation and thereby amplify cardiovascular responses.[144] It was shown that stress levels of adrenaline have an immediate[145,146] and delayed[147] amplifying effect on the pressor response during sympathetic stimulation, apparently by raising plasma noradrenaline.[145] The effect on both plasma noradrenaline and amplification of the pressor response was prevented by ß-blockade, suggesting that adrenaline exerts its effect through presynaptic ß-adrenoreceptors.[145] The delayed effect suggests neuronal uptake of adrenaline and subsequent co-release with noradrenaline.[147] Differences in frequency or quantity of adrenaline release or differences in uptake of adrenaline or stimulation of presynaptic ß-adrenoreceptors might be implicated in the development of hypertension.[148]

Sympathetic nervous system activity and blood pressure regulation

Repeated stimuli or chronically increased sympathetic nervous system activity could promote the development of hypertension in a number of ways. For instance through effects on cardiac output, peripheral resistance, sodium sensitivity, and renal mechanisms of sodium retention and renal vasoconstriction.

With regard to the fore mentioned hyperkinetic phase, the high cardiac output seen in certain borderline hypertensive subjects, disappeared after blockade with propanolol and atropine. This would suggest an increased autonomous nervous activation of the heart in borderline hypertensive subjects with a high cardiac output.[149]

Alpha adrenergic blockade after autonomic blockade by propanolol and atropine in high renin borderline hypertension significantly decreased peripheral resistance, but not in normal and low renin hypertension.[150] This suggests a neurogenic contribution to the increase of vascular resistance in high renin borderline hypertension. Stimulation of the

sympathetic nervous system, evoked during mental stress, appeared to increase forearm blood flow, vascular resistance and heart rate more in offspring of hypertensive parents than in offspring of normotensive parents.[151,152] Moreover, sympathetic neurotransmitters, especially adrenaline, may exert trophic stimuli on cardiovascular tissue through activation of adrenoreceptors, and induce early structural changes that increase peripheral resistance.[153,154,155]

With regard to sodium-induced development of hypertension a role of an increased sympathetic nervous system activity has also been postulated. In a number of experiments in young normotensive rats of a rat strain with salt sensitive hereditary hypertension, Oparil et al.[156] showed that a high sodium diet was accompanied by increased plasma noradrenaline levels relative to sodium excretion. Moreover, a positive association between plasma noradrenaline and blood pressure was present. It was also shown that stress tests in these young rats fed a high sodium diet compared to a normal sodium diet led to a more pronounced increase in noradrenaline and that the depressor response to ganglion blockade was increased.[156] Such an interaction of a high sodium diet with sympathetic nervous system activity and blood pressure, was not present in older established hypertensive rats. It was postulated that an increased sodium intake in these young rats could enhance sympathetic nervous system activity, resulting in an earlier and more severe blood pressure rise. From a further series of experiments it appeared that the high sodium diet reduced noradrenergic activity in the anterior hypothalamic area, thereby decreasing inhibition of sympathetic outflow resulting in an increased peripheral sympathetic nervous system activity and blood pressure rise.[156]

Several studies in man support an interaction of sodium intake, sympathetic nervous system activity and blood pressure in primary hypertension. Falkner et al. showed that a high sodium diet exaggerated the blood pressure response to a mental stress test in offspring of hypertensive parents, but not in offspring of normotensive parents, without a difference in plasma catecholamine levels.[157] In studies in normotensive youngsters of either hypertensive or normotensive parents, further classified as salt sensitive and salt resistant subjects, Skrabal et. al. reported that salt sensitive subjects showed a more pronounced pressor response to infused noradrenaline compared to salt resistant subjects, both at high and low salt intake.[137] Moreover, platelet α_2-adrenoreceptor density was higher in salt-sensitive compared to salt resistant subjects[134] and α_2-adrenoreceptor density decreased

during sodium restriction.[134] Furthermore, in a group of normotensive male subjects, it was shown that a reduction of sodium intake from 200 to 50 mmol/day lowered platelet α_2-adrenoreceptor density by 14% and increased lymphocytic β_2-adrenoreceptor density by 16%, resulting in a 53% decrease of the cellular α_2/β_2-adrenoreceptor ratio.[158] The change of α_2/β_2-adrenoreceptor density ratio, but not of absolute α_2- or β_2-adrenoreceptor density, was positively correlated to the change in blood pressure during the shift from low to high sodium intake.[158] These findings in normotensive youngsters appear to indicate that a high sodium intake may increase blood pressure through changes in cardiovascular responsiveness to sympathetic nervous system activity. Findings in established hypertensive subjects have shown that a high sodium intake decreases sympathetic nervous activity to a lesser degree in sodium sensitive subjects compared to sodium resistant subjects.[159,160] This suggests that a high sodium intake may sustain hypertension through a reduction in the usual suppression of the sympathetic nervous system activity.

Finally, the sympathetic nervous system is thought to be involved in renal mechanisms that may relate to hypertension.[97,98,133] Renal sympathetic nervous system activity and reactivity may influence renal haemodynamics and renal excretory function. During mental stress, youngsters at risk for hypertension with a high heart rate response showed a substantial reduction in sodium and fluid excretion. However, youngsters at risk for hypertension with a low heart rate response during the mental stress test or youngsters at low risk for hypertension did not show this reduction.[161] Only in case of a combination of an increased risk for hypertension (defined as either borderline hypertension or parental hypertension) with an increased sympathetic nervous system activity during mental stress, renal sodium and fluid excretion appeared to be reduced.[161] In accordance with this finding it has been reported that a mental stress test in offspring of hypertensive parents, but not in offspring of normotensive parents, decreases renal perfusion.[85] However, isometric exercise, did not induce a difference in sodium and potassium excretion in offspring of hypertensive and offspring of normotensive parents.[162] A difference in renal reactivity to changes in sympathetic nervous system activity in subjects at different risk for hypertension may also be involved. For instance, in hypertensive subjects, neurogenic renal vasoconstriction at rest and after isometric exercise depended mainly on activation of α_2-adrenoreceptors.[163] These receptors exert a tonic inhibitory influence on renin release.[164] Moreover, renal α_2-adrenoreceptors on the proximal tubule, stimulate sodium reabsorption.[98] From the combined

findings of an increased density of renal α_2-adrenoreceptors in genetic hypertensive rats before hypertension develops,[135] the increased density of platelet α_2-adrenoreceptors in either offspring of hypertensive parents[133] or sodium-sensitive normotensive youngsters,[134] and findings on renal α_2-adrenoreceptor function,[163,164] the following can be postulated: changes in renal α_2-adrenoreceptor characteristics in certain subjects in the early phase of primary hypertension affect renal reactivity to sympathetic stimulation which alters renal haemodynamics and renal sodium and fluid excretion which in turn causes blood pressure to rise (figure 2).[98]

Figure 2 Schematic representation of a hypothetical pathway that relates renal adrenoreceptor changes to blood pressure increase in the early phase of primary hypertension.

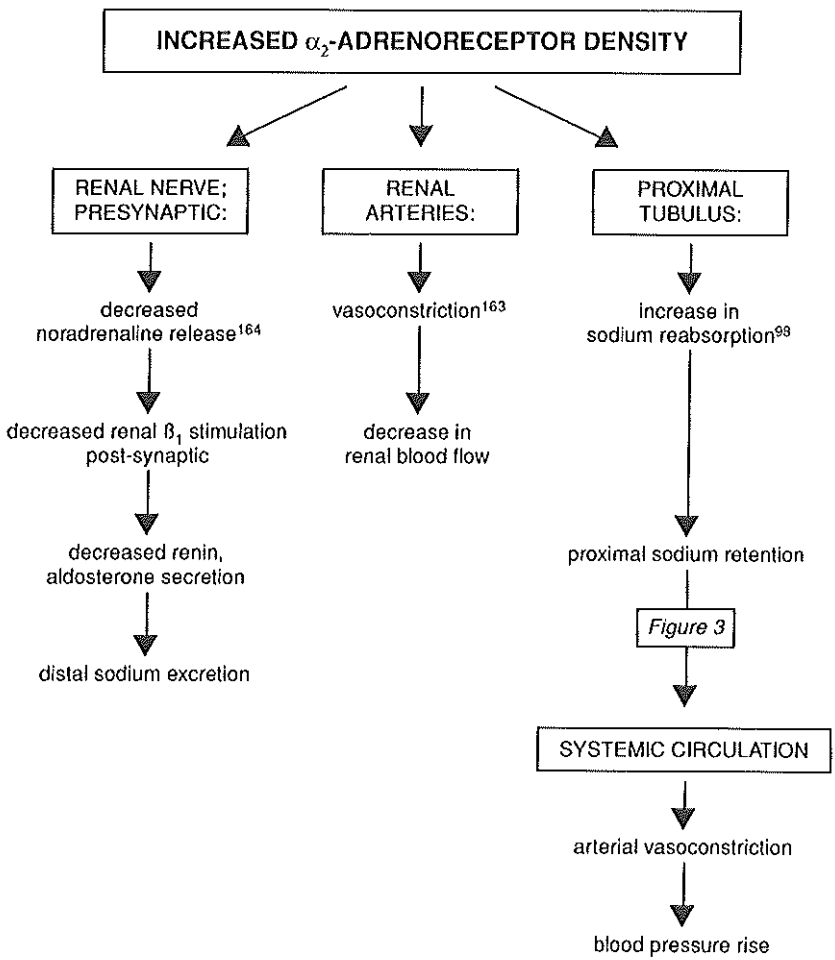

Table 3 The sympathetic nervous system in the prehypertensive state; review of family history studies.*

Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Venous Catecholamines at rest							
- noradrenaline, adrenaline	questionnaire	unknown	24.0	48	SBP 0, DBP 0	no,	Bianchetti[44]
- noradrenaline, adrenaline	BP, 2 parents	>150/95 vs <140/90	23	91	SBP 5 (s), DBP 5 (s)	no,	Bianchi[90]
- noradrenaline, adrenaline	BP, 2 parents	>150/95 vs <140/90	23	108	SBP 8 (s), DBP 5 (s)	no,	Bianchi[91]
- noradrenaline, adrenaline	questionnaire	unknown	23.8	50	SBP 4 (ns), DBP -1 (ns)	no	Bianchetti[120]
- noradrenaline, adrenaline	questionnaire	unknown	22.8	23	SBP 1 (ns), DBP 3 (ns)	no	Bianchetti[121]
- noradrenaline, adrenaline	questionnaire	unknown	20.0	59	SBP 4 (ns9, DBP 1 (ns)	no	Saito[122]
Arterial catecholamines, at rest							
- noradrenaline	questionnaire+BP	142/93 vs 123/75	26.5	22	SBP 6 (ns), DBP 0	yes	Ferrier[123]
- adrenaline	questionnaire+BP	142/93 vs 123/75	26.5	22	SBP 6 (ns), DBP 0	no	Ferrier[123]
Venous Catecholamines after stress							
- after mental stress	history	unknown	14.6	48	SBP 5 (ns), DBP 0	yes, +	Falkner[124]
- after physical stress	questionnaire+BP	204/119 vs 136/82	26	56	SBP 0, DBP -2 (ns)	yes, +	R-Nielsen[125]
Adrenoreceptors							
- platelet α_2 density	history	unknown	9.3	89	SBP ns, DBP ns	yes, +	Michel[133]
- lymphocyte β_2 density	history	unknown	9.3	89	SBP ns, DBP ns	no	Michel[133]
- platelet α_2 density	history	unknown	20 - 25	62	SBP 5 (s), DBP 3 (s)	yes, +	Skrabal[134]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Noradrenaline infusion							
- blood pressure response	questionnaire	unknown	24.0	48	SBP 0, DBP 0	yes, +	Bianchetti[44]
- blood pressure response	questionnaire	unknown	23.8	50	SBP 4 (ns), DBP -1 (ns)	yes, +	Bianchetti[120]
- blood pressure response	questionnaire +BP	R_x vs < 160/100	21 - 35	51	SBP 7 mmHg (s), DBP 3 (ns)	yes, +	Doyle[137]

* If three groups were studied, the data on normotensive groups at greatest contrast are presented.
+=increased in FH+ versus FH-
FH+=offspring with positive, FH-= offspring with negative, family history of hypertension. HT= hypertension, NT=Normotension, BP=blood pressure, R_x=treatment for hypertension.
s=significant difference, ns=nonsignificant difference between FH+,FH-.

1.2.4 ELECTROLYTE HOMOEOSTASIS

- *Sodium; introduction*
    - *Sodium retention and hypertension:*
        - *Infinite gain of pressure natriuresis*
        - *Circulating sodium transport inhibitor*
        - *Membrane abnormalities and sodium retention*
            - *Sodium-potassium ATPase activity and pump sites*
            - *Sodium-potassium co-transport*
            - *Sodium-lithium countertransport*
            - *Sodium-hydrogen exchange*

- *Calcium; introduction*
    - *Calcium metabolism in hypertension:*
        - *Serum calcium*
        - *Parathyroid hormone*
        - *Vitamin D*
        - *Urinary calcium excretion*
        - *Cellular calcium metabolism*
            - *Intracellular calcium concentration*
            - *Cellular calcium binding*
            - *Calcium fluxes*
    - *Calcium and hypertension*

- *Sodium and calcium interaction*

SODIUM

Introduction

From animal experiments, clinical observations, dietary trials and epidemiological studies evidence on a relation between sodium intake and blood pressure has been inferred. Although the range of sodium intake within populations is small, associations with blood pressure have been shown.[165,166,167] Across populations a linear relation between sodium intake and blood pressure was found,[168,169] although weakly and particularly depending on some low-sodium-low blood pressure populations. Interestingly from a pathogenetic view an association has been described across populations between sodium intake and rise of blood pressure with age.[167] However, discussion remains to what extent associations between sodium intake and blood pressure within populations are confounded by for example body weight and alcohol consumption.[170,171] Considering the multifactorial control of blood pressure, it can not be excluded that other acculturate factors besides sodium intake influence the relation between sodium intake and rise of blood pressure with age across populations.[170,172]

Therefore, within populations the effect of sodium intake on blood pressure was studied at a relative early age, both in observational and experimental randomized studies. From these studies no consistent relation between sodium intake and blood pressure levels in children and adolescents was found.[173]

Supported by clinical observations in primary and secondary forms of hypertension, many hypotheses on the pathophysiology of primary hypertension link an increased peripheral resistance to sodium retention.[174] Sodium retention occurs due to an inappropriate renal excretion, that is influenced by blood pressure itself, aldosterone, sympathetic nervous system activity and the renin-angiotensin system.[172,174, 174] Animal experiments have shown a different tendency for development of hypertension on a high salt diet in two inbred strains of rats.[175] Changes in sodium intake in different individuals do not show consistent changes in either sodium retention or blood pressure, and differences can be observed between groups of hypertensive and normotensive subjects,[172] and within groups of hypertensive subjects.[176] In offspring of hypertensive parents a significant rise of blood pressure was observed during a high sodium diet compared to a low sodium/high potassium diet, that was not seen in offspring of normotensive parents.[177] However, no difference in urinary electrolyte excretion was present in these two groups of offspring. Therefore, in the search of sodium related hypertensive mechanisms, much emphasis has been put on characterisation of salt-sensitive and salt resistant subjects.[178,179] Emphasis is put on factors that might be involved in the relation between sodium retention and hypertension or factors that influence sodium retention.

Sodium retention and hypertension

Several hypotheses have been proposed to explain a possible link between sodium and hypertension based on the assumption that sodium retention occurs during the development of high blood pressure.

Infinite gain of pressure-natriuresis

According to the "infinite gain" theory, the pressure mechanism that controls renal output of sodium and water, determines in the long range the level of arterial pressure.[71] Sodium and water retention lead, by an increased venous return, to an increased cardiac output and increased blood pressure.[51] It has been postulated that the increased cardiac output results

through autoregulation of the circulation of the body tissues in a gradual rise of peripheral resistance.[51,71,180] Although some doubt has arisen concerning both the whole body autoregulation theory,[52] and the presence of an increased cardiac output as the initial haemodynamic characteristic of hypertension (see section 1.2.1), abnormal renal sodium handling is thought to be causally related to an increased vascular resistance in certain hypertensive subjects.[174, 174,180]

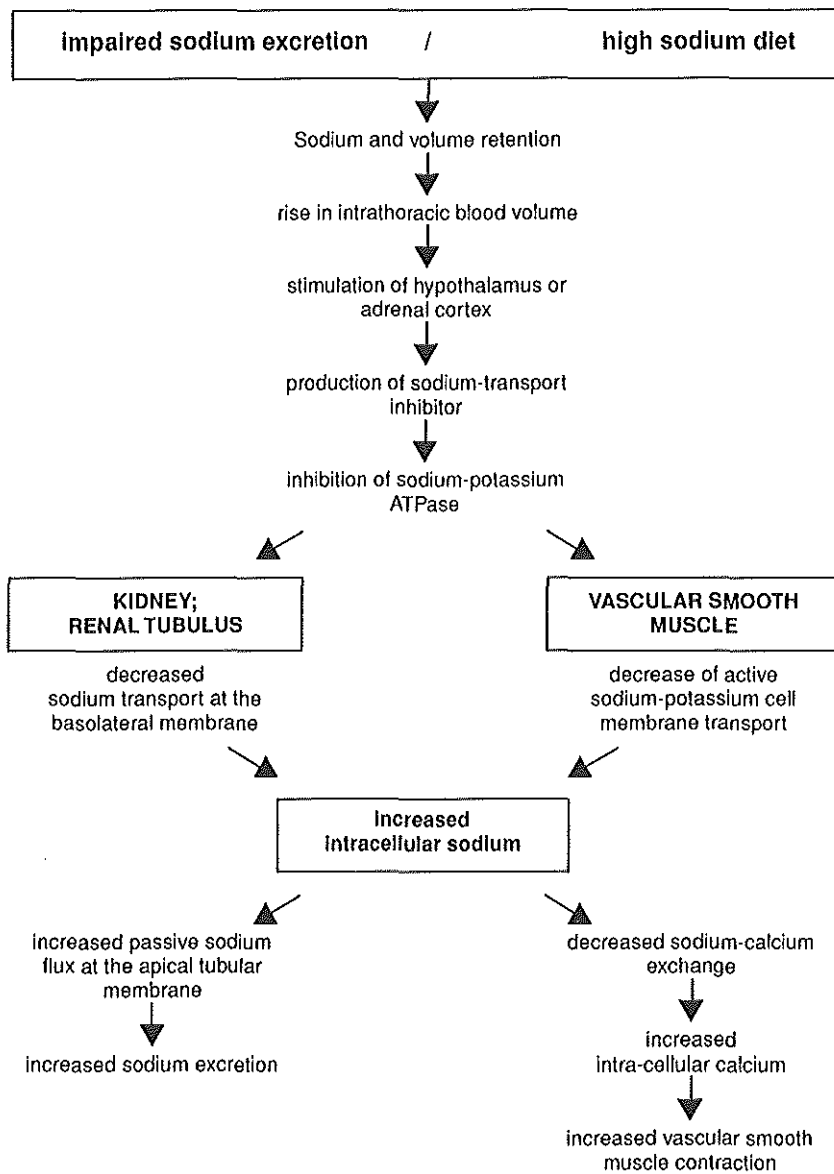
Circulating-sodium-transport inhibitor

Another hypothesis that links sodium and volume retention to an increase in blood pressure, assumes the existence of a circulating factor, or hormone, that has natriuretic properties. Such a mechanism was initially proposed by Dahl and subsequently elaborated by Blaustein, de Wardener and McGregor.[78,181] An excess sodium intake, or a primary defect in excreting sodium, may initially cause a transient rise in intrathoracic blood volume that stimulates either the hypothalamus and/or the adrenal cortex to secrete a circulating sodium-transport inhibitor which in the kidney adjusts sodium excretion so that balance returns to normal[182] (see below). However, as a sort of unwanted side effect, this circulating sodium-transport inhibitor also raises tone and vascular reactivity of smooth muscle of arteries and veins (see below), thereby increasing blood pressure and reducing venous compliance that again may cause a relative increase in intrathoracic pressure and a further enhancement of the secretion of the sodium-transport inhibitor (see figure 3).[78,183]

This postulated circulating sodium-transport inhibitor acts by inhibition of the cellular sodium pump.[184,185] In the kidney the inhibition of the active transport of sodium at the baso-lateral cell membrane of the renal tubular epithelial cells into the peri-tubular fluid enhances intracellular tubular sodium content and decreases the passive flux of sodium from the tubular lumen into the tubular epithelial cells at the apical cell membrane, thereby increasing natriuresis.

Many mechanisms are involved to explain the postulated effect of the circulating sodium-transport inhibitor on smooth muscle tone and reactivity. Inhibition of the sodium pump decreases the active sodium-potassium transport across the cell membrane, thereby increasing the cellular sodium content or sodium/potassium ratio. Two hypotheses relate the increased cellular sodium content to an increased cellular calcium concentration. Haddy suggested that an inhibited sodium pump and increased intracellular sodium would result in

Figure 3 Schematic representation of the theory on circulating sodium transport inhibitor and blood pressure rise.[78,181-185]



a small reduction of the membrane potential, thereby allowing an increased calcium influx through voltage-dependent calcium channels.[184] Blaustein proposed that a raised level of intracellular sodium would increase intracellular calcium by means of a slowing down of the sodium-calcium exchange mechanism.[185] It is by an indirect increase of cellular calcium content in either vascular smooth muscle cells and/or sympathetic nerves that this circulating sodium-transport inhibitor is supposed to increase vascular reactivity and tone.[184,185]

De Wardener and MacGregor further refined this theory to explain the development of primary hypertension as a sequence of events from a genetic defect in the kidney's ability to excrete sodium, which becomes more apparent the higher the sodium intake.[78,183]

To empirically associate humeral sodium-pump inhibition to primary hypertension, several elements of the chain of events need to be demonstrated; the presence of a circulating inhibitor and a decreased sodium transport in the early and later phases of primary hypertension especially during high sodium intake, an increase in intracellular sodium and/or calcium concentration and a decreased sodium excretory capacity.[186]

Although a sodium-potassium ATPase inhibitor has been found in sera of volume expanded animals, identified as a natriuretic factor[187] and as non-esterified fatty acids,[188,189] linoleic acid and oleic acid,[190] and in secondary forms of hypertension in humans, as a digitalis-like factor,[191] little evidence is available to support an increased level in normo-volemic hypertensive subjects for digitalis-like factors,[192] or for unsaturated fatty acids.[188] In children with a blood pressure level in the upper quartile of the distribution a higher venous plasma level of an endogenous-digoxin-like substance was measured by radio-immuno-assay compared to children with blood pressure levels below the median.[193] The level correlated positively with blood pressure, intra-erythrocytic sodium concentration and an eight-hour overnight urinary output.

The supposed heritability of the appearance of a circulating sodium transport inhibitor, is supported by the findings of an increased inhibitory effect of plasma of young spontaneous hypertensive rats on sodium-potassium-ATPase activity in erythrocytes of normotensive rats of a hereditary normotensive rat strain.[194] However, to date, in humans no sufficient proof for the presence of a circulating inhibitor in the prehypertensive state of primary hypertension exists.

Tobian et al., starting with a different hypothesis as described above, were the first

to draw attention to an increased sodium content of the arterial wall in hypertensive subjects compared to normotensive subjects, using postmortem tissue measurements.[195] As the above described hypothesis assumes that the electrolyte changes are present in all cells, most studies on this subject have been done using blood cells.[196] Although one can argue whether this is appropriate, sodium transport characteristics of leucocytes were proved to correlate well with those of resistance vessels measured in vitro.[197]

In hypertensive subjects a raised intracellular leucocyte concentration of sodium has been found.[198,199] This was not confirmed by others using red blood cells.[200,201,202] It has been proposed that different results might be expected for enucleate erythrocytes and nucleate leucocytes as sodium transport in the red blood cell is much slower compared to leucocytes and other cell types.[196] Gray et all, using both leucocytes and erythrocytes to compare hypertensive and normotensive subjects, did show an increased intracellular sodium content in the hypertensive subjects using the leucocytes, but not when using erythrocytes.[202] In the same study, normotensive subjects with a family history of hypertension compared to normotensive subjects without parental hypertension, did not show an increased intracellular sodium content in either leucocytes, or erythrocytes. However, in four separate studies, evidence for an increased intracellular sodium concentration in offspring of hypertensive parents compared to offspring of normotensive parents has been found, one study using lymphocytes[203] and three using erythrocytes.[204,205,206] But five other studies could not confirm these findings in groups of youngsters at different risk for hypertension, and found similar negative results as Gray et al.,[202] either in leucocytes[207,208] or in erythrocytes.[209,210,211] Differences in results might occur from different selection methods of the subjects at risk for hypertension, different isolation and/or measurement techniques. If anything, results of all these studies did not document a decreased intracellular sodium concentration in subjects at risk for hypertension, rather a tendency towards an increased level could be found. Moreover, according to Blaustein,[185] a concentration increase of 5% only could be enough to modify vascular contractility. In conclusion, a small, but physiologically important increase in intracellular sodium concentration, might be present in certain predisposed subjects.

Details of electrolyte transport systems across the cell membrane that influence intracellular sodium concentration and might be controlled by the sodium transport inhibitor, will be discussed below.

Electrolyte Homoeostasis: Sodium

A decreased sodium excretory capacity during the prehypertensive state, to stimulate the postulated sodium-transport-inhibitor, together with an increased excretory sodium capacity once hypertension has been developed due to natriuretic properties of the sodium-transport-inhibitor are even more difficult to demonstrate. The problem is that sodium excretory capacity is related to blood pressure itself and to sodium-volume status before the sodium-load is given. Often exchangeable sodium as a measure for total body sodium is taken. In three studies, on normal sodium intake[94,95] or after a 7-day low sodium diet[95] or a 7[212] to 28-day[94] high sodium diet, no difference in body sodium was present between normotensive subjects with or without a family history of hypertension. In these studies a blood pressure difference between the groups of offspring was reported on the high sodium diet[212] and on the normal sodium diet,[95] but not on the low sodium diet,[95] or on the normal or high sodium diet in the third study.[94] It is concluded that high sodium diets of one week duration do not seem to alter total body sodium differently in subjects at different risk for hypertension, but might influence blood pressure.

With regard to the response to an acute saline load, in one study comparing sons of hypertensive parents to sons of normotensive parents on a free diet before the test, a greater and faster sodium excretion was found in the sons of hypertensive parents, together with a more depressed plasma renin activity.[96] In this study no relation between blood pressure and sodium excretion was observed in the sons of hypertensive parents, while a positive association was seen in the sons of normotensive parents.[96] However, in another study, after one day on a strict regular sodium-potassium diet, a decreased sodium excretion in first degree relatives of hypertensive subjects was observed, in spite of an increased baseline blood pressure level, compared to control subjects without a family history of hypertension.[93] This was confirmed in a more recent study in youngsters on a free diet; besides a blunted natriuretic response during salt loading the investigators also showed a significant rise in blood pressure during salt loading in subjects with a positive family history of hypertension compared to those without.[213] An experiment in which the natriuretic response to an acute sodium load in normotensive subjects with and without a family history of hypertension and in borderline and hypertensive subjects on a liberal diet was studied, could not demonstrate a difference between the two groups of offspring but did show an increased excretion in the borderline and hypertensive subjects.[214] Moreover, when the response to sodium loading after 7-days on a low or high sodium diet was studied,

no difference in renal sodium excretion on either diet between sons of hypertensive and sons of normotensive parents was found.[215] However, having reached a mean age of 47, the sons of hypertensive parents showed a higher systolic and diastolic blood pressure. Therefore, an impairment of renal sodium excretion in the sons of hypertensive parents could not be ruled out, as sodium excretion was relatively decreased in relation to blood pressure level. Yet, it was argued that if a pressure-natriuresis relation would be of any importance to regulate blood pressure in these sons of hypertensive parents, the difference in blood pressure between the groups should have become more clear during sodium loading or on the high sodium diet.[215] From these five studies, one showing an increased,[96] two a decreased[93,213] and two a similar[214,215] sodium excretion following a sodium load, it is difficult to draw any conclusion regarding sodium excretory capacity in the pre-hypertensive state. Findings may be inconclusive due to methodological differences between the studies, as diet and fluid intake before the sodium load, definition of family history of hypertension, age and blood pressure of the subjects.

Clearly, not all subjects with primary hypertension are sodium sensitive.[216] It might be difficult to find such a trait in groups of offspring, in particular if it is not known whether the parents of the offspring were sodium sensitive. Even between groups of subjects with primary hypertension with an equal percentage of family history for hypertension (60%), a difference in sodium excretion after an acute sodium load at 60 minutes was shown when the parental history for cardiovascular disease accidents was taken into account.[217] The group with a positive parental history for cardiovascular events showed an increased sodium excretion, a less depressed plasma renin activity and a smaller increase in atrial natriuretic factor. It might be concluded that renal sodium excretory capacity is dependent on many factors of which one appears to be family history of hypertension. Moreover, it is not clear whether a change in renal sodium excretory capacity in the early phase of primary hypertension leads to sodium retention and secondarily to activation of the so called circulating-sodium-transport-inhibitor.

The one natriuretic hormone found sofar in humans, the atrial natriuretic peptide, does not suppress sodium-potassium ATP-ase activity and, rather than having vasoconstrictive properties, leads to vasodilatation.[218] Therefore, it was postulated that hypertension could develop due to a diminished level or sensitivity for atrial natriuretic factor. However, in hypertensive subjects slightly increased[219,220,221,222,223] or

similar[224,225,226,227] levels of atrial natriuretic factor have been reported compared to normotensive subjects. The slightly increased level in hypertensive subjects can be viewed as compensatory to sodium retention, again focusing on abnormal renal sodium handling as a mechanism in hypertension. A primary role for atrial natriuretic factor in sodium excretion capacity was suggested, by the finding of a blunted increase of atrial natriuretic factor together with a blunted increase of sodium excretion after an acute sodium load in normotensive men with a family history of hypertension.[228]

Membrane abnormalities and sodium retention

The primary derangement in both the "infinite gain theory of pressure-natriuresis" and the "circulating-sodium-transport-inhibitor" hypothesis is sodium retention. Even without a circulating sodium transport inhibitor of sodium-potassium-ATP-ase activity, a change in intracellular sodium and secondary calcium as hypothesised by both Haddy and Blaustein, could occur from an increase in cellular sodium uptake. Combining the hypotheses on renal sodium retention and an increased cellular sodium content, it was postulated that hypertension arises from an intrinsic membrane defect in sodium transport that allows an increased sodium uptake in both vascular smooth muscle cells and renal tubular cells.[186,229]

The existence of a renal membrane abnormality that causes sodium retention has been postulated. Kidney cross-transplantation experiments (see 1.2.2) do support the existence of a primary renal defect.[74] From the findings on renal function in offspring of hypertensive parents[90,91] (see section 1.2.2) and in prehypertensive rats, Bianchi concluded that a primary, probably genetic, increase in renal sodium tubular reabsorbtion might be responsible for an increase in glomerular filtration, urinary output and decreased renin in the offspring of hypertensive parents.[91] When these secondary modifications are no longer able to compensate the primary increased sodium absorption, sodium retention will occur with a subsequent increase in blood pressure.[230]

To study both the possible primary membrane defect that leads to sodium retention and the membrane transport mechanisms that link sodium retention to an increased intracellular calcium and hypertension various tests for ion transport mechanisms have been developed. By studying these in groups of individuals that are either salt-sensitive and/or at high risk to develop hypertension information about the supposed membrane defect that leads to sodium retention and hypertension might be gained. The membrane ion-transport

characteristics that have been studied in the early phase of primary hypertension are

- sodium-potassium-ATPase activity and pump sites
- sodium-potassium co-transport
- sodium-lithium countertransport
- fractional excretion of sodium and lithium
- sodium-hydrogen exchange

These mechanisms are discussed further below.

- *sodium-potassium-ATPase activity and pump sites (Ouabain sensitive)*

The sodium-potassium-ATPase pump actively extrudes sodium from the cell, by exchange for potassium and use of ATP. It is measured in intact cells in vitro as the ouabain sensitive sodium-efflux rate, or rubidium uptake and in membranes as the difference of Mg-ATPase activity minus Na-K insensitive Mg-ATPase. According to the above discussed hypothesis (section 1.2.4) of a circulating inhibitor,[185] sodium-potassium-ATPase activity in hypertensive subjects should be decreased in vivo in vascular smooth muscle cells and renal tubular cells. Based on that hypothesis, it could be argued that in vitro measurements of sodium-potassium-ATPase activity in blood cells will show decreased activity if still suppressed by the inhibitor, but normal or increased activity if the inhibitor is not bound to the cell membrane. Others have postulated an in vivo increased sodium-potassium-ATPase activity as a compensation for another, primary, membrane transport abnormality. Results of many studies in primary hypertension or its early phase have not clarified this issue.

Garay et al. found an increased ouabain sensitive sodium-efflux rate in erythrocytes of six children of hypertensive parents that also had a decreased furosemide sensitive sodium-potassium-cotransport (see below).[231] This was confirmed by Woods et al., with an increased ouabain sensitive rubidium uptake in erythrocytes of hypertensive subjects and their normotensive first degree relatives.[232] Others however, reported a decrease in sodium-potassium pump activity in subjects with a family history of hypertension compared to those without, either in leucocytes,[207,233] or in erythrocytes.[206,234] Swales and coworkers could not confirm their earlier findings,[207] when they studied groups of offspring on strict low or high sodium diets.[208] On either diet no difference in sodium-potassium pump activity measures in leucocytes between the groups of offspring was found. Moreover, the absence of differences in sodium-potassium pump activity between groups of offspring

at different risk for hypertension was also reported by others.[202,205,209,235,236] However, Taylor et al.[236] did show a decreased number of sodium-potassium pump sites by ouabain binding in lymphocytes of normotensive subjects with a family history of hypertension. Another study did not report a difference in sodium pump binding sites, but a decreased affinity of ouabain to erythrocytes in those youngsters at highest risk for hypertension.[237] In hypertensive subjects classified as nonmodulaters (see section 1.2.2), with a strong family history of hypertension no difference in erythrocyte sodium-potassium-pump activity could be shown as compared to modulaters.[238]

To summarize, two studies have shown an increased, four a decreased and six a similar sodium-potassium pump activity in subjects at different risk for hypertension. It is unlikely that a clear difference in sodium-potassium pump activity exists in the prehypertensive period. The possibility, however, cannot be completely excluded.

- sodium-potassium co-transport (furosemide sensitive)

This transport system can passively transport sodium uphill, outside the cell, due to an outward directed potassium gradient, and an inward chloride flux. Bianchi et al. observed an increased activity of this transport system in the Milan hypertensive rat strain, before hypertension develops.[230] In contrast, Garay et al. observed a decreased activity of this furosemide sensitive sodium-potassium-cotransport in hypertensive subjects and their offspring.[231] This was confirmed for offspring of hypertensive parents compared to offspring of normotensive parents by both Lijnen[205] and Uchiyama.[239] Canessa and coworkers observed an increased activity of sodium-potassium-cotransport in hypertensive subjects and normotensive offspring with a positive family history for hypertension (see below),[240] more in line with findings of Bianchi's group in rats[230] and humans.[241]

In a large study of normotensive and hypertensive families, Bianchi et al. showed an increased sodium-potassium cotransport in hypertensive subjects and their offspring compared to respectively normotensive subjects and their offspring.[241] By calculating intrafamilial correlations of sodium-potassium-cotransport levels in all, and in normotensive and hypertensive families separately, a positive and significant correlation was found between offspring and parents in the hypertensive families but not between spouses. From these results and segregation analyses in these families Bianchi et al. argued that a major, recessive gene contributes to the phenotypic value of the sodium-potassium-cotransport.[241]

Apart from this strong hereditary component in sodium-potassium cotransport level, Heagerty et al observed a dietary influence of sodium intake in offspring of hypertensive parents, but not in offspring of normotensive parents.[208] A low sodium intake stimulated a rise of sodium-potassium cotransport activity in the former, but not in the latter group. On a free sodium diet, no differences in sodium-potassium cotransport between the two groups could be shown.

To summarize, in offspring of hypertensive parents compared to offspring of normotensive parents, in three studies a decreased sodium-potassium cotransport was observed,[205,231,239] and in three an increased level was shown,[208,240,241] that became apparent in one study only on a low sodium diet.[208] Moreover a strong hereditary component for the level of sodium-potassium cotransport has been shown in hypertensive families.[241]

It is concluded that the level of sodium-potassium cotransport might be altered in hypertension, although the direction of the change is not clear. Moreover, alterations in the sodium-potassium cotransport level might be an epiphenomenon for hypertension, or represent a factor closely related to a causal factor. In favor of the latter are the findings of Bianchi's group.[242] They observed that hypertensive subjects with a high level of sodium-potassium-cotransport compared to hypertensive or normotensive subjects with a low level of sodium-potassium-cotransport had a low fractional uric acid excretion and low plasma renin activity, with similar glomerular filtration rate and urinary sodium and potassium excretion. This led Bianchi to his original hypothesis[230] of the relation between a high sodium-potassium-cotransport level and an abnormal renal sodium handling as the role of the kidney in primary hypertension.[242]

- sodium-lithium countertransport

The sodium-lithium countertransport in erythrocytes is measured by calculating the difference in lithium efflux of lithium loaded erythrocytes in a sodium-free and a sodium enriched medium. This measures the exchange of sodium for lithium. It is an ouabain insensitive pathway that either changes sodium for sodium, sodium for lithium, or lithium for lithium.

In 1980 Canessa was the first to describe this pathway in relation to hypertension,[243] after others had described the possible heritability of this system. The mean maximum rate of sodium-lithium countertransport was increased in subjects with

primary hypertension, but not in subjects with secondary forms of hypertension. Moreover, in first degree relatives of patients with primary hypertension increased levels compared to control subjects could be found.[243] By the same group in 1982, the latter could not be confirmed; between normotensive subjects with and without a family history of hypertension, no difference was described for the mean maximum rate of sodium-lithium countertransport.[240] Yet, a higher level of sodium-potassium-cotransport was observed.[240] These first results in youngsters at risk for hypertension were confirmed by Woods et al.; sons of two hypertensive parents compared to sons of two normotensive parents had an increased red-cell sodium-lithium countertransport.[244] This difference was observed again during a follow-up measurement after 25 to 40 months, when some of the offspring of hypertensive parents had became hypertensive.[245] Moreover, an increased mean maximum rate of sodium-lithium countertransport in subjects at risk for hypertension was also observed in Chinese youngsters,[235] but not in Italian schoolchildren with a family history of hypertension.[211] In the Italian study, an apparent association of blood pressure with sodium-lithium countertransport was lost after taking height and weight into consideration. In men with a high normal diastolic blood pressure, sodium-lithium countertransport was associated with the maximal systolic blood pressure during a submaximal exercise test, and therefore assumed to be a risk factor for hypertension.[246]

In summary, in five reports[235,243,244,245,246] an increased and in two[211,240] a similar level of sodium-lithium countertransport was described for youngsters at high risk compared to youngsters at low risk for hypertension. However, as promising as these findings appear, extensive work in hypertensive subjects and population based surveys has shown that the relation of sodium-lithium countertransport with primary hypertension is less strong than initially anticipated (see for review[247]). A distinction from secondary forms of hypertension did not hold, and considerable overlap was found between normotensive subjects and hypertensive subjects.[247] Moreover, although population based surveys did show a positive association of this marker with blood pressure and prevalence of hypertension,[248,249,250] it was not always prominent in each sample, and the association was affected by body weight[248,249] and other factors such as cholesterol, glucose, alcohol intake.[249] In several surveys, at all ages and in both sexes, two different, overlapping sodium-lithium-countertransport distributions have been observed, that supports the assumption that this marker is a presymptomatic predictor.[247,251] In a 7-year prospective

study with normotensive adults from Utah pedigrees an elevated baseline sodium-lithium countertransport level did not increase the risk of future hypertension.[252] Only if the information from the segregation analysis on the baseline levels of the pedigrees was used, subjects inferred by that analysis to carry the recessive major gene for high sodium-lithium countertransport were at high risk for hypertension (relative risk compared to low sodium-lithium countertransport genotype group was 4.6 [1,6-13.9]).[252] This was confirmed by others.[253] Although interesting from a genetic point of view, it does not support the clinical use of sodium-lithium countertransport level as a marker to define individual risk for hypertension, as genotypes can only be inferred from segregation analysis using pedigrees. Moreover, it might also mean that the level of sodium-lithium countertransport itself is not related to primary hypertension, but is linked to another possibly causally related marker.

This possible non-causal relation of the sodium-lithium countertransport level with hypertension is also stressed by the problem to define the pathophysiologic importance of an increased level of sodium-lithium countertransport in hypertension.[247] It is difficult to propose a physiological role for an ion-transport-pathway that acts as a sodium-sodium exchange mechanism. However, it has been suggested that the red-cell sodium lithium countertransport is analogous to the sodium-hydrogen ion exchange (see below) in the brush border of the proximal renal tubules.

Departing from that assumption, Weder observed in hypertensive subjects and their offspring who had an increased sodium-lithium countertansport level a diminished renal fractional lithium clearance.[254] Fractional lithium clearance is thought to be an inverse measure of proximal tubular sodium reabsorbtion. However, the observation of Weder[254] of a decreased renal fractional lithium clearance in hypertensive subjects or in offspring of hypertensive parents compared to appropriate controls was not confirmed by Bianchi's group,[214] neither unstimulated nor after a sodium load[214] (see above), and not by Weinberger et al.[255] or Strazullo.[256] The observation by Hollenberg et al.[238] of an increased sodium-lithium countertransport in hypertensive subjects with the nonmodulator characteristic compared to hypertensive subjects with the modulator trait (see section 1.2.2), focused on a possible link between sodium-lithium countertransport characteristics and hypertension in subjects known to have a strong family history of primary hypertension and salt sensitivity. Bianchi et al. observed that the increased level of sodium-lithium countertransport in a

group of hypertensive subjects compared to normotensive subjects, arose largely from those subjects with an increased level of sodium-potassium cotransport.[241] As discussed above, according to Bianchi et al., an increased sodium-potassium cotransport coincides with a decreased fractional uric acid excretion.[242] The findings of Hollenberg and Bianchi stress the possible association between the level of sodium-lithium countertransport and renal sodium handling in a subset of primary hypertension.

In conclusion, it appears that an increased sodium-lithium countertransport is partly genetically determined and weakly associated with future risk for and prevalence of primary hypertension. Although the nature of this association is not understood the evidence available does not suggest a causal relationship. Rather, it might provide a marker of another factor that is important for renal sodium handling.

- sodium-hydrogen exchange

The sodium-hydrogen exchange is a transport system across cell membranes that transports sodium into cells by exchange for a proton. It is driven by the inwardly directed sodium gradient established by the sodium-potassium-ATPase pump. It is activated indirectly by a decrease in intracellular pH, that promotes an increase of intracellular calcium and activates second messenger systems to stimulate the sodium-hydrogen exchanger.

Attention to this transport system in hypertension arose from different observations (see review[257]). For instance, similarities of the sodium-hydrogen exchanger with the sodium-lithium countertransport (besides amiloride sensitivity), and the finding of an increased platelet volume by acidification in platelets of hypertensive subjects (swelling assay).[257] Other techniques measuring direct fluxes of the sodium-hydrogen exchange have shown a consistent pattern of an increased activity in erythrocytes, platelets and leucocytes of subjects with primary hypertension compared to normotensive subjects.[257] Although a significant overlap was seen between the two groups, mean values of the sodium-proton exchange activity in hypertensive subjects were up to 2.4 times increased. In keeping with findings of the above discussed ion-transport markers, a bimodal distribution of the sodium-hydrogen exchange activity appears to exist in hypertensive patients. This suggests that the sodium-hydrogen exchange activity is increased in a subset of hypertensive subjects.

To define this subset of hypertensive subjects one has to focus on characteristics or

mechanisms that might be related to the level of sodium-hydrogen exchange activity in hypertension. Factors such as intracellular buffer capacity or intracellular calcium were hypothesised to be involved. This was, however, thought to be unrealistic by others as unphysiologic levels of protein or calcium respectively would have to be present to influence sodium-hydrogen exchange activity.[257] In hypertensive subjects, but not in normotensive subjects, a negative correlation was found between intracellular pH and serum cholesterol and a positive association between plasma triglyceride level and sodium-hydrogen exchange activity.[258] A decrease in serum cholesterol by HMG-CoA reductase inhibitor treatment of hypercholesterolaemic patients diminished platelet sodium-hydrogen exchange activity.[259] It was hypothesised that changes in serum lipids influence membrane lipid composition and fluidity. The activity of other ion-transport systems, however was not changed.

No data on sodium-proton exchange activity in the early phase of primary hypertension are available. If sodium-proton exchange is of pathophysiological influence in hypertension, differences in cellular acidification might be present between normotensive and hypertensive subjects. In vitro results on isolated blood cells do not show a difference in pH between hypertensive and normotensive subjects (see for review[257]). In isolated human resistance vessels no difference in pH of noncontracting smooth muscle cells of normotensive and hypertensive subjects was seen.[260] However, when stimulated by noradrenaline, the intracellular pH in smooth muscle cells of hypertensive subjects became more alkaline, indicative for an increased activity of the sodium-hydrogen exchange after agonist stimulation or during contraction. It is doubtful whether the sodium-proton exchange is involved in proximal renal sodium reabsorption,[257] as has been suggested.

Finally, a positive association between sodium-hydrogen exchange activity and smooth muscle cell proliferation of isolated cells in culture was seen in spontaneously hypertensive rats, but not in control rats.[261] Moreover, in hypertensive subjects a positive association between Na^+/H^+ exchange in lymphocytes and left ventricular hypertrophy was found.[262] As lymphocytes have no growth properties, it was concluded that the increased sodium-hydrogen exchange activity was not secondarily to an increased growth tendency, but could be primarily involved in cell proliferation.

In conclusion, no definite explanation or role for the observed increase of sodium-hydrogen exchange activity in hypertension is available. In particular no evidence is

available for a causal relation between sodium-hydrogen activity and hypertension. However, an increased activity might be important in cellular proliferation, and thereby inducing structural changes that themselves may be involved in the early pathogensis of hypertension (see 1.2.1). Concerning sodium homeostasis no clear mechanistic role for the sodium-proton exchanger is known; neither for sodium reabsorption nor for stimulation of the exchanger by sodium loading.

CALCIUM

- *Calcium; introduction*
    - *Calcium metabolism in hypertension:*
        - *Serum calcium*
        - *Parathyroid hormone*
        - *Vitamin D*
        - *Urinary calcium excretion*
        - *Cellular calcium metabolism*
            - *Intracellular calcium concentration*
            - *Cellular calcium binding*
            - *Calcium fluxes*
    - *Calcium and hypertension*

Introduction

Calcium ions play a central role in smooth muscle cell contraction.[263] With an increase of vascular smooth muscle tone peripheral resistance rises. An increase in vascular peripheral resistance is considered the main characteristic of primary hypertension.[22] The final common pathway of pathophysiologic mechanisms involved in primary hypertension, therefore, must include a disorder of cellular calcium metabolism.[264]

In the mid seventies, Blaustein[185] and Haddy[184], reasoning from sodium and volume retention as the primary problem, hypothesised an increase of intracellular calcium (see above, section on circulating sodium transport inhibitor). Long before that time a discussion of the role of calcium in blood pressure regulation had started with 'the water story':[265] an observation of an association between drinking water and cerebrovascular accidents,[266] followed by the report of an inverse relation between water hardness, calcium, magnesium and coronary heart disease,[267] and finally by the inverse association between blood pressure and water hardness.[268] The findings were confirmed by others, but not generally accepted, as the results from these geographical studies might have been easily confounded by other characteristics linked to the water districts studied.[265] In the early eighties, an association of low calcium intake and high blood pressure was supported by a small observational study on the difference in dietary intake of calcium between normotensive and hypertensive subjects.[269] Later this finding was confirmed in up to 23 reports of observational studies on diet and blood pressure from all over the world (see for review[270]). A prospective observational study of 4 years in 58218 women showed a relative risk for development of hypertension of 0.78 [0.69 - 0.88] in those women with a

calory adjusted calcium intake of at least 800 mg per day compared to less than 400 mg per day.[271] In this study a range of potential confounders could be controlled for.

In light of these promising observations, many trials have been executed to study the response of blood pressure to calcium supplementation (see for review[272]). The results of these studies are at first sight disappointing; in most studies no significant decrease in blood pressure could be observed. However, two third of the studies showed a tendency of calcium to lower blood pressure and it was argued that several factors might have influenced the results.[272] Among those factors are duration of the calcium supplementation, doses used, other dietary factors as sodium intake and cholecalciferol, and characteristics of the subjects such as blood pressure status, body weight and level of plasma parathyroid hormone or other calciotrophic hormones.[272]

After the contrasting findings on dietary calcium, calcium supplementation and blood pressure, research has focused on elements of calcium metabolism as modifiers of the blood pressure response to calcium supplementation and the definition of characteristics of individuals susceptible to develop hypertension on low calcium intake.

Calcium metabolism in hypertension

Several disturbances of calcium metabolism have been associated with primary hypertension. Markers of changes in calcium metabolism that have been studied in hypertension are serum calcium, parathyroid hormone, vitamin D, urinary calcium excretion and cellular calcium metabolism.

- Serum calcium

Lower serum ionized calcium levels in untreated essential hypertensive subjects compared to normotensive subjects have been described.[273] This was accompanied by a lower serum phosphorus value. In a subsequent report, a low serum ionized calcium level was confined to hypertensive subjects with a low renin profile,[274] and accompanied by an increase of the serum magnesium level in that group. The opposite was found for subjects with primary hypertension and a high renin profile. No difference in calcium and magnesium between normotensive and hypertensive subjects would have been detected if hypertensive subjects with a low, normal and high renin profile had been taken together. These findings were not confirmed by Kesteloot et al.,[275,276] in two large observational studies on the relation

between serum electrolytes and blood pressure. In these studies total serum calcium correlated positively with blood pressure. No results on ionised calcium were reported. Others reported no differences between hypertensive and normotensive subjects for both total and ionized serum calcium,[277] or decreased ionized calcium with similar total serum calcium levels,[278,279] or decreased total serum calcium levels[280] in hypertensive subjects. In conclusion a tendency towards a lower serum ionised calcium in hypertension exists, except for high renin hypertension.

- Parathyroid hormone

Parathyroid hormone is an important regulator of calcium metabolism. Moreover, hypertension is more common in patients with primary hyperparathyroidism than in the general population.[281] Vice versa, hyperparathyroidism is more prevalent in hypertensive than in normotensive men.[282] In several studies comparing hypertensive with normotensive subjects, an increased mean level of parathyroid hormone (PTH) was found in the former group.[277,279,283] In these studies total PTH was measured, comprising both active and inactive metabolites. It cannot be ruled out that this increase in total PTH level in hypertensive subjects is related to a reduced renal clearance of inactive PTH fragments, that may accumulate in hypertensive subjects with reduced glomerular filtration. A study on intact-(1-84)-PTH did not show an increased level in hypertensive subjects aged 20 to 69 years.[284] However, in a subgroup of hypertensive subjects from this study, aged 20 to 40 years of age,[284] and in youngsters with mildly elevated blood pressure,[280] an elevated (1-84)-PTH was found compared to age-matched normotensive subjects. These findings support the view that PTH may be involved in the early phase of primary hypertension. However, a recent study on calcium metabolism in offspring of hypertensive parents did not show a difference in plasma level of (1-84)-PTH with offspring of normotensive parents, either on a high or low salt diet (see below).[285] In view of these findings one could argue that an increase of plasma PTH is present during the early phase of hypertension, when blood pressure already has risen. An alternative explanation, put forward by Pang and coworkers, is that PTH is a marker for another factor excreted by the parathyroid gland with shows vasoactive properties (Parathyroid Hormone Related Peptide or Parathyroid Hypertensive Factor, PHF).[286] The consequences of a possible increased PTH on blood pressure regulation are not clear (see below).[287]

- Vitamin D

Active vitamin D is an important regulator of calcium metabolism and might therefore be related to changes in calcium metabolism in primary hypertension. Active vitamin D facilitates calcium and phosphate absorption from the intestine.[288] Inactive Vitamin D is activated by parathyroid hormone in the kidneys to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).[288] Low levels of ionised serum calcium stimulate parathyroid hormone secretion, and indirectly $1,25(OH)_2D$. From the above discussed aspects on calcium metabolism it follows that a low serum calcium and an increased parathyroid hormone might be involved in primary hypertension. Theoretically $1,25(OH)_2D$ could also be involved in hypertension, either as an epiphenomenon due to an increased activation by a relatively low serum calcium and high parathyroid hormone level, or as a causal factor if a relatively low level of $1,25(OH)_2D$ exists despite a relatively low serum calcium and high parathyroid hormone level. The latter would be suggestive for either a decrease of total vitamin D or a decreased sensitivity to parathyroid hormone. In favour of a reduced $1,25(OH)_2D$ in hypertension, are the results on blood pressure lowering effects of treatment with active Vitamin D, alphacalcidol. A decrease in blood pressure after treatment, compared to placebo, was seen in normocalcaemic hypertensive subjects,[289] and in mildly hypercalcaemic hypertensive subjects with primary hyperparathyroidism.[290] In favour of the former theory of an increased $1,25(OH)_2D$ as an epiphenomenon in hypertension, are observations of an increase in $1,25(OH)_2D$ in normotensive offspring of hypertensive parents during a change from a low to a high sodium diet, that was not apparent in the offspring of normotensive parents. This was accompanied in the offspring of hypertensive parents by a decrease of serum ionized calcium and an increase of urinary calcium excretion, without a change in (1-84)-PTH.[285] Therefore, no conclusion can be drawn on the role of $1,25(OH)_2D$ in human primary hypertension.

- Urinary calcium excretion

An increased urinary calcium excretion has been found in groups of hypertensive subjects compared to normotensive subjects.[277,279,283] In two of three studies results were calculated relative to urinary sodium excretion.[277,283] In one study, the increased calciuresis was reported for both a 24-hour period and during a fixed period in a fasting state.[277] In the third study, urinary calcium excretion was calculated relative to glomerular filtration.[279] In a

recent report on calcium metabolism in offspring of hypertensive parents,[285] no difference between the groups in 24-hour calcium or sodium excretion existed on the low sodium diet. During one week on the high sodium diet, a similar increase in sodium excretion for the two groups of offspring was seen. However, on the third to fifth day of the high sodium diet, 24 hour calcium excretion was significantly increased in the offspring of hypertensive parents, compared to the offspring of normotensive parents.[285] This was thought not to occur from the increase in blood pressure seen on the high sodium diet in the offspring of hypertensive parents, as the blood pressure difference between the groups of offspring was at its highest on the seventh day of the high sodium diet when 24-hour calcium excretion was not different between the groups. As natriuretic responses were equal between the groups, natriuresis could not explain the difference in calciuresis. As described above,[285] no difference in PTH was found. A decreased plasma ionized calcium and an increased $1,25(OH)_2D$ at day seven of the high sodium diet in the offspring of hypertensive parents compared to offspring of normotensive parents, could be secondary to the increased calcium leak on day three to five. Moreover, the decrease in ionised calcium was inversely related to the percentual increase in mean blood pressure during the high sodium diet in the offspring of hypertensive parents.[285] These data support the presence of hypercalciuresis in hypertension and in subjects at risk for hypertension. In the former, hypercalciuresis on liberal sodium diets may have existed for a longer time leading to the increased level of PTH. The data in the latter group suggest that the hypercalciuresis does not exist in youngsters at risk for hypertension on a low sodium diet. Because of the relative short period of the high sodium diet, after one week on the low sodium diet, differences in PTH between the groups off offspring might not have occurred due to the short duration of the stimulus for hypercalciuresis in this group.

- Cellular calcium metabolism

As described above, a rise in intracellular calcium is the final common pathway for increased vascular smooth muscle contraction and tone. Although an increased intracellular calcium does not have to be directly related to calcium metabolism it is relevant to discuss the available evidence for an increased intracellular calcium in hypertension. Moreover, if present, the question is whether it is related to extracellular calcium metabolism and/or membrane characteristics.

- Intracellular calcium

As a model for vascular smooth muscle cells intracellular calcium in platelets has been studied in hypertension. Because of similarities between vascular smooth muscle cells and platelets, such as the alpha$_2$-adrenoreceptor cyclase system and the calcium-dependent contraction-coupling, changes in platelet cytosolic calcium were thought to reflect changes in vascular smooth muscle cells.[291] Platelet intracellular free calcium concentration was found to be increased in essential hypertensive subjects, and to show a close correlation with both systolic and diastolic blood pressure level. Moreover, after treatment of hypertension with either calcium-entry blockers, beta-adrenoreceptor blockers or diuretics, a reduction of platelet calcium was seen that correlated to the blood pressure lowering effect.[291] The increased intracellular calcium in subjects with primary hypertension compared to normotensive subjects has been confirmed by others in platelets,[292,293,294] erythrocytes,[295,296] and lymphocytes,[297] but not in leucocytes.[298] These findings show a rather consistent increase in cellular calcium in primary hypertension. This, however, is not specific for primary hypertension as intracellular calcium is also increased in renal hypertension[295] and in experimental mineralocorticoid hypertension.[294] Furthermore, as the increased intracellular calcium level in hypertensive subjects diminishes after successful pharmacological[291,296] or nonpharmacological treatment[299] (weight reduction) of blood pressure, an increased intracellular calcium level may not be a primary causal pathogenetic factor for hypertension, but a final state closely related to blood pressure level. If true, one would not expect the intracellular calcium level to be increased in subjects at risk, before hypertension develops. The findings of Bing et al. of a similar leucocyte intracellular calcium in offspring of hypertensive parents compared to offspring of normotensive parents, do indeed suggest that intracellular calcium is not increased before hypertension develops.[298] In that study, however, leucocytes of the hypertensive subjects also did not show a significant increase of intracellular calcium. In the study of Yamayaka,[285] described above, an increased platelet intracellular calcium was found in the offspring of hypertensive parents, compared to offspring of normotensive parents. This was seen both on a low salt diet when no difference in blood pressure existed between the groups of offspring, and on a high sodium diet with a blood pressure difference.[285] Another study reported similar results of an increased platelet intracellular calcium in offspring of hypertensive compared to offspring of normotensive parents, on a liberal diet.[300] A genetic analysis of platelet

intracellular calcium concentration and blood pressure in 109 twin pairs,[301] showed that, after adjustment for confounding factors such as age and body-mass index, no relation existed between blood pressure and intracellular calcium. It was calculated that the additive genetic influence on variance of platelet cytosolic calcium concentration was 48% in females and 37% in males. The additive genetic influence on variance of total plasma calcium concentration was 78%. These results stress the possible confounding effect of environmental factors on intracellular calcium levels. However, in the groups of offspring studied by Yamakawa[285] no difference in age or body weight existed. In conclusion, from these data it cannot be ruled out that intracellular calcium is increased both in the early phase and during hypertension. The causal implication is doubtful.

Much discussion remains as to how an increased cellular calcium concentration in hypertension and the prehypertensive state might occur. Some argued that it should result from a blood-borne, circulating factor,[291,293] in line with the Blaustein[185]/Haddy[184] hypothesis. Lindner even found that plasma from hypertensive subjects increased intracellular calcium in isolated platelets of normotensive subjects,[293] as later confirmed by others.[302] Yet, the circulating factor in the Blaustein hypothesis first increases intracellular sodium and secondary calcium by the sodium/calcium exchange. As a sodium/calcium exchange does not exist on human erythrocytes findings of an increased intracellular calcium in erythrocytes could not be explained by this theory.[296] According to the theory by Haddy, the increased intracellular sodium (arisen by the circulating inhibitor) could decrease membrane potential, and thereby increase calcium influx through voltage-dependent calcium channels.[184] Lindner, on the other hand, proposed a circulating inhibitor of the calcium-ATPase pump,[296] that was found in patients with chronic renal failure.[303] Others, have suggested that an increased intracellular calcium results from an intrinsic membrane abnormality, that might either influence the calcium-ATPase pump, or the passive calcium influx by a change in calcium binding.[304,305]

- *Calcium membrane binding*

Calcium membrane binding may be measured in erythrocytes or platelets. The first studies on this subject used a calcium depletion method by removing calcium from the erythrocyte by EDTA.[306] In this way, more calcium ions could be removed from erythrocytes of hypertensive patients, compared to normotensive subjects. This was interpreted as an

increased calcium binding in the outer membrane of the erythrocyte from hypertensive subjects. This was confirmed later.[307] Other methods used were binding of ^{45}Calcium to intact erythrocytes (measure for outer part of the membrane) and binding of ^{45}Calcium to erythrocyte ghosts after repeated washing, a measure for binding to the inner part of the erythrocyte membrane.[308] With this method in hypertensive subjects, a decreased calcium binding ability by 28% of the inner part of the membrane of the erythrocyte compared to normotensive subjects was found.[308] In that study, Postnov et al., could not confirm their earlier finding of an increased binding capacity at the outer membrane. The reduced calcium binding capacity of the inner part of the erythrocytic membrane in hypertension has been confirmed by others,[309,310,311] but also an increased inner membrane binding capacity was reported.[312] In platelets, the membrane-bound calcium was reported to be increased in hypertensive compared to normotensive subjects, all of the black race.[313] Although a tendency to a decreased calcium binding capacity of the inner erythrocytic membrane in primary hypertension is not reported by all investigators, some relevant findings must be highlighted. The decreased calcium binding appeared to be specific for primary hypertension, as it was not found in renovascular hypertension.[309] Treatment of hypertension did not influence this membrane characteristic as similar values were detected for treated and untreated hypertensive subjects.[310] And finally, in offspring of hypertensive parents a decreased erythrocyte membrane calcium binding capacity was found, similar to that in hypertensive subjects, compared to offspring of normotensive subjects.[311] Both groups of offspring had similar blood pressure values. A decrease in calcium binding could destabilize the cell membrane, with consequent changes in passive calcium influx, by partial depolarization.[314] An increase in calcium binding might facilitate calcium related signal transducing pathways, important in activation of the cell.[312]

- Calcium fluxes

Calcium fluxes in hypertension are measured to search for changes in calcium transport that might be related to the postulated increase of intracellular calcium and the difference in membrane characteristics. Moreover, calcium fluxes are measured to test the above mentioned theory of circulating inhibitors. Various experiments have been developed to measure calcium influx, calcium efflux and activity of the calcium-pump by measuring calcium-magnesium-ATPase activity. In membraneous parts of erythrocytes, calcium-

stimulated, magnesium-activated ATPase appeared to be reduced in subjects with primary hypertension compared to normotensive subjects.[315] As the number of pump units did not differ between the groups, it was concluded that the difference in activity arose from a difference in membrane environment. By using intact erythrocytes, others could not detect a difference in calcium pump activity between groups of hypertensive and normotensive subjects.[316] However, the distribution of the calcium pump dissociation constant in the hypertensive group appeared to be bimodal, and a subgroup of hypertensive subjects was defined with a decreased affinity for internal calcium, a high maximal pump rate, but a calcium-ATPase activity depending on the intracellular calcium concentration. Therefore, it is rather difficult to compare results from different studies using intact, broken or ghost cells and various methods influencing intracellular calcium and apparent calcium-ATPase activity. Definite studies are awaited for.

Calcium and hypertension

From the above described alterations in calcium metabolism in primary hypertension it appears that serum ionized calcium is lower, parathyroid hormone might be increased, and an increased urinary calcium excretion might exist on a liberal or high sodium diet. Much debate remains, how these alterations could be related to hypertension, and whether a change in calcium intake could interact with these alterations. Several hypotheses have been put forward to explain parts of the possible chain of events.

McCarron argued that a low serum calcium concentration destabilizes the membrane of the vascular smooth muscle cell, possibly by a change in calcium binding, with a subsequent increase in intracellular calcium and contractile status.[273] McCarron postulated that the low serum calcium occurs secondary to an increased urinary calcium leak through which provides a stimulus for an increase in parathyroid hormone.[283] The increased calciuresis either occurs from an increased intestinal absorbtion of calcium, or a decreased renal tubular calcium reabsorption. According to this view, an increased parathyroid hormone level can be expected as secondary to the slightly lower ionised serum calcium and because of its' observed direct hypotensive action at normal to low calcium concentrations,[317] might even compensate part of the blood pressure increase. The increased urinary calcium excretion is supposed to be the primary phenomenon. It cannot be excluded that changes in the renal tubular cell membrane increase urinary calcium excretion

and that similar changes in the vascular smooth muscle cell membrane, destabilised by the lower serum calcium concentration, influence calcium transport. The primary phenomenon could then be a change in cellular membrane composition by which calcium handling is influenced.

Resnick et al. postulated that the low serum ionized calcium in low renin essential hypertension is secondary to a primary increase in intracellular calcium levels (from whatever reason, possibly membrane related). The increased cellular calcium concentration does not only account for the increased vasoconstriction, but also suppresses both renin secretion and parathyroid hormone, the latter reflected in the low serum ionized calcium.[274] In this way, the changes in renin, parathyroid hormone, and serum calcium can be viewed as secondary to the mechanism that increases intracellular calcium. As opposed to low renin hypertension, Resnick et al. found that high renin hypertension is accompanied by high serum ionized calcium levels, and low serum magnesium levels.[274] It was postulated that in high renin hypertension, renin was the driving force for the increased angiotensin mediated intracellular calcium concentration. Angiotensin could increase intracellular calcium by release from intracellular stores. A high aldosterone level could explain the depletion of magnesium.

These different views may be tested by measuring both parathyroid hormone and renin levels in primary hypertension. An increased parathyroid hormone level with a normal or low renin level favours McCarrons hypothesis. If an increase or a decrease in the level of parathyroid hormone and renin go together, Resnicks' hypothesis would be in favour. McCarron did not measure renin in his studies. Resnick however, 3 years after the first report, did find an increased parathyroid hormone level in hypertensive subjects with a low renin profile,[318] more in line with McCarrons' hypothesis. In the study of Yamayaka et al., between the groups of offspring at different risk for hypertension on either a low or high salt diet, no difference in plasma renin level or parathyroid hormone level was seen, while in the offspring at high risk of hypertension an increase in intracellular calcium and urinary calcium excretion, and a decrease in serum ionized calcium concentration was seen on the high salt diet.[285]

McCarron further emphasised his findings by postulating the calcium deficiency theory.[319] This theory departs from the profile in hypertension of an increased calciuresis, a low serum ionised calcium, a high intracellular calcium and a high parathyroid hormone

level. Quoting findings of Grobbee and Hofman,[320] and Strazullo et al,[321] McCarron reasoned that hypertensive subjects with a high parathyroid hormone level[320] or an increased urinary calcium excretion[321] respond with a clear blood pressure decrease to calcium supplementation. Hypertensive subjects with these characteristics might therefore be considered calcium-deficient.

Others have objected to the calcium deficiency theory, especially to the relation between extracellular calcium and vasoconstriction-relaxation, and the supposed protective role of the increased parathyroid hormone level.[322] A warning has been sounded against the general use of calcium supplementation in hypertension.[322] The debate as to whether vascular contraction can arise by only small decreases in extracellular calcium concentration together with the role of parathyroid hormone in blood pressure regulation, might be resolved by postulating that the increased parathyroid hormone acts as an ionophore, and increases the intracellular calcium concentration. This could be in agreement with the longterm effects of parathyroid hormone on blood pressure. To illustrate this, the possible relation between parathyroid hormone and blood pressure regulation will be discussed.

PTH infused in pharmacological doses acts as a vasodilator.[317,323] However, longterm infusion of PTH may induce hypertension.[287] PTH might also increase calcium influx in various tissues, e.g. cardiac muscle,[324] and smooth muscle cells.[325] Some findings suggest that a PTH-stimulated calcium influx in neurons, could lead to an increase of noradrenaline release.[287] Furthermore, effects on structural cardiovascular alterations by PTH have been described. In one study on the relation of (1-84)PTH level and aspects of the microcirculation in newly diagnosed mild hypertensive subjects, a positive association between level of PTH with the diameter of afferent and efferent capillary loops, and a negative association with the number of capillaries was observed.[326] By the same investigators, a positive association was described for plasma (1-84)PTH and left ventricular mass index, before and after adjustments for blood pressure.[327] Whether or not PTH is increased in the early phase of primary hypertension, an elevated PTH in mild hypertension might, by the various mechanisms described contribute to the persistence of high blood pressure and influence structural effects of hypertension.

In conclusion, in subjects with a tendency to an increased urinary calcium excretion, a slightly decreased ionized calcium concentration could provide a longterm stimulus for the secretion of parathyroid hormone. Parathyroid hormone could, with or without a possible

interaction of a low calcium concentration and/or change in cellular membrane characteristic, lead to a small increase in intracellular calcium concentration. This modified calcium deficiency theory of hypertension attributes a direct role of parathyroid hormone to the blood pressure increase. The possible role of a permissive effect of plasma parathyroid hormone on the vasoconstrictive and hypertensive action of extracellular calcium in hypertension was proposed by Lau and Eby.[328] Indirect evidence comes from a placebo controlled trial on calcium supplementation in young mildly hypertensive subjects, that showed an increased response on calcium supplementation in those subjects with an elevated parthyroid hormone level.[320] More important, the decrease in diastolic blood pressure in the group on calcium supplementation, was positively associated with the small observed decrease in plasma intact (1-84)PTH in the calcium treated group, but not in the placebo treated group.

Lau et al., however did not agree with the starting point of the calcium deficiency theory; the increased urinary calcium leak. Their objection was based on various experiments in spontaneous hypertensive rats.[329] They did observe changes in calcium transport characteristics along the renal and intestinal epithelia, but could not find a negative calcium balance in young animal, or an augmentation or attenuation of the blood pressure increase in rats fed respectively a calcium deficient diet or magnesium and calcitriol enriched diet. They postulated that the observed changes in calcium transport in these rats occurred by a genetic membrane defect, that eventually leads to an increased intracellular calcium concentration. The possible changes in membrane characteristics and transport were discussed above.

To summarize, all hypotheses seem to agree on an increase in intracellular calcium in hypertension. Even, all hypotheses agree with changes in calcium metabolism in hypertension. Both Resnick et al. and Lau et al., consider these changes secondary to alterations that are causally related to the increased intracellular calcium. Both suggest that this causal factor might be a membrane alteration. McCarron et al. do not exclude membrane alterations, but include them in the postulated effect of the low serum calcium on increase in intracellular calcium. Moreover, they point to an increased urinary calcium excretion as the primary phenomenon, to which the other observed changes in calcium metabolism are related. Only in McCarrons' hypotheses a link can be made with calcium intake. Subjects with an increased calcium excretion are postulated to be more sensitive to a

deficient calcium intake. Although the profile of hypertensive subjects often agrees to the derangement in calcium metabolism, including an increased calciuresis, evidence on this subject is not conclusive. Part of the problem might arise from the relation between calcium and sodium homeostasis, especially on the level of membrane transport and renal excretion.

SODIUM AND CALCIUM INTERACTION

The postulated interaction of monovalent (Na) and bivalent (Ca and Mg) cations in hypertension stems from the observations that a change in intake of one might influence the excretion of the other, and thereby influence blood pressure response. Moreover, calcium metabolism has been found to be related to renin, important for both sodium homeostasis and blood pressure regulation. Observations are abundantly available, without, however, evidence of the unifying regulating mechanism that links the alterations in metabolism of both ions to hypertension (see below).

In an observational study on dietary sodium and calcium in relation to urinary calcium in normotensive subjects, a positive association was found between dietary sodium and urinary calcium excretion[330] whilst an inverse association existed between dietary potassium and urinary calcium. In untreated hypertensive subjects studied one week on a low and one week on a high sodium diet, on the latter diet an increase in blood pressure was seen, together with a decrease in serum calcium, magnesium and phosphate concentration and an increase in both sodium and calcium 24-hour urinary excretion.[331] A positive association was found between the level of serum total or ionized calcium concentration during the low salt diet and the blood pressure response on the high salt diet. In other words, the serum calcium level was related to the magnitude of blood pressure increase seen on the high sodium diet. In another study among hypertensive subjects, a blood pressure increase observed between change from a low to a high sodium diet was accompanied by an increase in urinary sodium, calcium and magnesium excretion, platelet calcium concentration, and a decrease in serum calcium, magnesium, potassium, ionized calcium concentration, erythrocytic magnesium concentration and plasma renin activity.[332] Moreover, changes in blood pressure were inversely related to the change in serum ionized calcium and erythrocytic magnesium. Finally, the change in blood pressure was positively related with an increase in platelet calcium concentration and with an increase of parathyroid hormone concentration. In an earlier study by Resnick et al.[333] sodium sensitivity was defined as an increase of 5% in diastolic blood pressure between a 5 day

low and a 5-day high sodium diet. The sodium sensitive subjects experienced a significantly lower increase in urinary calcium excretion, while showing a similar sodium excretion. Moreover, the sodium sensitive subjects had a decrease of serum ionized calcium concentration and a significantly lower decrease in parathyroid hormone concentration. The findings of these studies combined suggest that during sodium loading the level of blood pressure increase might be related to calcium homoeostasis present before[331] and that sodium loading changes calcium homoeostasis.[332,333] Therefore, it is concluded that sodium sensitivity may be related to calcium homoeostasis. However, it cannot be inferred stated that a change in calcium homeostasis causes sodium sensitivity. The sodium sensitivity might be caused by a similar defect in electrolyte metabolism as the change in calcium homeostasis. The described relations of both calcium and sodium homeostasis with blood pressure might be causal for one, both or none of these.

Because of the observed associations between sodium and calcium homeostasis, and the notion that a liberal high sodium diet is often accompanied by a low calcium intake, it was tested whether calcium supplementation attenuates the blood pressure response on a high sodium diet.[334] In a group of hypertensive subjects receiving (more than 2 gram) calcium supplementation, no significant rise in blood pressure was observed during a high sodium diet. In the placebo treated group, the blood pressure rise was more prominent and significant. The diminished blood pressure increase in the calcium treated group, might have occurred due to the increased natriuresis that was observed during the high sodium diet in the calcium treated group, compared to the placebo treated group.[334] However, in another study, no difference in natriuresis was seen between calcium supplementation or placebo in normotensive subjects on a strict, but normal sodium diet during an acute saline load.[335] One cannot exclude that the interaction between calcium supplementation and the natriuretic and blood pressure response to sodium loading is specific for hypertensive subjects, and might even be confined to salt-sensitive subjects or those at risk for hypertension.

Findings by Resnick et al. have further emphasised the relation of calcium metabolism and salt sensitive hypertension, defined as low renin hypertension.[318] In low renin hypertension, in addition to earlier reported findings of an increased serum magnesium and a decreased serum ionized calcium,[274] a decrease in calcitonin and an increase in calcitriol and parathyroid hormone level were seen. Moreover, parathyroid hormone level was inversely associated with 24-hour sodium excretion, in line with a possible diminishing effect of parathyroid hormone on aldosterone secretion. Therefore, Resnick et al. postulated

that the renin-angiotensin-aldosterone system and calcium regulating hormones act together on the cellular calcium homeostasis, and may be influenced by dietary changes in either calcium or sodium or both.[318] This does not clarify the mechanism that might be responsible for these alterations, although it is speculated that membrane alterations are involved and transport systems such as the sodium-hydrogen exchange.[336]

Table 4 Electrolyte homoeostasis in the prehypertensive state; review of family history studies.*

Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Intracellular Sodium							
- leucocytes	history	unknown	26(18-42)	45	SBP 2 (ns),DBP 2 (ns)	no	Gray[202]
- leucocytes	history (BP, if doubt)	unknown	28	28	SBP 2 (ns),DBP -1 (ns)	no	Milner[207]
- leucocytes	history (BP, if doubt)	unknown	25.5	31	SBP 1 (ns),DBP 3 (ns)	no	Heagerty[208]
- lymphocytes	history	unknown	22(14-35)	19	SBP 1 (ns),DBP 1 (ns)	yes, +	Ambrosioni[203]
- erythrocytes	history (HT pregnancy)	142/94 vs 115/78	12.7	29	SBP 11 (s),DBP 5 (s)	no	Svensson[209]
- erythrocytes	history	unknown	26(18-42)	45	SBP 2 (ns),DBP 2 (ns)	no	Gray[202]
- erythrocytes	history	unknown	50	37	SBP ns,DBP ns	yes,+(high salt)	Gudmundsson[204]
- erythrocytes	history	unknown	29	60	SBP 0,DBP 0	yes, +	Lijnen[205]
- erythrocytes	history + BP	171/102 vs <140/90	39	10	not given	yes, +	Mazanti[206]
- erythrocytes	history + BP	R_{xp} >160/95 vs <140/90	11	84	not given	no	Trevisan[211]
- erythrocytes	history	unknown	14(0 - 33)	58	SBP 0, DBP 3	yes, +	Deal[234]
Sodium-Potassium-ATP-ase activity							
- erythrocyte membrane	history + BP	171/102 vs <140/90	39	10	not given	yes, -	Mazanti[206]
- erythrocyte membrane	history	unknown	19 - 64	52	MAP 2 (ns)	yes, +, but ns	Woods[232]
- erythrocyte membrane	history	unknown	14(0 - 33)	58	SBP 0, DBP 3	yes, -	Deal[234]
- leucocytes	history (BP, if doubt)	unknown	28	28	SBP 2 (ns), DBP -1 (ns)	yes, -	Milner[207]
- leucocytes	history	unknown	27.5	42	SBP 3 (ns), SBP 2 (ns)	yes, -	Heagerty[233]
- leucocytes	history (BP, if doubt)	unknown	25.5	31	SBP 1 (ns), DBP 3 (ns)	no	Heagerty[208]
- erythrocytes	history (HT pregnancy)	142/94 vs 115/78	12.7	29	SBP 11 (s), DBP 5 (s)	no	Svensson[209]
- erythrocytes	history	unknown	26(18-42)	45	SBP 2 (ns), DBP 2 (ns)	no	Gray[202]
- erythrocytes	history	unknown	29	60	SBP 0, DBP 0	no	Lijnen[205]
- lymphocytes	history	unknown	32(24-54)	105	SBP 1, DBP 2 (ns)	yes, -	Taylor[236]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Exchangeable sodium							
- at normal sodium intake	BP father, History	R_x vs NT	31	32	MAP 5 (ns)	no	Gudmundsson[94]
- at high sodium intake	BP father, History	R_x vs NT	31	32	MAP 3 (ns)	no	Gudmundsson[94]
- at normal sodium intake	questionnaire+BP	141/84,R_x vs 126/78	similar	62	SBP 8 (s), DBP 4 (ns)	no	Beretta-Pic[95]
- at high sodium intake	questionnaire+BP	R_x vs <140/90	similar	62	SBP 13 (s), DBP 9 (s)	no	Beretta-Pic[212]
Sodium-Potassium co-transport							
- erythrocytes	history	unknown	not given	116	not given	yes, -	Garay[231]
- erythrocytes	history	unknown	29	60	SBP 0, DBP 0	yes, -	Lijnen[205]
- erythrocytes	questionnaire, BP	>150/90, vs ?	13 - 15	84	not given	yes, -	Uchiyama[239]
- leucocytes	history (BP, if doubt)	unknown	25.5	31	SBP 1 (ns), DBP 3 (ns)	yes,+(on low salt)	Heagerty[208]
- erythrocytes	history	unknown	not given	24	not given	yes, +	Adragna[240]
- erythrocytes	history and BP	not given	14.8	109	SBP 2(ns), DBP 2(ns)	yes, +	Cusi[241]
Sodium-Lithium countertransport							
- erythrocytes	history	unknown	not given	24	not given	no	Adragna[240]
- erythrocytes	history	unknown	29.5	52	SBP 3(ns), DBP0	yes, +	Lau[235]
- erythrocytes	history	unknown	12-20	39	SBP 0, DBP 2	yes, +	Woods[244]
- erythrocytes	history + BP	R_x or >160/95 vs <140/90	11	84	not given	no	Trevisan[211]
Lithium							
- renal fractional clearance	history	unknown	23(18-50)	31	not given	yes, -	Weder[254]
- renal fractional clearance	BP	>160/95 vs <140/90	23	24	SBP 2 (ns), DBP 4 (ns)	no	Niutta[214]

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Parameter of interest	Definition of "family history"	BP contrast between parents	Age of subjects	Number of subjects	Difference in BP between offspring (mmHg)#	Difference FH^+ vs FH^-	Ref
Calcium homoeostasis on low sodium diet							
- Plasma ionised calcium	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 1 (ns), DBP 1 (ns)	no	Yamakawa[285]
- Parathyroid hormone (1-84)	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 1 (ns), DBP 1 (ns)	yes, + (ns)	Yamakawa[285]
- 1,25-diOH-Vitamine D	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 1 (ns), DBP 1 (ns)	no	Yamakawa[285]
- Urinary calcium excretion	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 1 (ns), DBP 1 (ns)	no	Yamakawa[285]
Calcium homoeostasis on a high sodium diet and change from low to high sodium diet							
- Plasma ionised calcium	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 7 (s), DBP 4 (s)	< FH+(s),= FH-,no§	Yamakawa[285]
- Parathyroid hormone (1-84)	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 7 (s), DBP 4 (s)	= FH+,= FH-,no§	Yamakawa[285]
- 1,25-diOH-Vitamine D	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 7 (s), DBP 4 (s)	> FH+(s),= FH-,no§	Yamakawa[285]
- Urinary calcium excretion	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 7 (s), DBP 4 (s)	>> FH+,> FH-,yes+(s)§	Yamakawa[285]
Cellular calcium homoeostasis							
- platelet $[Ca^{2+}]_i$ (low sodium)	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 1 (ns), DBP 1 (ns)	yes, + (s)	Yamakawa[285]
- platelet $[Ca^{2+}]_i$ (high sodium)	history + BP	>160/90 vs <140/85	20 - 22	28	SBP 7 (s), DBP 4 (s)	= FH+, = FH-, yes+(s)	Yamakawa[285]
- leucocyte $[Ca^{2+}]_i$	history	unknown	25.5	38	SBP 3 (ns), DBP 2 (ns)	no	Bing[298]
- platelet $[Ca^{2+}]_i$	history	unknown	26.0	20	SBP 0, DBP 0	yes,+ (s)	Gulati[300]
- erythrocyte membrane calcium binding	history	unknown	30	40	SBP 2 (ns), DBP 2 (ns)	yes, - (s)	Bing[311]

* If three groups were studied, the data on normotensive groups at greatest contrast are presented.
FH+=offspring with positive, FH-= offspring with negative, family history of hypertension. HT= hypertension, NT=Normotension, BP=blood pressure.
R_x=treatment for hypertension.
Difference FH+ vs FH-: +=increased in FH+ versus FH-, -=decreased in FH+ versus FH-.
s=significant difference, ns=nonsignificant difference between FH+,FH-.
Change from low sodium diet on high sodium diet: > means increase, < means decrease, >>> means greater increase (relative to other group), = means no change.
§ Difference FH+ vs FH- on high sodium diet.

1.2.5 IMMUNOGENETIC FACTORS

The interest in immunogenetic factors, such as ABO-blood type, HLA-type, immunoglobulins and immune complexes in hypertension research originates in the hypothesis that auto-immune mechanisms might be involved in vascular damage in hypertension. Especially, the HLA-system had been found to be related to diseases that have an abnormal immunological profile. Moreover, part of these factors are hereditary and therefore of interest in hypertension research among families.

Ebringer et al. observed increased levels of IgG immunoglobulins in hypertensive subjects, compared to normotensive subjects.[337] Later this was confirmed by others,[338,339] but not in another report showing similar levels in normotensive and hypertensive subjects.[340] These discrepancies were explained by different selection criteria for the control group.

In presence of an increase in immunoglobulines, Kristensen found an increased frequency of 18% of HLA-B27 in hypertensive subjects,[339] compared to 8% in normotensive subjects. This was only significant when not corrected for numbers of antigens analyzed. In patients with a family history of hypertension an increased frequency of HLA-B15 was observed.[339] Others reported slightly increased frequencies of HLA-B12,[341,342] and HLA-B8.[343] In contrast to Kristensen,[339] a slightly decreased frequency of HLA-B15[343] in hypertensive subjects compared to normotensive controls has been reported. In a 1984 review of this subject, an increased frequency of the HLA-B15 antigen in hypertension was described, especially in hypertensive subjects with a positive family history of hypertension or malignant hypertension.[344] In a report published after this review, an increased frequency was found for HLA-B18, but not for HLA-B15.[345]

The inconsistent results on HLA-typing in hypertension in the various groups studied, were followed by two reports on segregation analyses of HLA haplotypes and ABO- and RH- and MN-phenotypes. In 195 families, the concordance between hypertensive sib pairs of HLA-haplotypes was increased compared to either hypertensive-normotensive or normotensive-normotensive sib-pairs.[346] The frequency distribution of HLA-haplotypes in the hypertensive subjects of these families, compared to the normotensive subjects, was increased for HLA-B18 and decreased for HLA-B5. More striking, however, was the difference in distribution of the M-phenotype and N-phenotype of the MN bloodgroup allele, with an increased frequency of expression of the N-phenotype in the hypertensive subjects.[346] This association of MN-phenotypes with hypertension was also described by

others for both primary and renovascular hypertension.[347] Another report on 96 hypertensive subjects from 31 families did show an abnormal segregation pattern of the HLA-haplotypes, as again the hypertensive siblings had shared haplotypes in a higher frequency than expected.[348] When hypertensive caucasian subjects were compared to available, normotensive caucasian controls, a higher frequency for HLA-DR2, HLA-DR4 and HLA-A2 and B12 was seen in the hypertensive subjects.[348] These results do suggest that hypertension is linked to blood group systems as MN-phenotypes and HLA-haplotypes. As the magnitude of the relative risk of a linkage disequilibrium is related to other factors than genetic susceptibility alone, such as frequency and localisation, one must take caution in focussing only on the exact locus of the haplotype that has the highest relative risk for association with susceptibility to a disease.[349] In doing so, other and possibly alleles with a causative role to the disease could be missed.

Other characteristics of the immune system have been studied sparsely in hypertension, among them complement factors, autoantibodies, circulating immune complexes, and immunoglobulins as described above. It was postulated that these immune mechanisms induce arterial injury and give rise to atherosclerosis and possibly hypertension. This view arose from binding studies showing deposits of immunological factors in renal arterioles of primary and malignant hypertensive subjects. The association of hypertension with factors as immunoglobulins, circulating immune complexes or autoantibodies that is especially seen in more severe forms of hypertension, do suggest that these factors arise secondarily to the vascular damage.[344] Therefore, it is rather unlikely that changes in these immunological factors are prevalent in the early phase of primary hypertension.

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2 FAMILY STUDIES ON PRIMARY HYPERTENSION: PROMISES AND PROBLEMS

2.1 INTRODUCTION

The interest in family studies on primary hypertension originates from observations of an increased occurrence of cardiovascular mortality and morbidity in relatives of patients with hypertension.[1] First, the family oriented approach in the field of hypertension research has been used as an instrument to focus on the heredity of primary hypertension an its transmission.[2,3] Nowadays the family oriented approach is used as a methodology to study the cause of this heredity either in selected groups of youngsters with a familial background of hypertension compared to youngsters without, or in large pedigrees of hypertensive patients.[4] Ironically issues that arose at the start, such as definition of hypertension, can nowadays be applied to the possible factors that might cause hypertension, i.e. the distribution of those factors. These issues will be discussed with a view from recent medical history in section 2.2. In section 2.3, family based approaches to study the early pathogenesis of primary hypertension will be reviewed. Selection criteria in the family history approach, and methodological issues in the interpretation of results, will be presented.

2.2 PLATT VERSUS PICKERING: THE BIMODAL - UNIMODAL CONTROVERSY

The hereditary nature of primary hypertension was one issue that Pickering and Platt agreed upon (see for review[5]). A classic debate arose, however, from controversy on the mode of transmission, that was based on the shape of the distribution curve of blood pressure.

2.2.1 PLATT VERSUS PICKERING: THE DEBATE

Platt argued that primary hypertension is a hereditary disease with a mendelian dominant mode of transmission.[2] This was inferred from observations of the prevalence of a positive family history for cardiovascular diseases in patients with primary hypertension of up to 76%, compared to 30% in control patients and 28% in patients with secondary hypertension, in combination with the results of Weitz.[1] Weitz measured blood pressure in older siblings of hypertensive patients and found that more than half of the older siblings had hypertension, defined as a systolic blood pressure greater than 150 mmHg or death from heart failure or apoplexy.[1] Thus, the ratio of transmission was thought to be over 50%. Moreover, hypertension appeared in three generations. Therefore, a mendelian dominant transmission was hypothesised.[2] Much of the hypothesis of Platt, however, depended on the definition of primary hypertension and the distinction with normotension, secondary hypertension and family history of cardiovascular diseases.

Pickering argued[6] that the data based on family histories of cardiovascular diseases provided only evidence for heredity of vascular diseases and not of high blood pressure. If blood pressure in family history studies of hypertensive subjects and their relatives was available, still a reference group was lacking (data on blood pressure in siblings from normotensive subjects). Moreover, Pickering argued that the division of subjects into normotensive or hypertensive subjects was arbitrary, but that it clearly influenced the incidence of hypertension in the families studied. Therefore Pickering et al. measured blood pressure in a population sample and in relatives of hypertensive and of control subjects.[3] According to their observations;

- blood pressure shows a normal distribution curve in a population sample, although a little skewed,
- a division between normotension and hypertension based on the population sample could not be made,
- the blood pressure of the relatives of normotensive subjects is not different from the

population based sample,

- blood pressure rises with age,
- the blood pressure - age relation is different for men and women,
- a relatively high or low blood pressure can therefore only be distinguished if age and gender, together with blood pressure, are used to compose scores for blood pressure,
- age and gender specific blood pressure scores in relatives of clinically hypertensive subjects were similar for siblings, offspring and parents of the proband, without evidence for sex-linkage
- mean blood pressure scores of first degree relatives rose with an increasing score of the proband, until very high scores were reached,
- if, with increasing age, the score of the probands diminished, so did the score of their relatives,
- for each age category, blood pressure of relatives of hypertensive subjects was increased compared to the blood pressure of the relatives of control subjects,
- the association of blood pressure between the hypertensive probands and first degree relatives could be expressed by a linear regression coefficient of 0.2 mmHg/mmHg.[3]

Later, this last observation of a linear association between blood pressure of probands and their relatives was reproduced by others between for randomly selected probands (not based on high blood pressure of the proband).[6]

Based on these findings Pickering came to four statements;[6]

- a genetic factor is involved in the pathogenesis of primary hypertension;
- the difference between high or normal blood pressure is quantitative,
- the heredity is probably multifactorial as blood pressure is inherited as a graded characteristic, through the whole range of blood pressure,
- the contribution of heredity on blood pressure level is relatively modest, and therefore environmental factors are likely of importance.

Platt could not agree with these statements as in his view primary hypertension was an entity with a strong hereditary background (opposed to secondary hypertension) that becomes apparent only during middle age. It was argued that the rise in blood pressure with age observed in the general population by Pickering et al. is resulting from an age - blood pressure relation in two groups; one group with a rather small increase of blood pressure with age in the population at large, and one group with a significant increase of blood

pressure with age in a subset of the population with primary hypertension. Based on this assumption (already proved wrong by Pickering), Platt argued that the bimodality of blood pressure distribution should clearly appear in distribution curves of siblings of hypertensive subjects that have reached middle age. By then blood pressure might have risen in those with a trait for hypertension. Therefore distributions of middle aged siblings (45 - 60 years of age) of hypertensive subjects were drawn to test bi-modality on the assumption that 50% of the siblings (see above) had, and 50% had not, inherited the same trait as their hypertensive sibling. The distributions curves showed no clear unimodality, and were suggestive for bimodality, especially for systolic blood pressure.[7]

However, the distributions Platt had calculated from the blood pressure data of Pickering (measured to the nearest 5 mmHg), might have been influenced by adding up the frequencies for values of blood pressure ending at 0 and next 5. Pickering argued that an unconscious digit preference might have overestimated measurements ending at 0, so that through adding up the measurements at 0 and next 5, the occurrence of blood pressure values ending at 5 might had been underestimated, which influences the modality of the distribution curve even more as small numbers are involved.[8] Moreover, Pickering refuted the possibility raised by Platt of two sub-populations, one showing no rise of blood pressure with age, and one that does, by pointing to their earlier findings of a similar blood pressure rise with age in relatives of hypertensive subjects compared to relatives of normotensive subjects and subjects from the population sample.[6]

After the arguments of Platt,[7] intended to refute the hypothesis of Pickering,[6] the core of the debate became centred on the distribution of blood pressure curves; whether it will be unimodal in favour of the multifactorial inheritance as hypothesised by Pickering, or bimodal in favour of mendelian dominant inheritance argued by Platt.

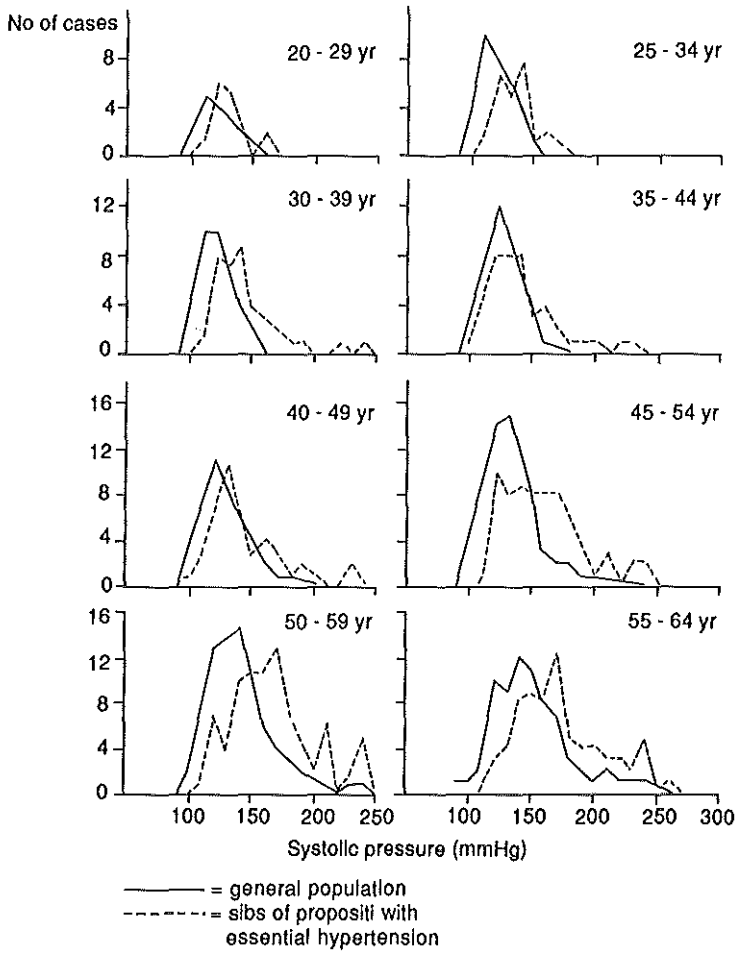
Pickering made no concessions and again explained all the evidence as before, but in greater detail with emphasis to the quantitative resemblance between hypertensive subjects and their relatives, using amongst others the age and gender adjusted blood pressure scores.[9] In the argumentation a resemblance with the graded inheritance of height and intelligence was made, pointing to the custom of using special gender and age adjusted scores.

Ironically, Platt did not seem to mind to accept the multifactorial inheritance of height, but pointed to the possible disturbance of other factors on height as rickets.[10] However, the discussion was primarily on mode of transmission for inheritance and not on

effect of environmental factors. Later, Platt started to accept that blood pressure shows a normal distribution in the general population and that it is probably determined by both multifactorial hereditary and environmental factors.[11] However, Platt remained focused on the hypertensive subjects and their families as a different population, because he found blood pressure of relatives of hypertensive subjects to rise more during middle age compared to relatives of normotensive subjects and that the distribution of blood pressure in the former group appeared to be bi- or even three-modal.

In his last contribution to the debate using new data, Platt focused on concordance of blood pressure in twins, in relatives of hypertensive and of normotensive subjects.[12] Various age-and gender specific blood pressure distributions were made and compared to the normal distribution from a population sample (the one of Pickering[6]). Platt noticed that the curves of the relatives of the hypertensive subjects to be more irregular with 2 or three bulges, and that the distribution of blood pressure in the relatives compared to the population sample, was shifted to the right (see fig 1).[12] The latter had been shown by Pickering before.[6] To account for the three bulges in the distribution in the relatives of hypertensive subjects, Platt argued that it would be tempting to relate this to multifactorial inheritance.[12] However, Platt refuted this possibility, as he argued that the individuals with a blood pressure in the middle bulb are relatives of those in the bulb at the end of the curve that was considered a separate group with primary hypertension. Therefore, the model of genetic transmission had to account for all three phenotypes, and not only the phenotypes with low and medium blood pressure levels. Platt also refuted the idea of multifactorial inheritance and interaction of environmental factors, as from his study in twins the genetic component appeared to cause severe hypertension.[12] Platt refuted the mendelian dominant transmission, and hypothesised transmission of an incompletely dominant gene, giving rise to severe hypertension if homozygous, and mild hypertension if heterozygous.[12] Pickering argued against these ideas making an analogy with the distribution of height.[13] It was argued that the samples that Platt had used to construct the age and gender specific blood pressure distributions in the relatives of hypertensive subjects were much smaller compared to samples used earlier to study the distribution of height in children. In the distribution of height in children three peaks were apparent, notwithstanding the fact that height was considered an example of polygenic inheritance.[13] Therefore, Pickering did not give any biologic meaning to the three bulges in the distributions of Platt, on which Platt based his theory of incomplete dominant inheritance.

Figure 1 Frequency distribution of systolic blood pressure of sibs of propositi with essential hypertension (malignant and benign) compared with that of the general population. Arranged in 10 year age groups (males and females together).[12]
The total number of sibs in each age category were:
20-29 years=8, 25-34 years=8, 30-39 years=12, 35-44 years=10,
40-49 years=25, 45-54 years=35, 50-59 years=36, 55-64 years=28.
(Source: J.D. Swales, Platt versus Pickering, 1986,[5] with permission).



In conclusion the debate dealt with the hereditary nature of hypertension, its mode of transmission and the distribution curve of blood pressure in the population at large. The controversy arose from clinical thinking in the assumption that primary hypertension is a separate entity. This binomial assumption led to all the important arguments of Platt:

- the assumption of a strict division between normotension and hypertension gave rise to the calculated transmission of 50% and more,
- the assumption of two populations differing in their behaviour of blood pressure to rise with age
- the assumption that the distributions of middle aged siblings of hypertensive subjects should be bimodal
- and the assumption that in siblings of hypertensive subjects the assumed appearance of three phenotypes with normal, mildly elevated and seriously elevated blood pressure could not arise from multifactorial inheritance as a similar mechanism had to explain both the hypertension in the proband (by assumption a different entity) and the blood pressure phenotypes in the siblings.

The view of primary hypertension as a separate entity from normotension, repeatedly activated the debate with Pickering who consequently described primary hypertension as a quantitative characteristic, of multifactorial origin, representing the extreme end of the age and gender specific distribution of blood pressure. Finally, Platt and Pickering seemed to agree on three issues[14]:

- primary hypertension is hereditary,
- the nature of the inheritance is probably multifactorial with influences of the environment,
- one cannot exclude single gene inheritance (among multifactorial inheritance) in certain small subsets of the population with essential hypertension, however no prove exists. The multifactorial nature of blood pressure regulation makes it rather unlikely to detect such a factor.

Most importantly it was agreed upon that the factors that might cause primary hypertension have to be studied, also in relation to environmental influences, and that these causes will not be found by studying the distribution of blood pressure itself. The fact that hypertension was assumed to have a multifactorial origin, did not diminish the need and interest to find certain causes, as Platt had been worried of before.[10] Later, the model of polygenic inheritance was proven with a likelihood pedigree analysis.[15] Although the theory of Pickering became

widely accepted and easily applicable to the quantitative nature of hypertension as a risk factor, in clinical medicine, a division of normotension and hypertension remained common practice.

2.2.2 PLATT VERSUS PICKERING: AFTER THE DEBATE

Research in human hypertension has developed from description of blood pressure patterns in families and population samples, to the comparison of blood pressure regulation in hypertensive and normotensive subjects. Many neural, hormonal and cellular characteristics known to be relevant in blood pressure regulation have been considered as causal factors in primary hypertension (see chapter 1.2.2 to 1.2.5). Due to the multifactorial nature of hypertension and the intricate relation between many blood pressure regulating mechanisms, it has not been possible to point at the one single marker of primary hypertension, and certainly not to causal factors.

However, in the age of molecular biology, it has become possible to search for abnormalities in protein synthesis and their related DNA alterations.[16] This gives a new opportunity to examine the multifactorial causes of primary hypertension, probably by definition of subsets of primary hypertension.[17] Much progress has been made already to identify genes for proteins related to blood pressure regulation and polymorphism of these genes.

As hypertension is a multifactorial defined characteristic with polygenic inheritance, one could try to define the gene involved with the highest relative effect (the major gene). This could easily be a different major gene in specified sub-groups with primary hypertension.[17] Moreover, known alterations in biochemical of physiological factors in primary hypertension can point to a certain major gene defect if one can dissolve the chain of events that lead from the major gene defect, by the factor studied (intermediate phenotype), to hypertension. If the parameter of interest, the intermediate phenotype, is close to the gene a clear bimodal distribution of the intermediate phenotype will be found in a population sample. If the phenotype is far apart from the gene in the chain of events, as is blood pressure as the ultimate phenotype in hypertension research, an unimodal distribution from the intermediate phenotype in a population sample may be found.[4] To search the major gene involved in primary hypertension, it might therefore be important to define those factors known to be relevant to blood pressure regulation, that show a clear bimodal distribution in

the population sample. Second, the linkage of this intermediate phenotype with blood pressure can be studied in large pedigrees to learn more. Third, the chain of events of intermediate phenotypes from molecular to organ level can be studied.[4]

As an example, sodium-lithium countertransport has extensively been studied as a possible intermediate phenotype in population samples and pedigrees (see section 1.2.4). Mathematical analyses of the age, height, and weight adjusted sodium-lithium countertransport distributions for males and females separately, showed a mixture of two overlapping distributions, not in favour of an unimodal distribution.[18] However, despite the assumed bimodal distribution, the level of sodium-lithium countertransport in the general population was not a clear determinant of blood pressure status.[18] Moreover, a biometrically inferred genotype of sodium-lithium countertransport (derived from pedigree analysis on segregation of sodium-lithiumcountertransport in 11 large pedigrees), did not add to the determination of blood pressure status in women, but did in men.[19] Future blood pressure status after seven years was not predicted by the level of sodium-lithium countertransport at baseline.[20] However, a prediction of future blood pressure could be made if the biometrically inferred genotype was used in the analysis.[20]

The example of the findings with sodium-lithium countertransport is presented here to stress some pitfalls in this line of research, that relate to the unimodal-bimodal controversy. A parameter of interest for determination of present and/or future blood pressure status must have a meaning to blood pressure regulation and not be unimodally distributed in the population at large. In the case of the sodium-lithium countertransport the relevance to blood pressure regulation has not yet been proven (see section 1.2.4). The considerable overlap of sodium-lithium countertransport between normotensive and hypertensive subjects[18] required age, height and weight adjusted values to compose gender specific distributions to which a bimodal distribution provided the best fitting, with a maximum likelihood method. Although mathematical methods are important in order to prevent discussion as in the debate of Platt versus Pickering, it might turn out that parameters that at first sight are not bimodally distributed, are of lesser value to predict hypertension than parameters who are. The quantitative level of sodium-lithium countertransport had no relevance to present and future blood pressure status.[18,19] However, if a qualitative distinction was used, i.e. the biometrically derived genotype estimated from relatively high and normal levels to perform segregation analysis in pedigrees, a better determination of present[19] and future blood

pressure status[20] could be made. However, this last finding might be biased by the assumption that sodium-lithium countertransport is a qualitative measure with a distinction in its distribution, between hypertensive and normotensive subjects.

From the above, it follows that in modern hypertension research the definition of the shape of a distribution curve has become again of importance, just as in the Platt-Pickering controversy. Moreover, bias might be introduced by qualitative methods and bimodal reasoning if the parameter studied actually has an unimodal distribution without a distinction between high and low values. Another analogy with the era of the Platt-Pickering debate is the need for family studies with large pedigrees, to perform segregation analysis of intermediate phenotypes.

2.3 FAMILY HISTORY APPROACH IN STUDIES ON THE EARLY PATHOGENESIS OF HUMAN HYPERTENSION

To study the early phase of primary hypertension determinants of primary hypertension are compared between subjects who will and who will not develop primary hypertension. However, to exclude secondary effects of sustained elevated blood pressure on the mechanisms involved, subjects should be selected at a young age, before expression of their future blood pressure rise.

A major limitation in such an approach is that to date, pre-hypertensive youngsters can not be defined with certainty. In several cohorts of youngsters the pattern of change in blood pressure during childhood,[21,22] from childhood to adolescence[23,24] during adolescence[25] and from adolescence to adulthood[26,27] has been studied with its determinants. Usually, the determinants studied were confined to anthropometric measures, stress tests, physical fitness and baseline blood pressure. Body size,[23] weight[24] and body weight gain,[21,25,26,27] and increase of skeletal age[21,24] have demonstrated to be important determinants of blood pressure level and rise in youngsters, as has baseline blood pressure for adolescents.[26,27] Furthermore, an initial decline of blood pressure during follow-up,[25] and a decrease in physical fitness during follow up[23] were related to blood pressure rise during follow-up. In this way information about the pattern of blood pressure and growth has been gathered. However, it remains difficult to select those youngsters that will develop hypertension later in life with certainty. If no adequate identification of youngsters that become hypertensive is possible, one may study determinants in a rather large initially normotensive cohort, to ascertain after follow up which determinants were related to the development of hypertension during follow up.[28]

To reduce the numbers of youngsters to be measured at baseline, a study population may be selected based on a contrast in risk to develop primary hypertension. Apart from the blood pressure level, this risk is best defined by a family history of hypertension.[29] The risk associate with a positive family history follows from the observation that:

- hypertension runs in families (see 2.1.1),
- blood pressure is correlated between family members from an early age onwards,[30,31,32]
- offspring of hypertensive parents more often has a blood pressure in the upper quintile compared to offspring of two normotensive parents.[33]

Moreover, familial risk for primary hypertension in offspring is better defined by the blood

pressure of both parents rather than of one parent, which follows from the observation that

- a higher percentage of children has a blood pressure in the top quintile if both parents have hypertension, compared to only one parent.[33]
- the risk for hypertension in an individual is higher if two relatives have hypertension compared to one affected relative.[34]
- the percentage of offspring with a blood pressure in the top quintile of the age and gender specific blood pressure distribution is highest (33%) if both parents have a blood pressure in the top quintile; intermediate (24.7%) if the blood pressure of one parent is in the top and of the other parent is in the bottom quintile and lowest (13.5%) when both parents have a blood pressure in the bottom quintile.[35]
- The relative risk (RR) for primary hypertension in offspring of hypertensive parents is higher, if the blood pressure levels of two parents are taken into account (RR 4.4), compared to one (1.57) (see figure 2, bar A versus C, derived from Data from Watt[29]).

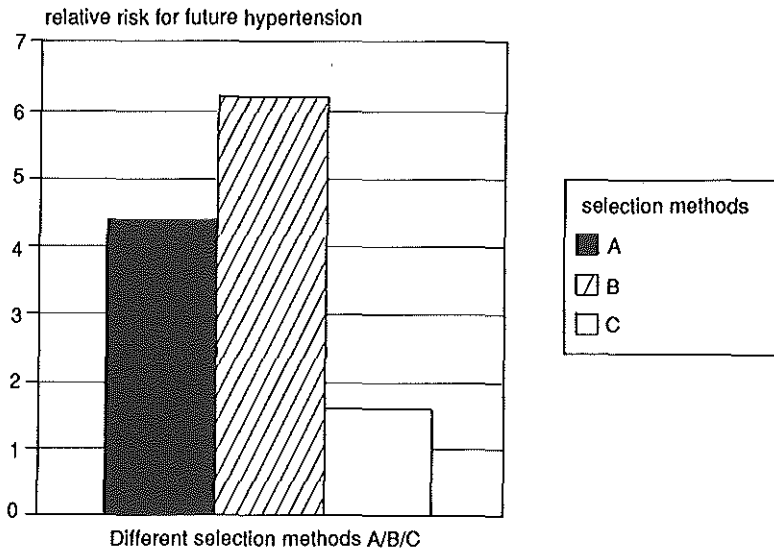
Therefore, in view of the hereditary and quantitative nature of blood pressure, the risk for hypertension of youngsters is best quantified by the blood pressure level of both their parents; the higher the blood pressure in both parents, the higher the risk; the lower the blood pressure level of both parents, the lower the risk. The blood pressure status of the parents is often referred to as the 'family history of hypertension'. However, to prevent misclassification by recall bias, and to be informed on the level of blood pressure also in presumed normotensive cases, the 'family history' preferably is ascertained by blood pressure measurements in both parents.[36]

As blood pressure is variable the measurement of the parental blood pressure should be repeated over time to prevent misclassification. Because of the blood pressure rise with age, and the relation between risk for hypertension in offspring with the blood pressure level of their parents, the criteria to select low and a high risk families for hypertension should not be based on clinically defined normotension versus hypertension. Rather, selection of parents should be based on their relative low or high position in the blood pressure distribution, using percentile cut off levels at the highest and lowest extreme. A selection based on one cut off level to divide the whole distribution in relative high or low blood pressure is suboptimal. This follows from data showing that the number of offspring with a blood pressure in the top or the bottom quintile does not differ between children from parents with

a blood pressure below the 80th-centile compared to children from parents with a blood pressure above the 20th-centile respectively.[37] Therefore, in stead of selection of the 'normotensive' families from all those outside the extreme top of the blood pressure distribution, the 'normotensive' control group is selected from the opposite extreme; the bottom of the blood pressure distribution. To account for the blood pressure rise with age and the difference in blood pressure between men and women, gender and age specific blood pressure distributions may be used.

A choice for the cut-off percentile at the top of the distribution and the opposite percentile at the bottom of the distribution must be made acknowledging both magnitude of contrast in risk and recruitment of adequate numbers of offspring.

Figure 2 The family history approach with several options to select offspring with a difference in risk for future hypertension. (derived from Watt,[29] based on data from Watt,[36] Deutscher et. al.,[37] and Higgins et. al.[33]).



Relative risk for future hypertension of

A = offspring of two parents with 'high blood pressure'* compared to offspring of two parents with 'low blood pressure'**,

B = offspring with 'high blood pressure' compared to offspring with 'low blood pressure'

C = offspring with 'high blood pressure' and 1 or 2 parents with 'high blood pressure' compared to offspring with 'high blood pressure' and 1 or 2 parents with 'low blood pressure'.

* High blood pressure = Fifth quintile of age and gender specific blood pressure distribution.

** Low blood pressure = First quintile of age and gender specific blood pressure distribution.

2.3.1 FAMILY HISTORY APPROACH, BLOOD PRESSURE DIFFERENCE BETWEEN OFFSPRING AND RISK FOR HYPERTENSION

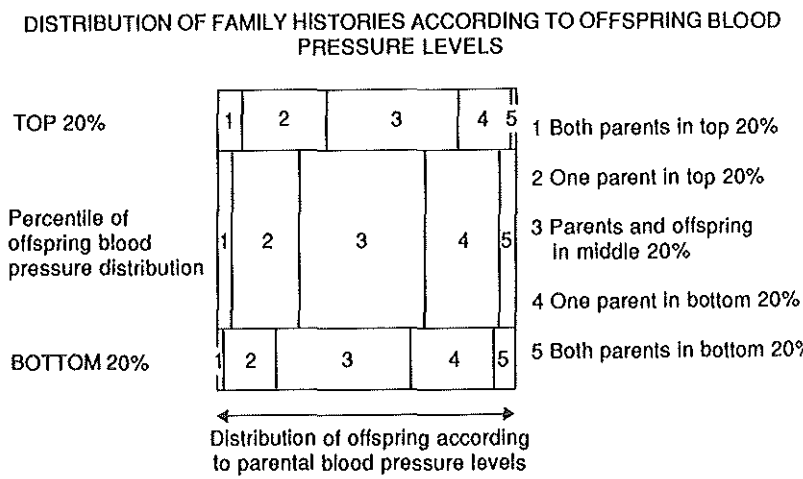
A hypothetical calculation of risk for future hypertension in children with a positive history for hypertension in one parent, showed a rather limited increase in risk.[38] The risk is diluted by lack of information on blood pressure in both parents, and by the use of absolute (clinical) criteria for hypertension and normotension rather than relative (percentile) cut off levels. Watt. et al,[39] calculated the positive predictive value for hypertension after 8-years of follow up, for groups of offspring according to various definitions of a positive family history of hypertension:

- if one parent had a blood pressure in the top decile, 29% of the offspring had a blood pressure in the top quintile after 8 years of follow up.
- if two parents had a blood pressure in the top quintile, 38% of the offspring had a blood pressure in the top quintile after 8 years.

Given that only 4% of the offspring would be selected from the population using the last criterium, and only 7% of the offspring with a blood pressure in the upper quintile after 8 years was identified by that selection criterium, the usefulness to select youngsters solely on parental blood pressure values for a high risk prevention strategy is limited. Projects such as the Dutch Hypertension and Offspring Study might indicate characteristics other than parental blood pressure to further improve prediction of hypertension in youngsters.

To study the early pathogenesis of hypertension, the absolute future risk for hypertension in the offspring groups of The Dutch Hypertension and Offspring Study, is not as important as the relative difference in predisposition for hypertension between the groups, as long as there is a large contrast in risk. This may be achieved by selection of groups of offspring from the extremes of the parental blood pressure distribution. As the predisposition for hypertension in youngsters is also influenced by their individual blood pressure level, one can not select youngsters with substantial difference in predisposition for hypertension, without the presence of a blood pressure difference between the groups selected.[36] On the other hand, one may increase the probability for a subject to develop hypertension in the future by a selection based both on the parental and individual age and gender specific blood pressure percentile, as is proposed with the "four-corner" method, discussed hereafter.

Figure 3 Distribution of family histories according to offspring blood pressure levels.[29] (Source: Hofman A, Grobbee DE, Schalekamp MADH, eds. The early pathogenesis of primary hypertension. Amsterdam: Elsevier; 1987, with permission).



2.3.2 SELECTION METHODS:

THE FOUR CORNER AND THE TWO CORNER METHOD

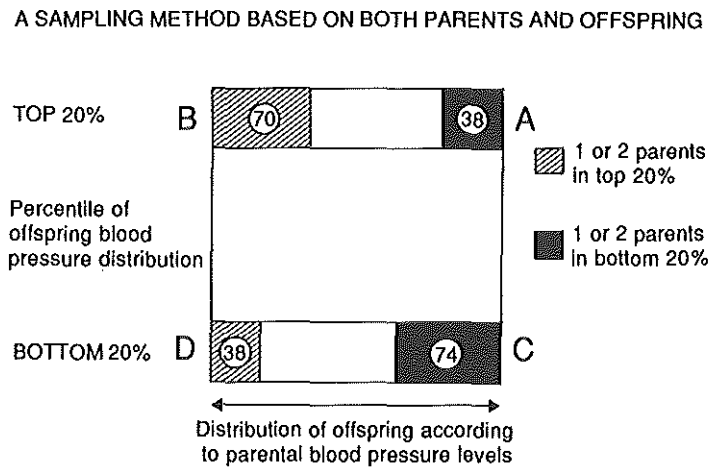
According to the four corner method, the selection from the population may either start with the children followed by selection of the parents or vice versa. Both blood pressure measurements in parents and offspring are needed. A model for this selection was suggested by Watt,[29] starting with selection of the offspring, based on data from the Tecumseh survey. By this method, eventually four groups of offspring become selected, from each corner of a blood pressure distribution square of the offspring. The square is divided in three rows, representing from top to bottom offspring with a blood pressure in the top quintile, in the middle three quintiles and in the bottom quintile (fig 3). Each row can be divided in 5 sections from the left to the right, representing two parents, one parent in the top quintile, two parents in the third quintile, one parent, and two parents in the bottom quintile respectively (see fig 4). As the number of offspring with a blood pressure in an extreme quintile with two parents in an opposed extreme quintile, is rather small, offspring with either one or two parents in the extreme are to be included. In offspring of the Tecumseh survey, Watt calculated that 220 offspring out of 1000 could become selected in the four corners (figure 4):[29]

- Offspring in top quintile with one or two parents in top quintile (n=70, Corner B),
- Offspring in bottom quintile with one or two parents in top quintile (n=38, Corner D),
- Offspring in top quintile with one or two parents in bottom quintile (n=38, Corner A),
- Offspring in bottom quintile with one or two parents in bottom quintile (n=74, Corner C).

If the objective is to compare groups based on parental blood pressure (B and D versus A and C) the contrast in predisposition for hypertension is diminished due to large difference in blood pressure of the children within one group, and the smaller difference in mean blood pressure that remains between the two groups. The blood pressure will still be higher in B and D versus A and C (in B and D, 66% has a relatively high blood pressure, in A and C 66 % has a relatively low blood pressure). Moreover, the blood pressure distribution is artificially bi-modal, which reduces the possibility to study the association between blood pressure and a parameter of interest.

If the objective is to compare groups based on blood pressure of the youngsters (A and B versus C and D), not only the large blood pressure difference between the groups of youngsters might interfere, but the contrast might also be diminished by the difference in family background wthin each group.

Figure 4 A sampling method based on blood pressure of both parents and offspring, calculated for a group of 1000 offspring, by Watt.[29] based on data from Deutscher et.al.[37]
(Source: Hofman A, Grobbee DE, Schalekamp MADH, eds. The early pathogenesis of primary hypertension. Amsterdam: Elsevier, 1987, with permission).



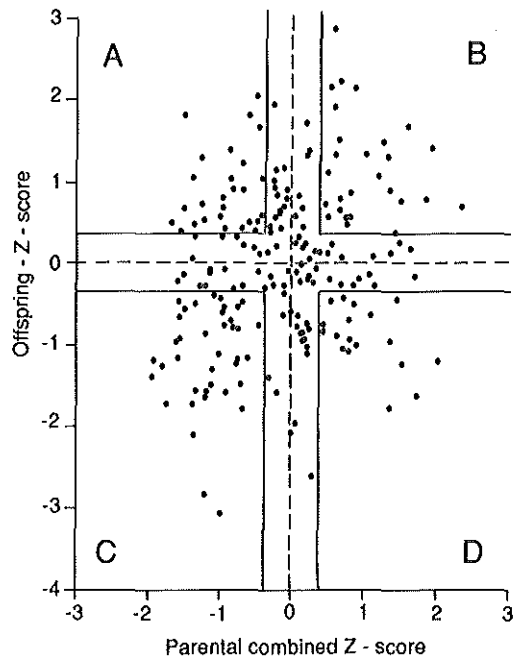
Description of A,B,C,D

Offspring in top quintile with one or two parents in top quintile (n=70, Corner B),
Offspring in bottom quintile with one or two parents in top quintile (n=38, Corner D),
Offspring in top quintile with one or two parents in bottom quintile (n=38, Corner A),
Offspring in bottom quintile with one or two parents in bottom quintile (n=74, Corner C).

Therefore, the four corner method seems most appropriate to compare groups of offspring with a similar blood pressure level, but with a difference in parental blood pressure (A versus B, or C versus D) or vice versa. In this way, the secondary influence of an already increased blood pressure level on the parameter of interest can be overcome. However, a problem occurs with regard to the size of the groups selected. If similar cut off values are used at both sides of the distribution for blood pressure of parents and children (i.e. the 80th centile at the top and the 20th centile at the bottom) group A will be much smaller than group B or group C, and group D will be much smaller than group C or B, as shown above (fig 4).[29] To select four groups of comparable magnitude, one must set the cut off value to select group A and D more close to the median of the blood pressure distribution either of the parents, or the children or both. The liberal use of cut off values will limit final contrast. In a study that used the four corner method,[40] Z-scores for the mean of systolic and diastolic blood pressure were used to select 70 offspring from each corner, according to a blood pressure Z-score greater than + or -0,35. This Z-score corresponded with the top and bottom 30th-centile of the blood pressure distribution. The blood pressure distributions were made specific for age (2-year groups for the offspring and 5-year groups for the parents) and gender, based on multiple blood pressure measurements (four of the offspring, and eight of the parents). With this method most offspring was selected in group C (163), a little less in group A (122) and B (118) and a small group in corner D (68). Therefore a random sample of 70 offspring was selected from A, B, and C and all 68 offspring of group D were invited (see figure 5).[40]

The selection based solely on blood pressure percentile of the parents selects offspring from the whole blood pressure range with a naturally occurring predominance of high blood pressure percentiles in the offspring of hypertensive parents, and of low blood pressure percentiles in the offspring of normotensive parents. Therefore, overlap in blood pressure can be found between the groups selected on the basis of parental blood pressure percentiles. This is important to assess whether differences in parameters of interest between the groups of offspring are influenced by the blood pressure differences between the groups of offspring. If offspring is selected from the whole range of blood pressure stratified analysis or regression methods are possible to study the effect of the blood pressure difference on the parameter of interest (figure 6), providing similar analysis as in the four-Corner.

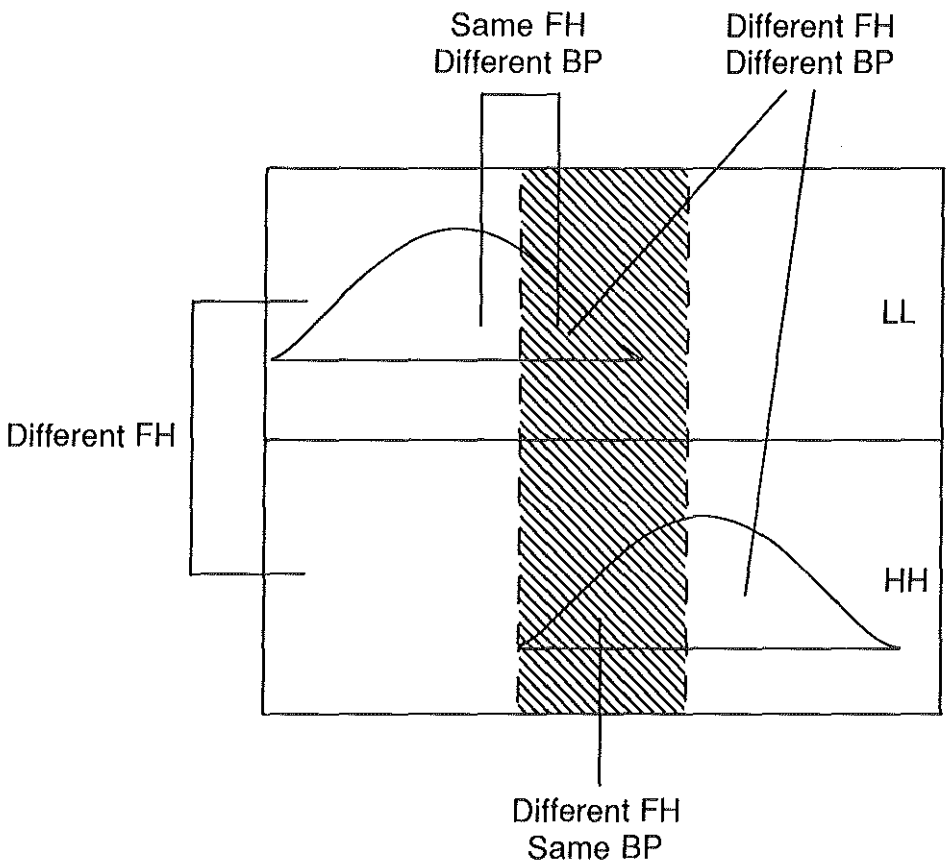
Figure 5 Scatter diagram of offspring and combined parental blood pressure Z-scores. For clarity, only a random sample of 25% was shown (Source: Watt et. al.[40], J Hypertens, 1992, with permission).



Description of A,B,C,D

Offspring in top tertile with one or two parents in top tertile (n=118, Corner B),
Offspring in bottom tertile with one or two parents in top tertile (n=68, Corner D),
Offspring in top tertile with one or two parents in bottom tertile (n=122, Corner A),
Offspring in bottom tertile with one or two parents in bottom tertile (n=163, Corner C).

Figure 6 Blood pressure distributions of the offspring in a family history study using a selection criteria based solely on the blood pressure of the parents with special attention to possible analyses for comparing groups of offspring, compared to the four corner method. Presented is the blood pressure distribution of the offspring of two parents with low blood pressure (LL), and of two parents with high blood pressure (HH).



To summarize, to study the early pathogenesis of primary hypertension using the family history approach, the following guidelines may be formulated

- use quantitative measures for "hypertension" and "normotension" (2.2.1),
- select families from a population based survey to define the reference group (2.3),
- use blood pressure measurements of the parents and not rely on history (2.3),
- use information of blood pressure in both parents, preferable more measurements and both systolic and diastolic blood pressure (2.3, figure 2),
- define families with contrasting risk for hypertension: for the control family a definition of low blood pressure must be used, instead of normotension (2.3).
- diminish secondary influences of blood pressure on the parameter of interest, no selection based on offspring blood pressure should be made (2.3).

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3 THE DUTCH HYPERTENSION AND OFFSPRING STUDY: DESIGN AND METHODS

3.1 DESIGN

The Dutch Hypertension and Offspring Study is a population-based family study on determinants of future hypertension.[1] It is designed to compare measures of circulatory and metabolic functions across groups of young subjects with a contrasting risk to develop hypertension in the future. Risk was determined by presence or absence of a parental hypertension, based on multiple blood pressure measurements over time. Hypertension and normotension of the parents were defined using cut off points according to age- and gender specific percentiles, for both systolic and diastolic blood pressure. Current use of antihypertensive medication was an inclusion criterium for hypertension, and excluded normotension.

3.2 METHOD OF SELECTION

The families were selected from a cohort participating in a study on cardiovascular risk indicators in the general population. The results of the blood pressure measurements in all participants aged 5 to 85 years of age were used to compute one-year-age- and gender-group specific distributions, for systolic and diastolic blood pressure separately. Hypertension was defined as a systolic and diastolic blood pressure equal to or greater than the age- and gender specific 75th centile, i.e. in the upper quartile of the distribution. Normotension was defined as a systolic and diastolic blood pressure less than or equal to the 25th centile. A subject taking anti-hypertensive medication, but with a systolic and/or diastolic blood pressure below the 75th centile, was also considered hypertensive. If in the one-year-age and gender-group specific blood pressure distributions, no blood pressure level corresponded with the exact 25th centile or the exact 75th centile, the nearest centile greater than 25th centile was taken, and the nearest blood pressure level corresponding with a percentile smaller than the 75th centile respectively. In other words, in those cases, the 25th centile estimate was rounded upwards, and the 75th centile estimate was rounded downwards.

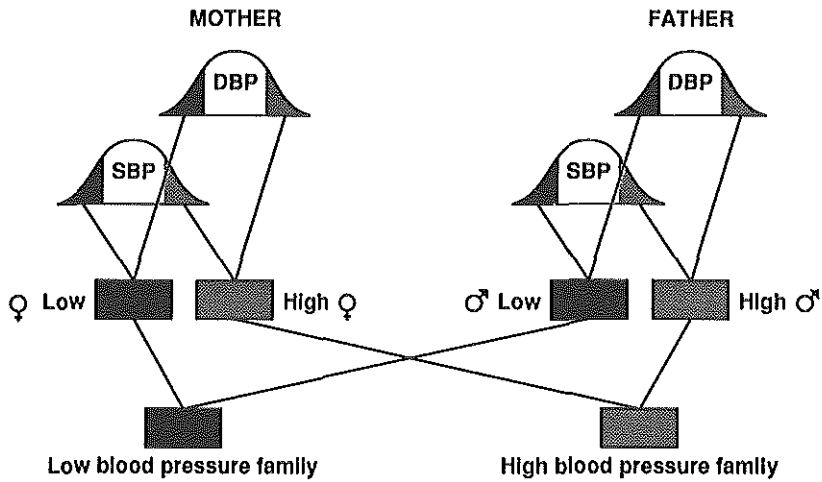
The procedure of selection of the families is outlined in figure 1. Groups of parental couples were selected comprising

- two hypertensive parents (both parents had a systolic- and diastolic blood pressure equal to or greater than the age- and gender specific 75th centile and/or were taking antihypertensive medication).

Figure 1 Dutch Hypertension and Offspring Study: selection method of the families.

The flow diagram of the selection method of the Dutch Hypertension and Offspring Study:

- blood pressure distribution for systolic- and diastolic blood pressure (SBP, DBP) in the parents (bell shaped curve),
- the selected parents with both SBP and DBP above the 75th centile (high) or both SBP and DBP below the 25th centile (low) (upper row of square blocks)
- the families composed of two hypertensive parents (high risk family), two low blood pressure parents (low risk family) and one hypertensive and one low blood pressure parent (mixed families with intermediate risk) (lowest row of square blocks).



- two normotensive parents (both parents had a systolic and diastolic blood pressure less than or equal to the age- and gender specific 25th centile without taking antihypertensive medication).
- one hypertensive parent and one normotensive parent.

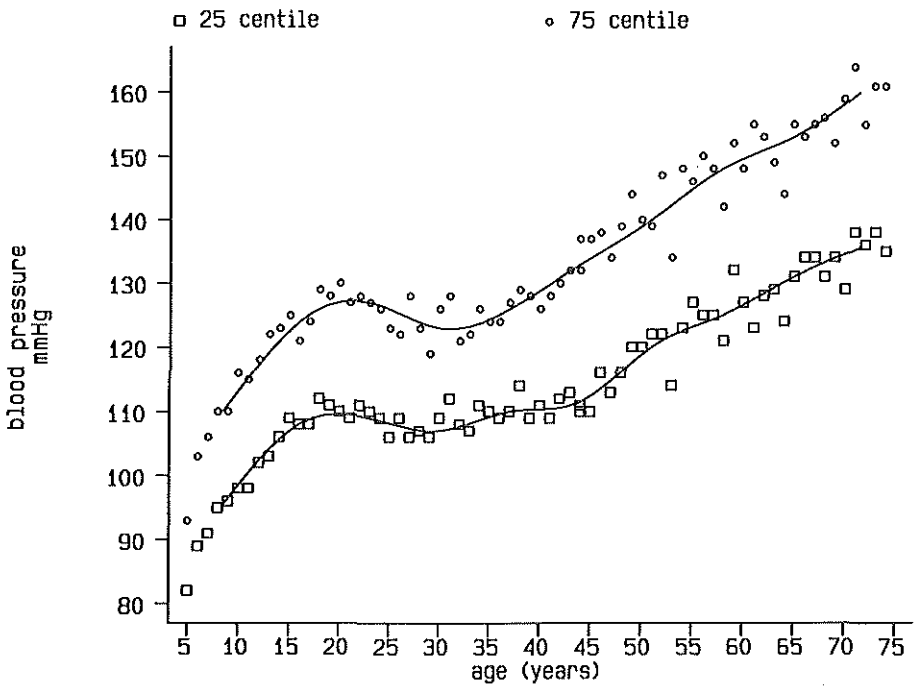
To assess whether blood pressure remained stable in the assigned range, parental couples that met either one of the three family-criteria had their blood pressure remeasured six to ten years after the measurement on which the initial selection was based. Eventually, a family became eligible for the Dutch Hypertension and Offspring Study if the blood pressure of both parents had stayed in the same category for the age- and gender specific systolic and diastolic blood pressure.

3.3 POPULATION

Families were selected from a cohort that participated in a study on cardiovascular risk indicators in the Netherlands between 1975-1978 (The EPOZ study: principal investigator Prof. Dr. H.A. Valkenburg). As part of this study, 10,532 (78%) of 13,462 eligible inhabitants aged 5 years and over from two districts of the town of Zoetermeer in the Netherlands had their blood pressure measured.[2] Blood pressure centiles were computed for one-year age- and gender-groups, for systolic and diastolic blood pressure separately. In figure 2 the 25th and 75th centile for systolic blood pressure in women aged 5 to 75 years of age is given. The cohort included 1,642 families, defined as father and mother and at least one child aged 5 years and older, living together at the time of the 1975-78 survey. Furthermore, potentially eligible for selection were 43 couples without children aged 5 years and older at the time of the 1975-1978 survey, if the mother aged less than 50 years of age at the time of the 1975-78 survey. In addition, families were selected from a cohort of 476 young parental couples (73% of those eligible), residents of Zoetermeer, that had infants born in 1980 and participated in a trial on sodium intake and blood pressure in newborns,[3] for which both parents had their blood pressure measured. In total, the selection was based on a group of 2161 parental couples.

Of the 2161 mothers and 2161 fathers, 18% had both systolic and diastolic blood pressure in the upper quartile of the age and gender specific blood pressure distribution and/or used antihypertensive medication. These subjects were considered hypertensive. 17% of the individual parents had a systolic and diastolic blood pressure in the lowest quartile without using antihypertensive medication. These subjects were considered normotensive.

Figure 2 The systolic blood pressure percentile chart for women, showing the 75th centile and 25th centile by age, for systolic blood pressure in females from the original EPOZ[29] in 1975-78. Drawn in it is the smoothed curve for 75th and 25th centile (line).



From these normotensive and hypertensive parents, 370 (17%) parental couples were selected with two hypertensive parents (n=125, 5.8%), one hypertensive parent and one normotensive parent (n=148, 6.8 %) or two normotensive parents (n=97, 4.5%).

At the start of the selection procedure of the Dutch Hypertension and Offspring Study (October 1986), 58 couples of the 370 selected parental couples turned out not to be eligible anymore (in 37 families one or both parents had died, 19 families without children aged between 5 and 30 years of age, and 2 families with adopted children only). The remaining 312 parental couples were invited for re-examination to assess whether their blood pressure had remained in the same age- and gender specific category as at the initial examination; 94 couples of two hypertensive parents, 128 of one hypertensive and one normotensive parent, and 90 of two normotensive parents. Remeasurements could not be performed in some parental couples; 34 refused, 26 moved away to far to participate, and 2 due to serious illness in one of the partners. A total of 250 couples (80%) had their blood pressure remeasured; 74 couples (79%) of two initially hypertensive parents, 106 couples (83%) of initially one hypertensive and one normotensive parent and 70 couples (78%) of two initially normotensive parents. Finally, after re-examination of the 250 parental couples, of 51 couples (69% of those who were re-examined) both parents remained hypertensive, of 35 couples (33%) one parent remained normotensive and the other parent remained hypertensive, and of 35 couples (50%) both parents remained normotensive. The 121 families selected comprised 291 healthy children aged 5 - 30 years of age in 1987; 111 offspring of two hypertensive parents, 98 of one hypertensive and one normotensive parent and 82 offspring of two normotensive parents.

3.4 METHODS

The protocol of selection and the various studies in the offspring groups will be discussed in section 3.4.1. The description of the measurements in section 3.4.2. will solely concern the methodology of studies in the offspring groups, with the exception of the blood pressure measurement. The procedures for blood pressure measurement apply to registration of blood pressure in parents and children during all phases of the study.

As outlined in chapter 1.1, the choice of measurements in the different phases of The Dutch Hypertension and Offspring Study was based on hypotheses regarding determinants that were well known at the time of preparation of this study. Moreover, measurements had to be confined not to stress the groups of youngsters too much and should be as non-invasive as

possible. The study protocol was approved by the Ethical committee of the Erasmus University/ University Hospital Dijkzigt, and informed consent was obtained from the children and their parents.

3.4.1 PROTOCOL

Selection

In the period between November 1986 and January 1987 all parental couples selected for re-examination of blood pressure were invited to visit the research centre in Zoetermeer between 02.00 pm and 09.00 pm. After 5 minutes of rest, anthropometric measurements, blood pressure, and pulse count were taken by a trained paramedical assistant, unaware of the initial blood pressure category of the parents. In the doctors office a microcomputer with data of the initial survey was installed. The observed blood pressure value was plotted against age on the gender-specific blood pressure chart depicting the 25th and 75th centile (see figure 2) for systolic and diastolic blood pressure in both parents for both the initial and present survey, and the use of antihypertensive medication at both occasions was registered. If blood pressure was in the same category as at the initial survey for both parents, a questionnaire on individual and familial cardiovascular risk indicators was taken and information on all their children concerning date of birth and home-address was obtained. Blood was sampled for measurement of sodium, potassium, creatinine and typing of HLA and ABO blood group. Urine was collected for qualitative analysis of glucose and albumin. If qualitative analysis of urine was positive, an aliquot of 3 ml was frozen for subsequent quantitative analysis, an blood was tested for glucose levels. The purpose and the protocol of the Dutch Hypertension and Offspring Study was discussed with the parents, and documentation was given. For children aged 5 to 12 years of age consent for participation was asked to the parents and the advice was given to discuss it with their children. Offspring aged 12 to 30 years received documentation either through their parents or by mail. Within a week, parents of offspring aged 5 to 12 years of age, and offspring aged 12 to 30 years of age were contacted by telephone to answer questions that had arisen, and to discuss participation in the Dutch Hypertension and Offspring Study.

First Phase

In the period between January 1987 and May 1987, offspring were invited for a short session of one hour duration that was scheduled between 08.00 a.m. and noon. The day before visiting

Table 1 Summary: measurements during selection of the parents in 1986.

Systolic and diastolic blood pressure	
Anthropometry	
Urine:	Qualitative albumin*, glucose**
Blood:	Sodium, potassium, creatinine
	HLA-type, ABO blood group

* if positive: quantitative albumin in urine was measured
** if positive: glucose in blood was measured

the research centre participants collected a 24-hour urine sample and refrained from smoking and coffee use, and from food and beverages starting from midnight. Participants visiting the research centre after 10.30 a.m. were allowed to take a light breakfast before 08.00 a.m. (bread with marmalade, honey, or syrup with water or fruit juice, without using butter, milk, thee or coffee). At the research centre, body weight and height were measured with the subject wearing indoor clothes, without shoes. Blood pressure was measured twice in sitting position, separated by a count of the pulse, and the procedure was repeated supine. An intravenous cannula was inserted, and blood was sampled for measurement of electrolytes, calcium, magnesium, phosphate, creatinine, parathyroid hormone, circulating immune complexes, complement C3 protein polymorphism and platelet α_2-adrenoreceptors. The participant remained supine during the next 30 minutes, after which blood was sampled for atrial natriuretic peptide, intra-erythrocytic sodium and potassium and plasma glucose. If two or more children of one family were seen blood was sampled for HLA-typing and ABO blood group. A maximum of 80 ml blood was sampled. All blood was handled at room temperature or on ice-water and immediately processed, according to specific requirements described in section 3.3.2. The intravenous cannula was removed and the participant was offered breakfast and asked to fill in a short questionnaire on general health and on use of medication, vitamin supplements, oral contraception and menstrual history. Subsequently, both the questionnaire and eating, drinking, smoking prescriptions of the day and morning before were checked. Furthermore the participant was briefly informed and invited for the second phase.

Second phase

In the period of October 1987 to July 1988, offspring were invited for an extended measurement session of 4 to 5 hours duration scheduled between 07.45 a.m. and 01.00 p.m.

Participants were visited at home to explain full details of the protocol and to give the 24-hour urine sampling device and lithiumcarbonate tablets. They were asked to refrain from smoking and coffee drinking during the day before visiting the research centre until after the examination. Subjects were asked to take the lithiumcarbonate tablets (8 - 10 mg/kg body weight) at bedtime, but no later than midnight, with water, and to drink only water afterwards. One hour before their visit to the research centre the following morning participants were asked to void, and register the time, and to collect all urine that might be voided after that time separately before arriving at the research centre.

Table 2 Summary: measurements in offspring during first phase.

Systolic and diastolic blood pressure
Anthropometry
24-hour urinary excretion of sodium, potassium, calcium
Blood: sodium, potassium, creatinine, phosphate, glucose, calcium, magnesium,
(1-84)intact-parathyroid hormone
circulating immune complexes
haplotype of complement C_3 (C_3F,C_3S,C_3FS)
atrial natriuretic factor
Platelet α_2-adrenoreceptors
Intra-erythrocytic sodium and potassium concentration
HLA-type and ABO blood group (only if two or more children of one family participated)

At the research centre deviations from the protocol were registered. The anthropometric measurements of phase 1 were repeated, followed by insertion of an intravenous cannula in the left arm. Blood was sampled for measurement of sodium-potassium-ATPase activity in the erythrocyte membrane, circulating inhibitor in plasma, intra-lymphocytic calcium and magnesium and for plasma insulin, somatomedin-C, 1,25-dihydroxyvitamin D_3, and parathyroid hormone. After 30 minutes of supine rest, in a quiet room, blood was sampled for measurement of catecholamines, renin, aldosterone, angiotensin-II, lymphocytic β_2-adrenoreceptors, plasma lithium, electrolytes, calcium, creatinine, phosphate and samples for renal function parameters. In total 110 ml of blood was taken. The second series of blood samples was taken about 1½ hour after voiding at home. Subjects were asked to collect some saliva for measurement of electrolytes, and subsequently were asked to drink at least 10 ml per kg body weight per hour.

Before the start of the renal function test, participants were once again asked to void.

An aliquot of 2 ml of this urine portion was frozen to determine baseline levels for renal function parameters, and the remainder was saved and added to the urine that was collected after the subject had voided at home. A second intravenous cannula was inserted in the right arm. Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were determined by from measurements of the clearance of para-aminohippuric acid (PAH) and polyfructosan S ('Inutest', Boehringer) (INU), using a constant infusion technique and timed urine collections.[4] After a bolus injection in the right arm, a continuous infusion was given for 2½ hours to achieve extracellular-fluid (ECF) and blood levels of 200 mg/l INU and 20 mg/l PAH. During the 2½ hours the participants were not allowed to eat and they were urged to drink as described before. To determine the starting dose of INU and PAH, ECF volume was estimated to be 20% of body weight. Doses for the subsequent infusion of INU were calculated from the clearance of INU based on the formula of Cockcroft which estimates the creatinine clearance from age, body weight, gender and serum creatinine.[5] To determine the infusion doses of PAH the clearance of PAH was taken as 5 times the calculated creatinine clearance. The amount of INU and PAH excreted by the kidneys was supposed to be in equilibrium with the amount infused after 1½ hour. Urine samples were collected by active voiding again after 1½ and 2½ hours of the infusion. Blood samples were collected from the left arm just after voiding. After an aliquot of 4 ml of the urine collected after 1½ hour was frozen for measurement of PAH and INU, the remainder was added to the portion sampled since the subject had voided at home, about three hours before. The total volume and exact duration of this approximate 3-hour clearance period were noted. Together with the blood sample taken during the second series of blood sampling in the middle of the 3-hour period, clearances and fractional excretion of lithium, sodium, potassium, calcium and phosphate could be calculated. Clearances of INU and PAH were calculated both from the rate of intravenous infusion and from the rate of urinary excretion for a one hour period between 1½ and 2½ hours after start of the infusion.

During the renal function test an echocardiogram was made. Before echocardiography the subject was positioned supine on the left side and connected to a paramagnetic O_2 analyzer and an infrared CO_2 analyzer (Oxycon-4, Mijnhardt, Bunnik, The Netherlands) by tube and mouthpiece, with nose clamped, to measure O_2 use and CO_2 production during echocardiography. At least five minutes were allowed to accommodate while the Oxycon-4 measured and registered mean gas exchange during each 30 seconds. After five minutes and with a respiratory quotient at or below one, a 4-chamber echocardiogram was made. 2D-

echocardiography was performed (Toshiba Sonolayer SSH-60A, Woerden, The Netherlands) using a 3.75 mHz transducer to estimate cardiac output. Cardiac dimensions were registered using the para-sternal view with M-mode echocardiography. In case the participant was asthmatic, had a severe cold or was otherwise not able to breath normally, the tube was disconnected and the echocardiogram was made without measurement of oxygen consumption.

After echocardiography and during renal function measurement, an extensive dietary assessment was made by a supervised dietician in training to register dietary intake in the preceding month using a dietary history recall with a cross check list to estimate daily intake of various nutrients (carbohydrates, protein, saturated and unsaturated fatty acids, calcium, phosphate, fibre, alcohol). Before the one-month recall a short 24-hour dietary history recall was recorded by the participant to get used to the questions, and for the dietician to assess the eating habits. With younger children, the dietician started interviewing the child but frequently had to turn to the mother also. If necessary, preparation of food was checked with the subject in the household that attended cooking, i.e. either a parent or partner of the participant. Estimates of the amount of food and beverages used were facilitated by presence of numerous numbered cups, glasses and spoons, of which the dietician knew the volume. As use of butter on bread may account for a large part of fat intake in persons eating many slices of bread each day, as in youngsters, participants were asked to butter a piece of bread that was weighted before and after buttering, without notice of the participant, to estimate fat intake per slice of bread.

On a day of their own choice, participants were invited to carry an ambulatory blood pressure measurement device (Spacelabs 90202 monitor, Redmond, Washington, USA) during a 24-hour period for measurement of blood pressure and heart rate. The ambulatory blood pressure monitor was applied to the non-dominant arm to take measurements at a frequency of one reading per hour between midnight and 6.00 a.m., three times per hour between 6.00 a.m. and 6.00 p.m., and twice per hour between 6.00 p.m. and midnight. The results of the readings were not disclosed to the participant. During this day the participant was asked to register physical activities for each hour on a scale from 0 (bedrest, sleeping) to 1 (sitting, reading, watching television, eating), 2 (walking at normal pace, bicycling quietly) and 3 (sports activities, running, bicycling fast).

On a separate occasion a subgroup of male offspring aged 18 years and older of two hypertensive parents and of two normotensive parents was invited to participate in echographic

Table 3 Summary: measurements in offspring during the second phase.

24-hour Ambulatory blood pressure	
Anthropometry	
Saliva:	Sodium and potassium
Oxygen use and carbondioxyde production at rest during echocardiography	
Dietary questionnaire	
Cellular characteristics:	
-	Sodium-potassium ATP-ase activity in erythrocyte membrane
-	Intralymphocytic calcium and magnesium
-	Lymphocytic $ß_2$-adrenoreceptors
Plasma:	Plasma circulating inhibitor
	Insulin
	Somatomedin-C
	1,25-dihydroxy Vitamin D_3
	[1-84]-parathyroid hormone
	Catecholamines (adrenaline, noradrenaline, dopamine)
	Renin (APRC and PRA)
	Aldosterone
	Angiotensine-II
	Sodium, potassium, calcium, creatinine, phosphate, uric acid.
	Samples for renal function (Inuline, para-amminohippuric acid)
	Samples for lithium clearance (Lithium, creatinine)
Urine:	Samples for renal function (Inuline, para-amminohippuric acid)
	Samples for lithium clearance (Lithium, creatinine, uric acid)
	24-hour urinary excretion of sodium, potassium, calcium
Echography:	cardiac dimensions, stroke volume
	carotid artery, with doppler measurement (flow velocity profile in subgroup)

and doppler evaluation of the common carotid artery. These measurements were performed in Maastricht, at the department of Physiology and Biophysics, University of Limburg (Head Prof. Dr. R.S. Reneman). Before and during carotid artery measurements, subjects were in supine position and cuff blood pressure measurements were taken on both the right and left brachial artery with an automatic device (Dinamap), eight times after 15 minutes rest. Relative changes in diameter of the common carotid artery during the cardiac cycle were recorded on line with a high resolution multi-gate pulsed Doppler-system, together with recording of velocity profiles. From the width of the profile absolute internal diameter was determined.[6]

Third phase

In the period between September 1989 and February 1990 the offspring groups were invited for the third time. The offspring of one hypertensive and one normotensive parent came once in the morning for anthropometric and blood pressure measurements. The other groups of offspring visited the research centre twice. One visit for anthropometric and blood pressure measurements followed by mental and physical stress tests in a 2½ hour period in the morning. The protocol and methods used for the mental and physical stress tests are described elsewhere.[7] During a second visit urinary calcium excretion and related parameters were measured in response to an oral calcium load, described hereafter.

The day before the second visit to the research centre, participants refrained from smoking and coffee use, and collected a 24-hour urine sample for measurements of electrolyte excretion. The 24-hour sampling period had to end the evening before coming to the research centre. Participants refrained from food and beverages starting from midnight, except water. On the day of the visit to the research centre, participants were instructed to awake at 06.00 am to void. This portion did not have to be sampled. However, urine voided afterwards was sampled in a separate container until arrival at the centre at 07.00 a.m. At 07.00 am, after insertion of an intravenous canula, blood was sampled for determination of plasma levels of sodium, potassium, calcium, parathyroid hormone, creatinine, phosphate and erythrocyte calcium membrane binding. Subsequently participants stayed in the research centre and were instructed to drink distilled water at least 50 ml/kg.hour. At 8.00 h urine was collected by active voiding and sampled for the two-hour period between 06.00 and 08.00 a.m. After voiding, subjects took a powder dissolved in distilled water. The powder contained 1000 mgr elementary Calcium (6,81 gr calciumlactogluconate and 0,3 g calciumcarbonate with 20 mgr aspartate in two sachets Calcium Sandoz forte). This formula does not contain sodium. The first sampling period of two hours after calcium intake, ended with active voiding at 10.00 a.m., with a blood sample at 09.00 a.m. for measurements of electrolytes and parathyroid hormone. A second sampling period after calcium intake between 10.00 and 12.00 am followed. The volume of the urine voided during each sampling period was noted, and an aliquot of each portion was frozen for determination of electrolytes. The exact time of urine collection and blood sampling was noted to calculate urinary clearances. During the five hour stay in the research centre, subjects remained mobile and could watch video-films or read magazines.

Table 4 Summary: measurements in offspring during the third phase.

Systolic and diastolic blood pressure
Anthropometry
Stress reactivity tests: mental and physical[7]
24 hour urinary sodium, potassium, calcium and phosphate excretion
Erythrocyte membrane calcium binding
Calcium clearance before and after an oral calcium-load (1 gr) in three two-hour periods: - plasma calcium, creatinine, phosphate - urinary calcium, creatinine, phosphate - plasma [1-84]parathyroid hormone
Catecholamine excretion in urine at rest, and during mental and physical stress test

3.4.2. MEASUREMENTS

Blood pressure was measured with a random zero sphygmomanometer in a strictly standardized protocol without exception. Two readings were taken in time separated by a count of the pulse. The mean of the two readings was used in all analyses. Several cuffs were used for the different groups of participants. A cuff comfortable encircling the arm over a length of two thirds the length of the upper arm was chosen. In general, children aged 5 - 9 years were measured with a 23 x 10 cm^2 cuff. For children aged 10 years and over a cuff of 23 x 14 cm^2 was used, and for some rather obese adolescents and young adults a large-adult cuff of 30 x 14 cm^2 was used. The first, fourth and fifth Korotkoff sounds were noted. For diastolic blood pressure, the fifth Korotkoff sounds were used in the analysis. Two experienced paramedical assistants measured the parents during re-examination, and one other trained assistant measured the offspring.

Cardiac output was calculated as the mean from six 4-chamber echocardiograms by area-length method.[8] Cardiac index (CI) was estimated from cardiac output and calculated body surface area. Mean arterial pressure (MAP) was calculated from SBP and DBP measured in supine position, and was used to calculate total peripheral vascular resistance (TPR) by dividing MAP by CI. From ten consecutive oxycon registrations during five minutes in the middle of the echocardiography, oxygen consumption per minute was calculated. The arterio-venous

oxygen difference was estimated by dividing oxygen consumption by cardiac output.

Left ventricular mass was estimated from left ventricular end-diastolic diameter (LVED), intraventricular septum (IVS) and left ventricular posterior wall (LVPW) and indexed for body surface area.[8]

Ambulatory blood pressure and heart rate. To obtain the average 24-hour ambulatory SBP, DBP and heart rate, measurements of each of these parameters were calculated by period of the day (midnight - 6.00 a.m., 6.00 a.m. - 6.00 p.m., 6.00 p.m. - midnight) if at least 75% of the measurements per period were successful. Mean 24-hour ambulatory SBP, DBP and heart rate were averaged from these three periods, weighted for number of hours per period. As a measure of 24-hour variability the mean 24-hour SBP, DBP and heart rate were divided by their standard deviation. Categories of mean activity scores per hour were used to calculate blood pressure by activity level.

Carotid artery vessel wall characteristics were evaluated at both left and right side, and results of the measurements were averaged. A combined technique of ultrasound and doppler registrations were used to measure velocity profiles at the common carotid artery.[9] Brachial pulse pressure was calculated from systolic and diastolic blood pressure measured eight times on each arm, and averaged for left and right arm. Formulas of calculations used are given in table 1. Carotid artery diameter changes during the cardiac cycle were expressed relative to pulse pressure. The distensibility of the common carotid artery, the change in diameter per unit of pressure, was calculated according to formula 1. The cross sectional compliance, the change in cross sectional area per unit of pressure, was calculated according to formula 2.

Table 5 Calculation of carotid artery vessel wall characteristics.

Formula 1	$DC = ((\delta D/D) / PP) * 15.037.$
Formula 2	$CC = ((\delta D/D) / PP) * D^2 * 118.1.$

D = diameter in mm
δD/D = change in diameter in %
DC = distensibility coefficient in 10^{-3}/kPa
CC = cross sectional compliance in $10^{-7}m^2$/kPa

Renal function INU and PAH were measured in the infusate, in the blood samples drawn before and after 1½ and 2½ hour of the infusion and in the urine samples collected before and between 1½ and 2½ hour of the infusion. INU was measured indirectly; after

deproteinisation with 0.6N $HClO_4$, endogenous fructose and glucose were measured (A_1). After a subsequent incubation period of 15 minutes at 70° C, INU was converted into fructose and measured (A_2). The difference between A_2 and A_1 is the amount of fructose originating from the acid hydrolysis of INU. For determination of fructose levels an enzymatic method (Boehringer Mannheim 716260) adapted to a Cobas Bio (Hoffman la Roche, Basel) was used, in which the conversion of NADP to NADPH is measured.

PAH was measured by the method of Bratton and Marshall modified by Smith, after hydrolysation of conjugated PAH by heating the sample with 4N HCl during 1 hour in a boiling waterbath.[10] In this way the rate of intravenous infusion may also be used to calculate ERPF from total PAH clearance.[4]

GFR or ERPF were calculated from the rate of intravenous infusion, if blood levels of INU or PAH for individual blood samples were within 5% of the mean INU or PAH level, respectively. A calculation from the rate of urinary excretion of INU and PAH was performed if adequate urine samples were obtained at the three sample times.

Renal blood flow (RBF) was estimated from the ERPF divided by (1 minus the haematocrit). There are no reasons to suspect differences in PAH extraction between the groups, and no adjustment was made for incomplete extraction of PAH.[11] The filtration fraction (FF) was calculated by division of the GFR by the ERPF. All calculations were standardized for body surface area, according to Dubois-Dubois.[12] Renal vascular resistance (RVR) was estimated by dividing the calculated mean arterial pressure by the RBF.

Serum electrolytes sodium, potassium, phosphate, uric acid and total calcium were measured by standard laboratory methods by an autoanalyzer (SMAIII). Serum Magnesium was measured by ionselective flame-photometry.

Urinary electrolytes as sodium and potassium in 24 hour and three (phase 2) and two (phase 3) hour urine portions were measured by flame-photometry. Creatinine, calcium, uric acid and phosphate in urine were measured by autoanalyzer (Kone specific).

Fractional excretion of electrolytes was calculated from the concentration in the three-hour (phase 2) or two-hour (phase 3) fasting urine portion (U_x), with the concentration of plasma creatinine (P_{Cr}) sampled after 1½ (phase 2) or 1 (phase 3) hour, the urinary concentration of creatinine (U_{Cr}) and the plasma concentration of the electrolyte (P_x) according to the following formula: $((U_x * P_{Cr}) / (U_{Cr} * P_x))$. Lithium in serum and urine was measured by atomic photometric absorption.

Urinary calcium excretion in a 2-hour urine portion (phase 3) was calculated from urinary calcium concentration times the volume voided recalculated for an exact two-hour period.

Plasma intact parathyroid hormone ([1-84]PTH) was determined by a two step immunochemical method,[13,14] measuring the intact 1-84 molecule, without metabolized fragments.

1,25-dihydroxyvitamin D_3 was measured by radioimmunoassay (Incstar Corp., Stillwater, MN).

Circulating immune complexes were measured by C1Q binding assay and expressed in µg equivalents of aggregated IgG. The F, S and FS haplotype of C3 protein polymorphism were measured in EDTA-plasma by agarose-electrophorese.

Adrenoreceptors Of 20 ml of blood containing EDTA, platelets were isolated from platelet-rich plasma, immediately frozen and stored at -80°C. Platelet α_2-adrenoreceptors were measured by Scatchard analysis of ^{3}H-Yohimbine binding on cell membranes.[15] To measure lymphocyte $ß_2$-adrenoreceptors, lymphocytes were isolated with lymphopreb from 20 ml of heparinized blood, and washed with 0.9 % NaCl. To keep the cells intact, the pellet was resuspended in 2 ml 20% FCS/RPMI, and with the tube on ice, 2 ml of 20% DMSO in FCS/RPMI was added. This pellet was gradually frozen; first in isolation boxes 24 hours at -20°C, then without isolation boxes, followed by freezing the pellet in isolation boxes at -80° by 24 hours, and finally without boxes at -80° C. The pellet was kept frozen until binding assay of ^{125}I-cyanopindolol to lymphocytic membranes, after counting cell numbers.[16]

Catecholamines Plasma from 10 ml of immediately chilled, heparinized blood, containing 12 mg reduced glutathione, was frozen and kept at -80°C for analysis of catecholamines using high-performance liquid chromatography with electrochemical detection.[17] An aliquot of 100 ml of the timed 24-hour urine sample was acidified and frozen for analysis of sodium and catecholamine excretion.

Renin 6 ml blood was sampled in a tube containing di-sodium-EDTA, 5mmol/l, processed at room temperature, and frozen at -20°C, until assayed. Plasma renin activity (PRA) was measured by radioimmunoassay of angiotensin-I generated from endogenous renin substrate. Renin concentration was measured by its capacity to generate angiotensin-I from saturating amounts of purified sheep renin substrate. Angiotensin-I was measured by radioimmunoassay. Prorenin was activated for measurement by adding Sepharose-bound

trypsin. Renin and prorenin were expressed as milliunits of the MRC human kidney renin standard (MRC 68/356, WHO International Laboratory for Biological Standards, Holly Hill, Hampstead, London, United Kingdom).[18]

Immunoreactive angiotensin-II 6 ml blood was sampled in a chilled tube containing di-sodium-EDTA, final concentration 5 mmol/l, and O-phenantroline 1.25 mmol/l, and immediately centrifuged at 4°C. Plasma was frozen by -80°C until measured by radioimmunoassay.[19]

Aldosterone 6 ml blood was sampled in a chilled tube containing di-sodium-EDTA 5 mmol/l, processed immediately until plasma was frozen. Aldosterone was measured by radioimmunoassay.[20]

Atrial natriuretic peptide 10 ml of blood sampled in a chilled tube containing aprotinin-trypsin, was immediately centrifuged at 4°C, and plasma was frozen with 200 KIU/aprotinin per ml plasma at -80°C . Plasma atrial natriuretic peptide was measured with radio-immunoassay after extraction.[21]

Intra-erythrocytic sodium and potassium Erythrocytes were isolated from blood in tubes containing 5 mmol/l di-sodium-EDTA, and washed three times with ice-cold 140 mM choline chloride. Isolated cells were kept at 4°C until lysed with Aqua-dest and Triton X100. Subsequently, sodium and potassium concentration in the lysate were measured by flame-photometry. Haemoglobin levels were measured both in cell suspension and in the lysate and the haematocrit in cell suspension, to express obtained sodium and potassium lysate concentration in mmol/l cells.

Glucose was measured in fasting serum by standard laboratory methods by autoanalyzer (SMAIII).

HLA-typing Of parents and children of families with two or more participating children, 10 ml heparinized blood was sampled and kept at room temperature. Within 24 hours mononuclear cells were isolated and kept until HLA-A, HLA-B and HLA-Dr typing using leuco-agglutination and a lymphocytotoxicity technique.[22]

Sodium-potassium ATP-ase activity and circulating inhibitor 20 ml blood was sampled in a chilled tube containing sodium-heparin and centrifuged for 10 minutes at 2000g at 4° C. The supernatant was discarded, and kept in a tube under gaseous nitrogen, immediately frozen at -180° C until assayed for circulating inhibitor. As a measure for the ouabain-like inhibitor, plasma total free fatty acids and fatty acids composition were measured by extraction with thin-

layer chromatography and gas-liquid-chromatography respectively.[23] After removal of the buffycoat from the erythrocyte pellet, cells were washed three times with ice-cold 0.3 M sucrose, 2mM EDTA buffer (pH 7.4) and subsequently suspended in buffer and frozen by -180°C. For isolation of the erythrocyte membrane, cells were lysed with 35 ml 20 mM Tris-HCl, 2mM EDTA (pH 7.4), centrifuged and washed three times with Tris-EDTA. A fluffy-pink sediment was suspended in a small volume of water and freeze dried, and stored at -20°C until assay. Na-K ATPase activity was calculated from the difference between Mg-ATPase activity and Na-K ouabain insensitive ATPase activity, measured by spectrophotometry at 700 nm absorbency, as described.[24] Activity was expressed in µmole of phosphate released per mg of protein per hour (i.e. µmole Pi/hr.mg protein). Protein in the membrane preparations was measured by means of the method of Lowry.[25]

Somatomedin C was measured by slight modifications of the non-equilibrium method,[26] as described,[27] in 2 ml of di-sodium-EDTA plasma, sampled and processed at 4° C, and frozen at -20° C until assayed.

Intra-lymphocytic calcium and magnesium 10 ml of RPMI was added to 15 ml of heparinized blood at room temperature, carefully mixed and brought on 12 ml of Lymphopreb. After 30-40 minutes of centrifugation at 400 g at room temperature, the lymphocyte ring could be isolated and was subsequently washed several times with RPMI. Eventually the pellet was resuspended in excess ice-cold 140 mM choline chloride, centrifuged at 160 g, resuspended in a small volume choline chloride and transferred to a preweighed polytene tube. This tube was only handled by a pair of tweezers and transferred in a larger tube for final centrifugation during 10 min. at 400 g. Supernatant was removed and the tube was dried and reweighed. Ash was suspended in 0.1mHCl and $LiCl_2$ (2g/l) to leach out calcium and magnesium, measured by atomic absorption photometry. Electrolytes were expressed in mmol per gram of dry weight.

Erythrocyte membrane calcium binding 10 ml of lithium-heparinized blood was directly centrifuged by 2000 g at 4°C for 10 minutes. The sediment was washed with a phosphate buffered (pH7.4), 0.9% NaCl solution and again centrifuged by 2000 g at 4°C for 10 minutes. The pellet was divided in 8 portions and washed with a sodium-phosphate buffer at pH 8.0 to haemolyse the cells, and centrifuged 10 minutes at 22000 g at 4° C. This washing procedure was repeated 2 to 3 times until the membrane pellet was white. After resuspension, the pellet was frozen to -20°C and transported within a month to Leicester UK for determination of calcium binding, by atomic absorption spectrophotometry.[28]

3.4.3 DATA ANALYSIS

Descriptive data for the three groups of parents and for the three groups of offspring are presented as means and standard deviations. For comparison between groups, means and standard errors are given with the difference and 95% confidence interval of the difference between groups, using a t-test for unpaired observations.

Adjustments for differences in age, height, body weight and proportion of males between the groups were made using a model for multiple linear regression. Differences in blood pressure were treated in a similar way. To examine confounding by blood pressure differences that appeared to exist between the groups, adjustments were made for age, height, body weight, proportion of males and blood pressure using a multiple linear regression model. However, one must be aware that those with the highest blood pressure at a young age, are likely to be at highest risk to develop hypertension (chapter 2, figure 2). In the analysis with blood pressure as an independent variable, the true difference between the groups of offspring in the parameter of interest (dependent variable), might be obscured as the blood pressure difference is evened out.

Trend analysis of the mean of a parameter across the three groups of offspring, was performed with a model for linear regression, after checking for nonlinear trends and weighted for possible differences in variance between groups. Adjustments were made for differences in age, height, weight and proportion of males.

Associations between study variables were studied across groups, adjusted for group characteristics (using indicator variables), and age, height, weight, and gender by multiple regression analysis.

3.5 GENERAL CHARACTERISTICS OF THE STUDY POPULATION

As indicated above, 121 families were found eligible for participation to the Dutch Hypertension and Offspring Study; 51 families with two hypertensive parents, having 111 children aged 5 - 30 years of age, 35 families with one hypertensive parent and one normotensive parent (98 children), and 35 families with two normotensive parents (82 children). In table 6 the general characteristics of the three groups of selected parents are given.

During the first phase of the study 180 (62%) offspring of the 291 eligible subjects participated. These 180 children came from 97 (80%) of the 121 families; 69 (62% of 111) children from 38 (75% of 51) families with two hypertensive parents, 58 (59% of 98) children from 30 (86% of 35) families with one hypertensive and one normotensive parent, and 53 (65%

Table 6 Blood pressure and related characteristics of the parental couples from the 121 eligible families of the Dutch Hypertension and Offspring Study.*

	couples of two normotensive parents	couples of one hypertensive and one normotensive parent	couples of two hypertensive parents
Number of couples	35	35	51
Age (years)	46.5 ± 9.2	51.1 ± 10.0	51.8 ± 9.0
Height (cm)	168.5 ± 8.3	169.1 ± 8.9	169.6 ± 8.8
Body weight (kg)	67.3 ± 12.3	70.9 ± 12.0	77.4 ± 11.9
SBP+ sitting (mmHg)	113.3 ± 9.7	132.6 ± 23.8	152.9 ± 19.9
DBP+ sitting (mmHg)	68.8 ± 6.6	80.1 ± 14.6	90.1 ± 9.5
SBP§ sitting (mmHg)	116.5 ± 8.2	132.9 ± 22.3	150.3 ± 19.1
DBP§ sitting (mmHg)	70.3 ± 6.2	80.4 ± 13.8	88.6 ± 9.0

* Values are means ± SD.
\+ SBP=systolic blood pressure, DBP=diastolic blood pressure
§ Adjusted for differences between the groups in age and body weight

of 82) children from 29 (83% of 35) families with two normotensive parents.

The general characteristics, measured during the selection procedure, of the 97 parental couples from families that participated, and the general characteristics of the 180 participating children during phase 1, are given in table 7.

The 97 families participating in the Dutch Hypertension and Offspring Study represent only a very small part of the original cohort of families (4.5%). It can be seen in table 7 that a high contrast in blood pressure between the participating parents was achieved. These differences were not affected by correction for differences in age between the groups of parents. The age difference between the groups of parents is reflected in their offspring. The difference in age between the offspring might influence the difference in weight and height between the groups of offspring. Moreover, a blood pressure difference between the groups of offspring was observed, with a similar pattern but of less magnitude compared to their parents. This difference in blood pressure between the groups of offspring was only slightly diminished after adjustments for differences in age, height, body weight and proportion of males were made.

Table 7 Blood pressure and related characteristics of the 97 parental couples and 180 of their children participating in the Dutch Hypertension and Offspring Study.*

	families with two normotensive parents	families with one hypertensive parent	families with two hypertensive parents
Parents			
Number of couples	29	30	38
Age (years)	46.6 ± 8.8	50.9 ± 8.9	52.1 ± 8.3
Height (cm)	168.8 ± 4.6	169.6 ± 4.4	169.6 ± 4.4
Body weight (kg)	68.2 ± 9.1	71.2 ± 6.8	77.3 ± 8.2
SBP+ sitting (mmHg)	113.3 ± 63	133.0 ± 11.4	151.8 ± 14.1
DBP+ sitting (mmHg	68.5 ± 5.4	80.6 ± 6.9	89.8 ± 6.4
SBP§ sitting (mmHg)	114.5 ± 12.0	133.0 ± 11.4	150.9 ± 12.1
DBP§ sitting (mmHg)	69.0 ± 6.7	80.7 ± 6.4	89.5 ± 6.8
Offspring			
Males/females (number)	34 / 19	29 / 29	40 / 29
Age (years)	18.7 ± 7.3	20.4 ± 6.4	22.1 ± 6.4
Height (cm)	166.0 ± 21.8	169.8 ± 15.9	172.7 ± 14.9
Body weight (kg)	57.7 ± 20.6	60.0 ± 14.9	66.1 ± 15.1
SBP+ sitting (mmHg)	116.1 ± 11.7	123.6 ± 13.1	127.2 ± 11.4
DBP+ sitting (mmHg)	70.3 ± 8.9	74.9 ± 9.8	78.9 ± 8.1
SBP# sitting (mmHg)	117.9 ± 9.2	124.2 ± 9.1	125.3 ± 9.2
DBP# sitting (mmHg)	70.3 ± 8.8	74.9 ± 8.8	78.4 ± 8.9

* Values are means ± SD.
\+ SBP=systolic blood pressure, DBP=diastolic blood pressure
§ Adjusted for differences between the groups in age and body weight.
\# Adjusted for differences between the groups in age, height, body weight and proportion of males.

3.6 DISCUSSION

The selection procedure chosen for the Dutch Hypertension and Offspring Study was based on population based blood pressure distributions using percentile cut-off values at the lower and upper quartile. This has distinctive advantages above

- family history based on questionnaire (recall bias and misclassification)
- parental hypertension and normotension defined according to fixed cut off points (not quantitative, see chapter 2).

In contrast with a selection based on either family history or fixed cut off points, the selection procedure of the Dutch Hypertension and Offspring Study used

- measurements of blood pressure from a population based survey
- measurements in both father and mother
- cut-off levels to define both relatively high and relatively low blood pressure levels, with using age and gender specific percentiles, in stead of absolute high blood pressure levels compared to normal blood pressure levels
- compliance of both systolic and diastolic blood pressure to the selection criteria
- compliance to the selection criteria twice in a 10 year period.

In this way a sufficient contrast in blood pressure between the groups of parents could be expected, depending on the choice of the percentile used as a cut-off point.

The choice for the percentile cut-off values at the upper and lower quartile, but not more to the extremes of the distribution was pragmatic. It was based on the numbers of parents and children selected at different cut-off levels from the population based survey in 1975-1978 (table 8). The number of children was supposed to diminish with 50 to 70 %, due to remeasurements of the parents in 1986, and due to non-compliance of families and children. Moreover, the contrast found in blood pressure between children of the parents selected at different cut-off levels was taken into account (figure 3). Also, numerical estimates of percentiles become less precise at the extremes of the sample distribution, in particular if one-year-age- and gender-specific distributions are used (i.e. 10,500 measurements divided equally across 140 one-year-age (from 5 to 75 years of age) and gender specific distributions, give 75 numerical values per distribution for 100 percentile estimates). Moving from the tail to the median of the distribution, more accurate numerical estimates of a percentile may be made.

The use of the upper and lower quartile as cut-off level to define relatively high and low systolic and diastolic blood pressure respectively, resulted in 18 % of the parents defined as hypertensive subjects, and 17% as normotensive. Consequently, of all couples 5.8% comprised two hypertensive parents, 6.8% one hypertensive and one normotensive parent and 4.5% two normotensive parents. The second measurement over time even further declines the number of selected families (from 370 to 121). However, in this way, only families with two parents with a stable relative high or relative low blood pressure were selected, minimizing misclassification, and optimising contrast in predisposition for high blood pressure in the offspring. Due to the substantial reduction in number of selected families by this method, the centile selection criteria

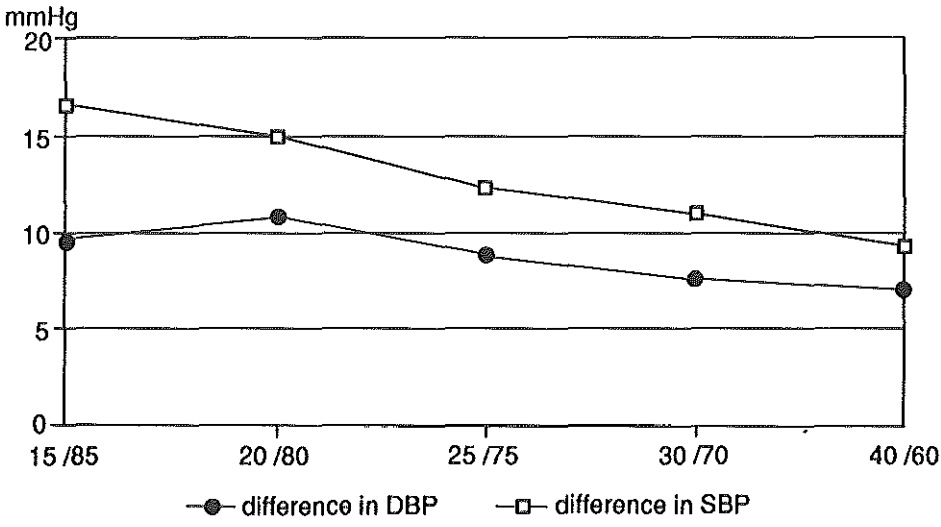
can not be too extreme.

Table 8 Number of children selected with various selection criteria in the High and Low risk families of the Dutch Hypertension and Offspring Study.

SELECTION METHOD Cut off centile for lowest and highest extreme of blood pressure distribution; *Lowest versus Highest extreme (Px versus P100-x)*		NUMBER OF CHILDREN SELECTED in high and low risk families *High (n)*	 *Low (n)*
P15*	P85 + med**	135	38
P20	P80 + med	176	56
P25	P75 + med	232	150
P30	P70 + med	314	196
P40	P60 + med	517	365

* P = percentile cut off at lowest and highest extreme for selection of the parents.
** Med = parent possible selected on bases of antihypertensive medication.

Figure 3 Selection of the parents with different blood pressure percentile cut off levels to define hypertensive and normotensive parents in the original EPOZ[29] in 1975-78; the difference in systolic blood pressure (SBP = square) and diastolic blood pressure (DBP = point) is given for the offspring of hypertensive parents compared to offspring of normotensive parents for the various selection methods, described at the X-axis; P = percentile at low P(100-X) and high P(X) cut-off level.



The results of the selection suggest that blood pressure at the extremes aggregates in parental couples; eligible families were found more frequently than expected based on individual definition of both hypertensive and normotensive parents alone (table 9).

Table 9 Prevalence of hypertension and normotension in the cohort of 2161 parental couples defined as a systolic and diastolic blood pressure in the upper respectively lower quartile of the age- and gender specific blood pressure distribution during the first survey (1975 - 1980).

Individual parents		Parental Couples:	Observed	Expected	O/E ratio
Hypertension	18%	Both hypertensive	5.8%	3.2%	1.8
		One hypertensive	6.8%	3.1%	2.2
Normotension	17%	Both normotensive	4.5%	2.9%	1.6

The occurrence of parental couples with a contrasting blood pressure status defined according to the selection procedure is the most prevailing and was observed twice as often as expected. However, also parental couples with similar blood pressure status for each parent were observed more often. Therefore, no conclusion can be drawn why more couples were found than expected from the individual blood pressure status. In the group with one hypertensive and one normotensive parent unassortative mating might have been of influence. In the couples of either two hypertensive or two normotensive parents, assortative mating or shared environment since the time of marriage might be considered.

One other problem appears from the results of the selection. In table 1 it can be seen that the age of the two hypertensive parents is older compared to the age of the two normotensive parents. This could have been expected, as the selection criterium for hypertension was not only based on one-year-age and gender specific percentiles, but also on use of anti-hypertensive medication. In the group of parental couples antihypertensive medication was more common among those aged 43 years and over (median age) (12%) compared to those aged below 43 years of age (3%). This higher frequency of treatment in the older parents can be explained not only by the increased incidence of hypertension according to clinical cut-off criteria in older people, but also by a lower percent treated hypertensive subjects in the younger age groups, as was described before for this cohort.[29] Therefore, young hypertensive parents more likely became selected on bases of a blood pressure percentile in the

upper quartile. On the contrary older hypertensive parents, more likely successfully treated for their hypertension, were selected based on their antihypertensive medication. This gives rise to the extra selection of older parents in the hypertensive group, that are successfully treated for hypertension.

Furthermore, as can be seen from table 1, hypertensive couples have on average a higher body weight compared to the normotensive couples, while no differences appear in height. This might have occurred due to the well known positive relation between body weight and blood pressure.

As expected from the selection method, the systolic blood pressure was 40 mmHg and the diastolic blood pressure was 21 mmHg higher in the hypertensive couple compared to the normotensive couple. The blood pressure differences became only slightly less when adjusted for differences in age and body weight. This suggest that factors other than body weight and age alone, give rise to the blood pressure difference between the selected groups of parents.

The results of the selection procedure for the groups of offspring and their parents, measured in 1987, are shown in table 7. The age difference between the parents is represented in their offspring; the offspring of the two hypertensive parents being older. The difference in height between the groups of offspring, with the offspring of hypertensive parents being taller, was not seen when adjusted for the age difference between the groups. However, the body weight difference between the offspring groups, with the offspring of hypertensive parents being more heavy, did not disappear totally when adjusted for the age difference. At a mean age of 20 years the three groups of offspring differed in both systolic and diastolic blood pressure with systolic blood pressure being 11.1 mmHg and diastolic blood pressure 8.5 mmHg higher in the offspring of two hypertensive parents compared to the offspring of two normotensive parents. These differences became 7.4 mmHg and 8.1 mmHg, respectively, when adjusted for differences in age, height, body weight and proportion of males between the groups. The blood pressure characteristics in the groups will be presented in more detail in section 4.1.

The clear blood pressure difference between the groups of offspring seem to contradict the purpose of the Dutch Hypertension and Offspring Study to study mechanisms in the early phase of primary hypertension. It is difficult to determine if differences in factors of interest present between the groups of offspring are the cause or the consequence of the differences in blood pressure that already exist.[30] However, the purpose of the design is to select groups of

youngsters with a contrasting predisposition to develop hypertension. As discussed in chapter 2, section 3, not all offspring of hypertensive parents become hypertensive, and some of the offspring of normotensive parents will become hypertensive. In order to create a reasonable chance that the proportion of the offspring developing hypertension in the future differs substantially between the types of family, a difference in blood pressure between the groups of offspring during adolescence and young adulthood is necessary and inevitable (chapter 2).[30] To select groups of youngsters at different risk for hypertension several methods might be applied, with a differential effect on the blood pressure contrast between the selected groups. Selection might be based on

- blood pressure level of the parents
- blood pressure level of the children
- a combination of blood pressure level of parents and their children (four corner method).

In chapter 2, section 3.1, it was discussed that a selection based solely on blood pressure of the children was not appropriate. Then a possible effect of the blood pressure level on the mechanisms studied cannot be accounted for as only a very small overlap will exist between the distributions of the blood pressure in the selected groups. In that case, no stratified analysis or regression techniques can be applied to even out the blood pressure difference, that can be applied if the offspring groups are selected based on parental blood pressure only (chapter 2, figure 6).

A selection with the four corner method[31] (see chapter 2.3.2) based on blood pressure of both parents and children has the disadvantage of a possible bias by the blood pressure of the offspring. The difference in risk for future hypertension between the offspring of hypertensive parents compared to offspring of normotensive parents is diminished due to large range of blood pressure in the children within one group. If determinants are compared for the groups of offspring based on their blood pressure, the contrast might be diminished by the difference in family background within each group, and the blood pressure difference might interfere with the determinant of interest. Therefore, the four corner method seems most appropriate to compare groups of offspring with a similar blood pressure level, but with a difference in parental blood pressure. However, than the problem occurs of the magnitude of the groups selected (see chapter 2.3.2).

The results of the selection of youngsters at different risk for hypertension in the Dutch Hypertension and Offspring Study based on blood pressure of their parents has some features

in advantage;

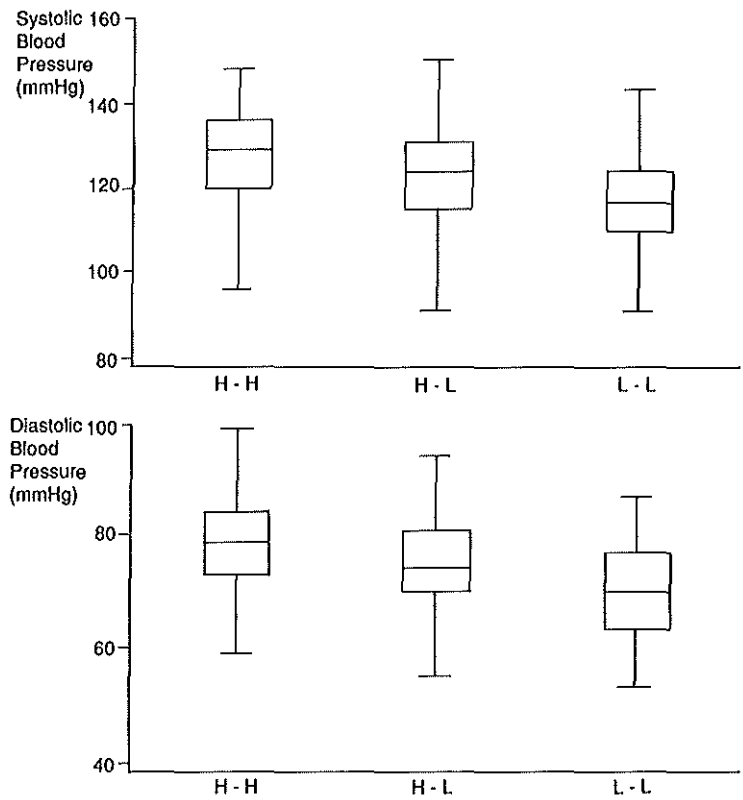
- the risk is not diminished by preferential selection of offspring with a blood pressure status opposite to their parents' blood pressure profile (table 10)
- although a difference in blood pressure between the selected groups of offspring is detected, the distribution of the blood pressure in each group shows substantial overlap, as was hypothesised in chapter 2, figure 6, and can be shown by the box plot of the distribution of blood pressure in each group of offspring, given in figure 4.

In this way the groups of offspring with contrasting risk for hypertension can be compared with and without taking into account the blood pressure difference, as outlined above (3.4.3).

Table 10 Percentage of the offspring with a relatively high, intermediate or low blood pressure from the three groups of families in the Dutch Hypertension and Offspring study.

Percentage of offspring with	offspring of two normotensive parents (n=53)	offspring of one hypertensive parent (n=58)	offspring of two hypertensive parents (n=69)
- SBP and/or DBP in upper quartile	20.8	37.9	50.7
- SBP and DBP in middle quartiles	24.5	27.6	31.9
- SBP and/or DBP in lower quartile	54.7	34.5	17.4

Figure 4 Box plots of systolic blood pressure in the upper diagram, and diastolic blood pressure in the lower diagram for each group of offspring during the first phase in 1987;
- Offspring of two hypertensive parents (H-H),
- Offspring of one hypertensive and one normotensive parent (H-L),
- Offspring of two normotensive parents (L-L).



Box plot of the blood pressure distribution:
- *middle line in square box* is the 50th centile,
- *upper an lower line of the square box* represent respectively the 75th and 25th centile,
- *highest and lowest horizontal line* represent respectively the 99th and 1th centile.

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4 DUTCH HYPERTENSION AND OFFSPRING STUDY; RESULTS AND DISCUSSION

4.1 HAEMODYNAMIC CHARACTERISTICS

Blood pressure

In section 4.1.1. results from blood pressure measurements are presented for the 121 parental couples and the 180 youngsters that participated to the survey in 1986-1987. At the time of measurement the mean age of the total group of offspring was 20.5 years of age. At that age blood pressure in offspring of two hypertensive parents, compared to offspring of two normotensive parents, was up to 11 mmHg [95% confidence interval 7 to 15] higher for systolic blood pressure (SBP) and 9 mmHg [6 to 12] for diastolic blood pressure (DBP). The differences in blood pressure remained and became only slightly smaller after adjustment for differences in age, height, body weight and proportions of males between the groups (SBP 7 mmHg [4 to 10], DBP 7 mmHg [4 to 10]). The blood pressure of the offspring of one hypertensive and one normotensive parent was intermediate for DBP, and just below SBP compared to the offspring of two hypertensive parents.

Moreover, the earliest data on 133 of the 180 offspring, those obtained in the EPOZ study between 1975 - 1978, are presented. From this first examination, at a mean age of 13 years of age, a blood pressure difference between the offspring of two hypertensive parents and the offspring of two normotensive parents was already apparent (SBP 8 mmHg [3 to 13] and DBP 10 mmHg [5 to 15]. Subsequently, at an interval of approximately 10 years between the first EPOZ measurement and the reexamination for the DHOS, no clear difference was found in the mean change in BP per year for the 133 offspring (section 4.1.1). However, if the mean change per year was compared after adjustment for differences in baseline characteristics (age, height, body weight, and BP in the 1975-1978 survey, table 1) between the groups, a more pronounced increase in SBP and DBP in mmHg per year for the offspring of two hypertensive parents compared to the offspring of two normotensive parents was found (table 2).

When the 133 offspring were analysed for blood pressure stratified for age, no differences in either SBP or DBP between the groups were present in the lowest tertile, at a mean age of 8 years (section 4.1.1). In the second and third tertile for age significant blood pressure differences at a mean age of 13.3 and 17.9 years of age respectively were found.

Using age and gender specific blood pressure distributions from the population in the 1975 - 1978 survey, blood pressure levels of the offspring in 1987 were expressed as

their age- and gender specific position in either the lower quartile for systolic and/or diastolic blood pressure, or the upper quartile. If the SBP and DBP of a subject were in the second or third quartile, the position of the blood pressure percentile of that subject was named intermediate. The distribution of the blood pressure position of the three groups of offspring in 1987 is given in table 3.

Table 1 Characteristics in 133 offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents, in the 1975 - 1978 EPOZ survey and the 1987 DHOS survey.*

	offspring of two normotensive parents	offspring of one hypertensive parent	offspring of two hypertensive parents
Males/Females (number)	21 / 14	21 / 20	31 / 26
1975-1978 EPOZ; 133 offspring			
Age (years)	12.3 ± 4.4	13.2 ± 4.0	13.5 ± 4.4
Height (cm)	150.2 ± 21.1	155.8 ± 19.3	157.3 ± 19.8
Body weight (kg)	42.9 ± 17.3	45.8 ± 16.1	48.3 ± 16.8
Systolic BP (mmHg)	108.5 ± 13.3	116.4 ± 15.2	119.4 ± 16.8
Diastolic BP (mmHg)	61.9 ± 10.8	68.3 ± 9.4	72.4 ± 10.6
1987 DHOS; 133 offspring			
Age (years)	22.2 ± 4.6	23.1 ± 4.2	23.6 ± 4.2
Height (cm)	176.4 ± 10.6	174.7 ± 10.2	176.7 ± 7.6
Body weight (kg)	67.8 ± 12.4	65.1 ± 9.6	69.5 ± 10.6
Systolic BP (mmHg)	120.9 ± 9.4	126.5 ± 12.2	129.9 ± 9.1
Diastolic BP (mmHg)	71.9 ± 9.4	76.0 ± 8.3	79.5 ± 8.3

* Values are means ± SD.

It is shown that more than 50% of the offspring of two normotensive parents have a SBP and/or DBP in the lowest quartile, while more than 50% of the offspring of two hypertensive parents have a SBP and/or DBP in the upper quartile. In the offspring of one hypertensive and one normotensive family, approximately one third of the offspring has a blood pressure in respectively the highest and lowest quartile for SBP and DBP.

Table 2 Blood pressure measurements in 133 of the offspring in the three groups, during the 1975-1978 survey, in 1987 and mean changes in blood pressure per year in this 9 to 12 year period.*

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
1975 - 1978 survey										
SBP (mmHg)**	110.4	(1.9)	116.5	(1.6)	118.2	(1.7)	6.1	[1.1, 11.1]	7.8	[2.8, 12.8]
DBP (mmHg)**	62.2	(1.8)	68.2	(1.5)	72.1	(1.3)	6.0	[1.3, 10.7]	9.7	[5.3, 14.1]
1987 survey										
SBP (mmHg)**	120.9	(1.1)	127.3	(1.7)	129.4	(1.1)	6.4	[1.9, 10.9]	8.5	[4.8, 12.2]
DBP (mmHg)**	71.8	(1.5)	76.1	(1.3)	79.4	(1.1)	4.3	[0.3, 8.3]	7.6	[3.9, 11.3]
Change per year										
SBP (mmHg/year)***	0.7	(0.15)	1.1	(0.18)	1.3	(0.12)	0.4	[-0.07, 0.87]	0.6	[0.22, 0.98]
DBP (mmHg/year)***	0.5	(0.15)	0.7	(0.12)	1.0	(0.10)	0.2	[-0.18, 0.58]	0.5	[0.14, 0.86]

* Given are means with standard errors in parentheses, and differences between means with 95% confidence interval between brackets.
** Adjusted for differences between the groups in age, height, body weight and proportion of males.
*** Adjusted for differences between the groups in age, height, body weight, proportion of males and blood pressure in 1975 - 1978 survey.

Table 3 Proportion of offspring with blood pressure levels during the 1987 survey in either the upper (≥P75) or lower (≤P25) quartile of the age and gender specific blood pressure distribution for systolic and/or diastolic blood pressure, or for neither, for the three groups of offspring.

	offspring of two normotensive parents	offspring of one hypertensive parent	offspring of two hypertensive parents
SBP and/or DBP ≥ P75	20.8%	37.9%	50.7%
SBP and DBP intermediate	24.5%	27.6%	31.9%
SBP and/or DBP ≤ P25	54.7%	34.5%	17.4%

24-Hour ambulatory blood pressure characteristics

Of 146 offspring one 24-hour blood pressure registration was obtained using a non-invasive ambulatory device. The mean 24-hour blood pressure was clearly higher in the offspring of two hypertensive parents, compared to offspring of two normotensive parents with a mean difference of 4.7 mmHg (95% confidence interval 1.8 to 7.6) for systolic and a difference of 4.8 mmHg (2.3 to 7.3) for diastolic blood pressure (section 4.1.3). The 24-hour blood pressure curve ran at a higher level for both systolic and diastolic blood pressure in the offspring of two hypertensive parents, with small differences compared to the offspring of two normotensive parents at night and more pronounced differences during the day period (section 4.1.2, table 1; section 4.1.3, figure 1). The largest differences in blood pressure levels between the groups of offspring were seen during daily physical activities (section 4.1.3, figure 3). No differences in mean 24-hour heart rate between the groups were found (section 4.1.3, table 2).

Cardiovascular characteristics

Echocardiographic examination with registration of oxygen consumption did not show a difference in cardiac index and arterio-venous oxygen difference. However, an increased left ventricular mass index (8.7 g/m^2, 95% confidence interval 2.4 to 15.0) was observed in the offspring of two hypertensive parents (section 4.1.3, table 3). The calculated total peripheral

vascular resistance was slightly, but not significantly, increased in the offspring of two hypertensive parents (3.1 mmHg/l/min/m^2, -2.0 to 8.1) compared to offspring of two normotensive parents, and showed a positive trend across the three groups (p-value for trend analysis 0.05).

Carotid artery characteristics

As described in section 3.3.2, in a subgroup of male offspring of either two hypertensive, or two normotensive parents, aged 18 years and over the common carotid artery was studied in detail. General characteristics of this subgroup, together with findings on the common carotid artery, are presented in section 4.1.4. While pulse pressure was comparable between the groups, no difference in dynamic vessel wall properties of the common carotid artery at rest were observed.

To summarize, from the results and discussion in section 4.1.1. to 4.1.4, and tables 4.1 to 4.3, it is concluded that the offspring of two hypertensive parents compared to the offspring of two normotensive parents

With regard to blood pressure

- have on average an elevated blood pressure of 7 mmHg at a mean age of 20.5 years,
- the difference in blood pressure is not yet present at 8 years of age, but becomes apparent at an age of 13 years of age,
- the increase in blood pressure per year is more pronounced when adjusted for baseline characteristics,
- more than half of the offspring has a SBP and/or DBP in the extreme quartiles of the age and gender specific blood pressure distribution in the same relative category as their parents,
- 20% or less of the offspring have a SBP and/or DBP in the extreme quartiles of the age and gender specific blood pressure distribution opposite to the blood pressure quartile of their parents,
- show ambulatory blood pressure with a similar circadian pattern but at a higher level during 24 hour, with the most marked increase during day-time and during physical activities,

With regard to myocardial and vascular characteristics

- have a similar stroke volume, cardiac output or index at rest with similar oxygen consumption at rest, i.e. no signs of a hyperkinetic circulation,
- have slightly increased cardiac dimensions and a clearly increased left ventricular mass and mass index,
- show an elevated (calculated) total peripheral resistance,
- do not show differences in vascular properties like arterial diameter, wall stiffness and cross sectional compliance of large arteries as assessed in the common carotid artery.

The parameters studied in the offspring of one hypertensive and one normotensive parent resemble either to the parameters in the offspring of two hypertensive parents, or take an intermediate position.

4.1.1 CHANGE IN BLOOD PRESSURE IN OFFSPRING OF PARENTS WITH HIGH OR LOW BLOOD PRESSURE: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

A family history of hypertension is an important predictor of high blood pressure. We investigated the question of whether this predisposition affects the level and change of blood pressure early in life, and in particular, at what age the familial aggregation of blood pressure occurs. Blood pressure and related characteristics were studied in children whose parents both had relatively high blood pressure ('high') and the results were compared with those from children whose parents both had a relatively low blood pressure ('low') and with children with one parent high and the other parent low ('mixed'). At the age of 8 years there were no clear differences in pressure but at the age of 20 years there was a difference of 7 mmHg for both systolic and diastolic pressure between the high- and low-risk offspring. These findings suggest that the magnitude of familial aggregation of blood pressure increases during childhood and adolescence.

Introduction

It is generally agreed that the level of blood pressure has strong genetic determinants. This view stems largely from family studies showing an increased risk of hypertension among relatives of hypertensive subjects.[1,2] Although the shared environment may be a non-genetic determinant of the familial aggregation of hypertension, the correlation of blood pressure is greater between monozygotic twins than between dizygotic twins, and greater between natural siblings than between natural and adopted siblings or between adopted siblings.[3-6] We investigated blood pressure levels and related characteristics in the offspring of three groups of parents with high or low blood pressure in order to determine the age at which the difference in parental blood pressure expresses itself in the children and how this expression is influenced by other determinants of blood pressure. We studied 180 offspring of parental couples with both low or mixed blood pressure levels, selected from 6864 Dutch subjects in 1642 families.

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* Journal of Hypertension 1988;6(Suppl 4):S594-6.

Methods

Population

Between 1975 and 1979, all residents aged 5 years and over in two districts of the Dutch town of Zoetermeer were asked to participate in a study of cardiovascular risk indicators.[7,8] Blood pressure was measured in 1642 families, comprising 6864 subjects. The subjects were selected from these families according to the parents' blood pressures.[9] If both parents had a systolic (SBP) and diastolic blood pressure (DBP) in the upper quartile of the distribution or were on antihypertensive medication, the family was considered a 'high' family. If SBP and DBP for both parents were in the lowest quartile and neither parent was taking antihypertensive medication, the family was considered a 'low' family. A 'mixed' family comprised one parent with SBP and DBP in the upper quartile or on antihypertensive medication and one parent with SBP and DBP in the lower quartile and not on antihypertensive medication. In this way 370 families were selected, of whom 125 were high, 148 mixed and 97 low. In 1986, 58 couples were not eligible for remeasurement as described elsewhere.[9] Out of the eligible 312 couples, 250 parental couples (80%) were remeasured. Of these, 121 couples (48%) were still in the same category (high 51, mixed 35, low 35), and therefore these couples met the criteria twice in a 10-year period. Of their offspring aged 5-30 years, 180 were evaluated in this study (69 high, 58 mixed and 53 low). Out of these 180, 133 children were examined during the initial family survey of 1975-1979; the remaining 47 offspring were either not born or were too young to be examined in the initial study.

Measurements

Blood pressure was measured in a standardized protocol described elsewhere,[7,8] using a random zero sphygmomanometer.[10] The mean of two readings was used in the analysis. Diastolic blood pressure was based on Korotkoff phase V. Body weight and height were measured with the subject wearing indoor clothes without shoes. In the children timed 24-hour urine samples were collected in order to measure 24-h sodium and potassium excretion.

Data analysis

The characteristics of the 121 parent couples and their 180 offspring were compared for the three groups giving means and s.e. Adjustments for the observed differences between the groups were made using a multiple linear regression model. We also compared the means in the study groups for the 133 offspring who were examined in the 1975-1979 initial survey. With these 133 children, the mean yearly increase in blood pressure was calculated for each study group.

Results

Table 1 shows blood pressure and related characteristics for the 121 parent couples and 180 of their offspring in the three study groups in the 1986-1987 examination. Similar differences in blood pressure were found for the three groups when the analysis was restricted to the 133 offspring who were examined in 1975-1979 at a mean age of 13 years [SBP adjusted for age, height, body weight and gender: high 118 mmHg (s.e. 1.7 mmHg), mixed 117 mmHg (1.7), low 110 mmHg (1.9); DBP adjusted: high 72 mmHg (1.3), mixed 68 mmHg (1.5), low 62 mmHg (1.8)]. In 44 out of these 133 offspring at a mean age of 8 years (lowest tertile for age), there were no significant differences in blood pressure among the three groups (SBP adjusted for height, body weight and gender: high 105 mmHg (3.3), mixed 103 mmHg (2.7), low 103 mmHg (3.1); DBP adjusted: high 69 mmHg (2.3), mixed 69 mmHg (1.8), low 62 mmHg (2.8)]. The yearly increase in blood pressure between 1975 and 1986 for the 133 offspring did not differ significantly among the three groups [SBP: high 1.03 mmHg/year (0.24), mixed 1.06 mmHg/year (0.26), low 1.24 mmHg/year (0.20); DBP: high 0.71 mmHg/year (0.14), mixed 0.75 mmHg/year (0.16), low 0.98 mmHg/year (0.20)].

Discussion

As a result of the selection procedures there were large differences in blood pressure between the three groups of parents. Differences in SBP and DBP in the offspring were independent of differences in age, height, body weight and gender. No difference in urinary sodium excretion was found and therefore we consider it unlikely that different sodium intakes were responsible for the differences in blood pressure. In an earlier study [11] we observed a difference of 3 mmHg in SBP between newborn infants of parents with high

Table 1 Blood pressure (BP) and related characteristics in 121 parent couples and 180 offspring in three group measured in 1986 - 1987.

	High BP	Mixed BP	Low BP
Parents (102 high, 70 mixed, 70 low)			
Age (years)	52 ± 0.9	51 ± 1.2	47 ± 1.1
SBP (mmHg)	153 ± 2.0	133 ± 2.8	113 ± 1.2
DBP (mmHg)	90 ± 0.9	80 ± 1.7	69 ± 0.8
Height (cm)	170 ± 0.9	169 ± 1.0	169 ± 1.0
Weight (kg)	77 ± 1.2	71 ± 1.4	67 ± 1.5
SBP adj*	150 ± 1.9	133 ± 2.7	117 ± 1.0
DBP adj*	89 ± 0.9	80 ± 1.7	70 ± 0.8
Offspring (69 high, 58 mixed, 53 low)			
Age (year)	22 ± 0.8	20 ± 0.9	19 ± 1.0
SBP (mmHg)	127 ± 1.4	124 ± 1.7	116 ± 1.6
DBP (mmHg)	79 ± 1.0	75 ± 1.3	70 ± 1.2
Height (cm)	173± 1.8	170 ± 2.1	166 ± 3.0
Weight (kg)	66 ± 1.8	60 ± 2.0	58 ± 2.8
Na (mmol/day)	134 ± 7.3	134 ± 9.3	128 ± 7.8
SBP adj**	125 ± 1.0	124 ± 1.3	118 ± 1.2
DBP adj**	78 ± 1.0	75 ± 1.2	71 ± 1.2

* Adjusted for differences in age and body weight;

** Adjusted for differences in age, height, weight and gender;

high: Both parents in upper quartile of blood pressure distribution or taking antihypertensive medication;

low: Both parents in lowest quartile of blood pressure distribution and neither taking antihypertensive medication;

mixed: One parent high, one parent low; SBP, systolic blood pressure; DBP, diastolic blood pressure.

blood pressure compared to those of the other parents. This suggests that differences between children selected on the basis of parental blood pressure should be detectable at a mean age of 8 years. However, the data presented here showed no clear difference at that age for SBP, and a non-significant difference for DBP. At a mean age of 20 years the difference was clear. There may be small differences in blood pressure early in life between children from hypertensive parents compared to children from normotensive parents, reflecting a different genetic make-up. As the child grows, and especially during puberty, this difference may become larger and more stable due to environmental influences in combination with differing genetic susceptibilities to these environmental factors.

Acknowledgements

We thank all the participating families and other members of the Steering Committee of this study: Prof. M.A.D.H. Schalekamp, Dr P.W. de Leeuw, Dr P. van Brummelen, Prof. K.H. Rahn and Dr Th. Thien.

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4.1.2 TWENTY-FOUR HOUR AMBULATORY BLOOD PRESSURE PATTERN IN YOUNGSTERS WITH A DIFFERENT FAMILY HISTORY OF HYPERTENSION: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

The offspring of hypertensive parents in general show higher casual blood pressure levels during adolescence than the offspring of normotensive parents. Comparative ambulatory blood pressure measurements might reveal the stability and pattern of this difference during circadian blood pressure variation. We studied the 24-hour ambulatory blood pressure (Space-Labs 90202 monitor, Redmond, Washington, USA) in youngsters with two hypertensive parents (high; n = 62), with one hypertensive parent (mixed; n = 51) and with no hypertensive parent (low; n = 42). The pattern for both systolic and diastolic blood pressure in the three groups ran parallel, with the high group continuously at a higher level. A clear difference in systolic blood pressure was observed during the day period (high minus low: 5.4 mmHg; 95% confidence range 2.5-8.3). Our data show that offspring of hypertensive parents differ in ambulatory blood pressure levels, but not in circadian pattern, from offspring of normotensive parents.

Introduction

With the availability of automatic ambulatory blood pressure measuring devices, comparison of circadian blood pressure patterns and levels during normal daily activities has become feasible[1] and might give more information on when and under what circumstances blood pressure increases.[2] The offspring of hypertensive parents tend to have higher casual blood pressure levels, starting in adolescence, than the offspring of normotensive parents.[3] Casual blood pressure measurements, however, might overestimate blood pressure differences due to the circumstances of the procedure.[4] Ambulatory blood pressure measurements might reveal a more valid and fundamental difference in the blood pressure level, pattern or variation in the different groups.[5] We therefore studied 24-h blood pressure patterns in three

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* Journal of Hypertension 1989;7(suppl 6):S66-67.

groups of offspring of either two hypertensive parents (high), one hypertensive parent (mixed) or no hypertensive parent (low) who took part in the Dutch Hypertension and Offspring Study.[3]

Methods

Population

Three groups of youngsters were defined on the basis of parental blood pressure values recorded on two different occasions. The families were selected from 1642 families taking part in a population-based study, according to the following criteria: both parents with a systolic blood pressure and diastolic blood pressure in the upper quartile of the age and gender-specific blood pressure distribution (high families); both parents with systolic and diastolic blood pressure in the lower quartile (low families); or one parent with a systolic and diastolic blood pressure in the upper and the other parent in the lower quartile.[3] From the parental couples, 121 were selected (51 high, 35 mixed, 35 low) and 155 of their offspring, aged 5-30 years of age, took part in the 24-h ambulatory blood pressure measurement (62 high, 51 mixed, 42 low).

Measurements

The monitor was applied to the non-dominant arm to take measurements at a frequency of one reading an hour between midnight and 6.00 a.m., three times an hour between 6.00 a.m. and 6.00 p.m. and twice an hour between 6.00 p.m. and midnight. The blood pressure values were not disclosed to the participant.

Data analysis

The mean blood pressure readings obtained in each period were calculated if at least 75% of the measurements per period were successful. All results were adjusted for differences in age, height, weight and gender between the groups using linear regression analysis.

Results

With proper instructions and attention to detail, ambulatory blood pressure was recorded in 146 (94%) of these youngsters at a mean age of 20 years. In 60 high, 46 mixed and 40 low offspring the measurements were of sufficient quality to be analysed. An activity score of

Table 1 Casual and 24-h ambulatory blood pressure (mmHg) characteristics.*

	Low	Mixed	High	
Casual SBP	119 (1.5)	126 (1.4)	125 (1.2)	**
Casual DBP	72 (1.3)	75 (1.3)	78 (1.1)	**
Mean 24-h SBP	116 (1.0)	118 (0.9)	120 (0.9)	**
Mean SBP at T1	110 (1.5)	109 (1.2)	112 (1.1)	
Mean SBP at T2	117 (1.0)	120 (1.2)	122 (1.1)	**
Mean SBP at T3	119 (1.2)	121 (1.1)	124 (1.1)	**
Mean 24-h DBP	67 (0.8)	70 (0.9)	72 (0.8)	**
Mean DBP at T1	59 (1.1)	60 (1.0)	62 (1.1)	
Mean DBP at T2	70 (0.8)	74 (1.0)	75 (0.9)	**
Mean DBP at T3	70 (1.1)	73 (1.1)	76 (1.1)	**

* Given are means ± s.e., adjusted for differences in age, weight, height and gender between the groups.
SBP systolic blood pressure.
DBP diastolic blood pressure.
T1 one reading per hour between midnight and 6.00 a.m.;
T2 three readings per hour between 6.00 a.m. and 6.00 p.m.;
T3 two readings per hour between 6.00 p.m. and midnight.
** $P < 0.05$ versus low.

daily activities showed a good range of different activities during the day, indicating a normal life style in all groups of youngsters.

Table 1 shows the 24-h blood pressure characteristics for the three groups of normotensive offspring. A plot of the hourly pattern of the three groups of youngsters over 24 h showed parallel curves, with the high group continuously at a higher level.

Conclusion

We conclude that normotensive youngsters at high risk of hypertension compared with youngsters at a lower risk of hypertension show a similar circadian pattern of 24-h blood pressure but a higher level of systolic and diastolic blood pressure at each time of the day. The most marked differences in systolic and diastolic blood pressure are present during the daytime.

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4.1.3 HAEMODYNAMIC CHARACTERISTICS OF THE EARLY PHASE OF PRIMARY HYPERTENSION: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

Background The haemodynamic characteristics of the early phase of primary hypertension are subject to debate. In particular, it remains unclear whether an increased vascular peripheral resistance or a raised cardiac output is involved as the primary haemodynamic alteration in hypertension.

Methods and Results We studied haemodynamic characteristics and oxygen consumption, in relation to 24-hour ambulatory blood pressure measurements, in three groups of normotensive children with a different familial predisposition for hypertension. Selection of participants was based on parental blood pressure levels. Mean 24-hour blood pressure was higher in the offspring of two hypertensive parents, compared to offspring of two normotensive parents; there was a difference of 4.7 mmHg (95% confidence interval [CI], 1.8 - 7.6) for systolic blood pressure and a difference of 4.8 mmHg (CI, 2.3 - 7.3) for diastolic blood pressure. The 24-hour blood pressure pattern was consistently at a higher level for both systolic and diastolic blood pressure in the offspring of two hypertensive parents, compared with the offspring of two normotensive parents. The smallest differences in blood pressure were seen at night, and the largest differences in blood pressure between the groups of offspring were seen during periods of physical activity. Echocardiographic examination combined with registration of oxygen consumption did not show a difference in cardiac index and arteriovenous oxygen difference between the study groups. However, differences in cardiac dimensions were apparent, with an increased left ventricular mass index (8.7 g/m^2, CI 2.4 to 15.0) in the offspring of two hypertensive parents.

Conclusions These findings do not support the existence of a hyperkinetic circulatory phase but may indicate the presence of an increased left ventricular mass in early primary hypertension.

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* Circulation 1993;87:100-6.

Introduction

In the past decades our understanding of the pathophysiological correlates of primary hypertension has increased greatly. By contrast, however, the mechanisms of initiation and development of high blood pressure and the hemodynamic characteristics of its early phase remain controversial. There are two major hypotheses: one hypothesis suggests that the initial phase of the development of high blood pressure is characterized by an increased peripheral resistance. The other suggests that it is characterized by a raised cardiac output.[1] The first hypothesis assumes that the peripheral resistance rises gradually with age when hypertension develops. The second hypothesis suggests that after a period of a high cardiac output, the so-called hyperkinetic circulatory phase, a secondary increase in peripheral resistance develops.[1] This theory rests on the postulate that disproportionately increased blood flow results in a decreased arteriovenous oxygen difference under normal oxygen consumption.[2,3] In 'early,' 'mild,' 'borderline,' or 'labile' young hypertensives, an increased cardiac output has been reported, at least in a sizable proportion of these groups.[4-7] However, others could not confirm this[8] or have even reported a decrease in cardiac output in young borderline hypertensive subjects compared to normotensive subjects.[9,10] Although follow up of borderline hypertensive subjects with an initial high cardiac output showed a decrease in cardiac output and an increase in peripheral resistance over years, blood pressure increased less than in borderline hypertensive subjects initially showing a normal cardiac output and an increased peripheral resistance.[3] Blood pressure in either young hypertensive or young normotensive subjects did not show a relation with cardiac output.[11,12] However, in children with a wide range of blood pressure, cardiac output was weakly related to blood pressure, but the level of cardiac output did not predict future blood pressure during follow up.[13]

We have studied haemodynamic characteristics including cardiac output, cardiac dimensions, oxygen consumption, and 24-hour ambulatory blood pressure in 155 young normotensive males and females, aged 6-31 years, in three groups; the subjects had two hypertensive parents, one hypertensive and one normotensive parent, or two normotensive parents.

Methods

Population

The Dutch Hypertension and Offspring Study is a collaborative study of four Dutch Universities, and is conducted in Zoetermeer, a suburban residential area near The Hague in the Netherlands. From 1975-1979, residents of two districts of this town were invited to participate in a study of blood pressure and other cardiovascular risk indicators (EPOZ Study).[14] Blood pressure was measured in 10,532 (78%) of 13,462 eligible subjects. This group included 1,642 parental couples. A stringent selection procedure was applied to these couples to select groups of offspring with a maximal contrast in familial predisposition for hypertension. The procedures for selection have been described elsewhere.[15] In brief, individual parents with both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the upper ("hypertensive") or lower ("normotensive") quartile of the age- and sex-specific blood pressure distribution were selected. Those on antihypertensive medication were included in the hypertensive group. Couples of two hypertensive parents, one hypertensive and one normotensive parent, and of two normotensive parents were invited for remeasurement of blood pressure in 1986. At this occasion, the same criteria for hypertension and normotension were applied as for the initial screening. Of 250 parental couples who were remeasured (80% of those invited), 51 remained in the group with two hypertensive parents, 35 in the group with one hypertensive and one normotensive parent and 35 in the group with two normotensive parents. Together, these parents had 291 healthy biological children, aged between 5-30 years, who were invited to take part in this study. Of these, 155 gave signed informed consent and participated. For the present analysis, only subjects were considered from whom complete data from 24-hour ambulatory blood pressure measurements and echocardiography could be obtained; 40 children of two normotensive parents, 46 children with one hypertensive and one normotensive parent, and 60 children of two hypertensive parents.

The study protocol was approved by the ethical committee of the University Hospital Dijkzigt and informed consent was obtained from the children and their parents.

Protocol and Measurements

Blood pressure was measured on the left arm with a random-zero sphygmomanometer, by a trained paramedical assistant. A series of two readings was made with the subject sitting

and the mean of these readings was used in the analysis. The procedure was repeated with the subject in supine position. Body weight and height were measured with the subject wearing indoor clothes without shoes. Before echocardiography, the subject was positioned supine on the side and connected to a paramagnetic O_2 analyzer and an infrared CO_2 analyzer (Oxycon-4, Mijnhardt, Bunnik, The Netherlands) by tube and mouthpiece, with nose clamped, to measure O_2 use and CO_2 production during echocardiography. At least five minutes were allowed to accommodate while the Oxycon-4 measured and registered mean gas exchange during each 30-second interval. After five minutes and with a respiratory quotient at or below 1, a four-chamber echocardiogram was made using two-dimensional echocardiography (Toshiba Sonolayer SSH-60A) and a 3.75 mHz transducer to measure cardiac output. Cardiac dimensions were registered using the para-sternal view with M-mode echocardiography. In case the participant was asthmatic, had a severe cold, or was otherwise not willing or able to breath normally, the tube was disconnected and the echocardiogram was made without measurement of oxygen consumption. Oxygen measurements could be obtained only in a subset of 60 participants equally distributed across the offspring groups (offspring of two hypertensive parents, 22; of one hypertensive parent, 19; and of two normotensive parents, 19).

The day before visiting the examination centre, participants collected one 24-hour urine sample and refrained from smoking and coffee use during that day until after the examination. On a day of their own choice, participants were invited to carry an ambulatory blood pressure measurement device (Spacelabs 90202) during a 24-hour period for measurement of blood pressure and heart rate. The ambulatory blood pressure monitor was applied to the non-dominant arm to take measurements at a frequency of one reading per hour between 12:00 midnight and 6:00 AM, three times per hour between 6:00 AM and 6:00 PM, and twice per hour between 6:00 PM and 12:00 midnight. The results of the readings were not disclosed to the participant. During this day, the participant was asked to record physical activities for each hour on a scale from 0 (bedrest, sleeping) to 1 (sitting, reading, watching television, eating), 2 (walking at normal pace, bicycling quietly) and 3 (sports, running, bicycling fast).

Cardiac output was calculated as the mean from six four-chamber echocardiograms by the area-length method.[16] Cardiac index was estimated from cardiac output and body surface area according to Dubois and Dubois.[17] Left ventricular mass was estimated from

left ventricular end-diastolic diameter, intraventricular septum, and left ventricular posterior wall and indexed for body surface area.[16] Mean arterial pressure was calculated from SBP and DBP measured in the supine position and was used to calculate total peripheral vascular resistance by dividing mean arteril pressure by cardiac index. From 10 consecutive Oxycon registrations during 5 minutes in the middle of the echocardiography, oxygen consumption per minute was calculated. The arteriovenous oxygen difference was estimated by dividing oxygen consumption by cardiac output.[18]

To obtain the mean 24-hour ambulatory SBP, DBP and heart rate, the parameters were calculated per period of the day (midnight to 6:00 AM, 6:00 AM to 6:00 PM, and 6:00 PM to midnight) if at least 75% of the measurements per period were successful. Of the 155 participants 146 (95%) (60 offspring of two hypertensive parents, 46 offspring of one hypertensive and one normotensive parent and 40 offspring of two normotensive parents) had sufficient blood pressure data to be analyzed. Most of the offspring had normal blood pressures although there was a large distribution of blood pressure levels (SBP: P10, 106 mmHg; P90, 140 mmHg) that can in part be explained by variation in age. Mean 24-hour ambulatory SBP, DBP and heart rate were averaged from the three periods, weighted for number of hours per period. As a measure of 24-hour variability, the standard deviation of mean 24-hour SBP, DBP and heart rate were divided by their mean. Categories of mean activity scores per hour were used to calculate blood pressure by activity level.

Data-analysis

Descriptive data for the three groups are presented as means and standard deviations. For comparisons between groups, means and standard errors are given with the difference and 95% confidence intervals (CI) of the difference between groups. Adjustments for differences in age, height, weight and proportion of males between the three groups were made by entering these variables simultaneously with indicator variables for each group in a model for multiple linear regression.

Associations between study variables were studied across groups adjusted for group characteristics (using indicator variables) and age, height, weight and gender by multiple linear regression analysis. To further assess the differences across groups, data were analyzed by two blood pressure groups and two age groups, based on median cutoff points.

Results

General Characteristics

Table 1 gives a general description of the three groups. At a mean age of 21.6 years, a difference in sitting SBP and DBP and supine MAP between the offspring of two hypertensive parents and the offspring of two normotensive parents was already present. The blood pressure difference remained after adjustments for differences in age, height, body weight and proportion of males between the groups; the difference between the offspring of two hypertensive parents and the offspring of two normotensive parents was 6.4 mmHg (95% CI 2.6 - 10.2) for SBP, 6.3 mmHg (CI, 2.9 - 9.7) for DBP, and 5.8 mmHg (CI 2.8 - 8.8) for mean arterial pressure (Table 1).

Table 1 Casual blood pressure and other characteristics in offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents.**

	offspring of two normotensive parents	offspring of one hypertensive parent	offspring of two hypertensive parents
Males/Females (number)	24 / 16	25 / 21	37 / 23
Age (years)	19.5 ± 7.6	21.8 ± 6.1	22.8 ± 6.5
Height (cm)	166.9 ± 21.3	172.7 ± 14.0	173.5 ± 13.9
Body weight (kg)	59.0 ± 21.1	63.3 ± 13.9	66.5 ± 15.2
Body Mass Index (kg/m^2)	20.3 ± 3.9	21.0 ± 2.9	21.8 ± 3.1
Body Surface Area (m^2)	1.65 ± 0.40	1.75 ± 0.26	1.79 ± 0.27
SBP sitting (mmHg)	116.4 ± 12.9	125.7 ± 13.4	126.9 ± 11.6
DBP sitting (mmHg)	71.0 ± 9.2	76.2 ± 9.4	78.7 ± 7.9
MAP supine (mmHg)	78.5 ± 8.3	83.6 ± 8.4	86.7 ± 8.4
SBP sitting (mmHg)*	119.0 ± 9.4	125.5 ± 9.4	125.4 ± 9.4
DBP sitting (mmHg)*	71.9 ± 8.4	76.0 ± 8.4	78.3 ± 8.4
MAP supine (mmHg)*	79.9 ± 7.3	83.7 ± 7.2	85.7 ± 7.2

** Values are means ± SD, SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial blood pressure.

* Adjusted for differences between the groups in age, height, body weight and gender.

The number of hours at different levels of physical activity during the 24-hour ambulatory blood pressure measurement did not differ among the groups and showed a good range of different activities throughout the day.

Ambulatory blood pressure measurements

In figure 1, the SBP and DBP for each hour of a 24-hour period are given for each of the three groups. At each time of the day, the DBP of offspring of two hypertensive parents was higher than that of the offspring of two normotensive parents, with the offspring of one hypertensive parent between the two levels. For SBP, a similar pattern of differences was seen during the 24-hour period, except at 12:00 midnight and 3:00 AM, when SBP was the

Figure 1 Twenty-four-hour ambulatory blood pressure pattern for systolic and diastolic blood pressure in offspring of two normotensive parents, of one hypertensive and one normotensive parent and of two hypertensive parents. Hourly values were calculated as the mean of 3 measurements per hour between 06:00 AM and 06:00 PM, of two measurements per hour between 06:00 PM and 12:00 midnight, and of one measurement between 12:00 midnight and 06:00 AM. All values were adjusted for differences in age, height, body weight and sex among the groups.

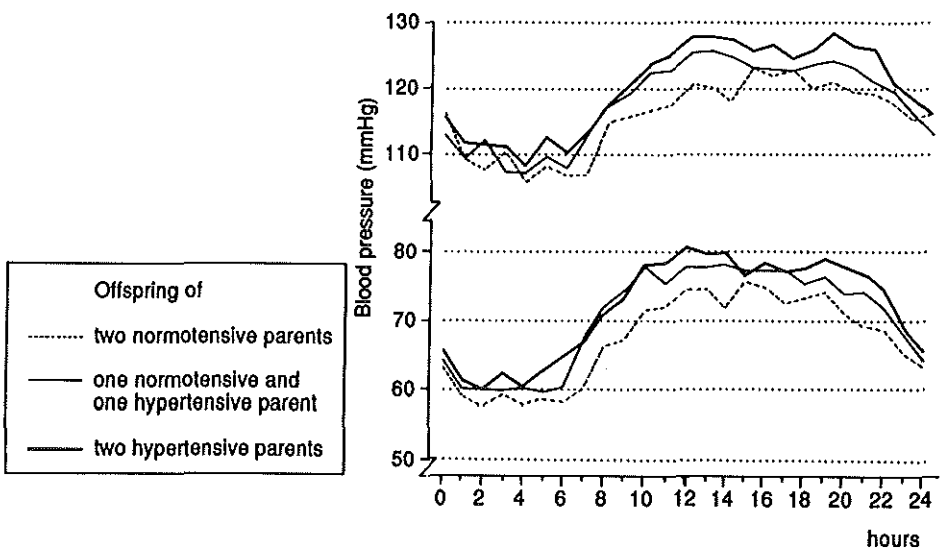


Table 2 Mean 24-hour ambulatory blood pressure measurements in the offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents; 24-hour mean systolic (SBP), diastolic (DBP) blood pressure, heart rate (HR), and variability of the 24-hour mean SBP, DBP and HR are given.*

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
24-hour SBP (mmHg)	115.6	(1.02)	117.9	(0.99)	120.3	(0.99)	2.3	[-0.5, 5.1]	4.7	[1.8, 7.6]
24-hour DBP (mmHg)	67.2	(0.82)	70.4	(0.88)	72.0	(0.85)	3.2	[0.8, 5.6]	4.8	[2.3, 7.3]
24-hour HR (beats/min)	75.1	(1.51)	76.5	(1.36)	73.7	(1.19)	1.4	[-2.6, 5.4]	-1.4	[-5.2, 2.4]
SBP variability (%)	8.9	(0.3)	9.1	(0.3)	9.1	(0.2)	0.2	[-0.6, 1.0]	0.2	[-0.6, 1.0]
DBP variability (%)	15.8	(0.6)	14.4	(0.6)	15.0	(0.5)	-1.4	[-3.1, 0.3]	-0.8	[-2.4, 0.8]
HR variability (%)	17.4	(0.9)	17.7	(0.8)	17.8	(0.7)	0.3	[-2.1, 2.7]	0.4	[-1.9, 2.7]

SBP systolic blood pressure
DBP diastolic blood pressure
HR heart rate
bpm beats per minute
* Values are means with SEM in parentheses, and differences between means with 95% confidence intervals in brackets; adjusted for differences in age, height, body weight and sex among the groups.

Table 3 Cardiovascular parameters in the offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents.*

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
Heart rate (bpm)	72.5	(1.8)	71.3	(1.6)	68.5	(1.4)	-1.2	[-5.9, 3.5]	-4.0	[-8.4, 0.4]
Stroke volume (mL)	44.6	(2.0)	43.8	(1.9)	45.1	(1.8)	-0.8	[-6.2, 4.6]	0.5	[-4.7, 5.7]
Cardiac output (L/min)	3.09	(0.12)	3.01	(0.12)	3.05	(0.11)	-0.08	[-0.43, 0.27]	-0.04	[-0.38, 0.30]
Cardiac index ($L/min/m^2$)**	1.82	(0.07)	1.77	(0.07)	1.80	(0.06)	-0.05	[-0.24, 0.14]	-0.02	[-0.20, 0.16]
Oxygen consumption (mL/min)	253	(6)	261	(4)	257	(5)	8	[-6, 22]	4	[-11, 19]
AVO_2 difference (mL/L)	79	(5)	87	(5)	83	(4)	8	[-5, 21]	4	[-8, 16]
AVO_2 difference ($mL/L/m^2$)**	46	(3)	48	(3)	50	(3)	2	[-4, 8]	4	[-2,10]
LVPW (mm)	8.8	(0.1)	8.8	(0.1)	9.0	(0.1)	0.0	[-0.3, 0.3]	0.2	[-0.1, 0.5]
IVS (mm)	9.0	(0.1)	9.1	(0.1)	9.2	(0.1)	0.1	[-0.3, 0.5]	0.2	[-0.1, 0.5]
LVED (mm)	48.0	(0.4)	48.8	(0.4)	49.3	(0.4)	0.8	[-0.4, 2.0]	1.3	[0.2, 2.4]
LVM (g)	189.7	(4.3)	193.9	(4.0)	202.0	(3.5)	4.2	[-7.5, 15.9]	12.3	[1.3, 23.3]
LVMI (g/m^2)**	106.1	(2.5)	110.4	(2.3)	114.8	(2.0)	4.4	[-2.4, 11.0]	8.7	[2.4, 15.0]
TPR ($mmHg/L/min/m^2$)**	45.1	(1.9)	49.4	(2.1)	48.2	(1.7)	4.3	[-1.3, 9.9]	3.1	[-2.0, 8.1]

bpm Beats per minute

LVPW Left ventricular posterior wall thickness, IVS; intra-ventricular septum thickness, LVED; left ventricular end-diastolic diameter

LVM left ventricular mass, LVMI; left ventricular mass index, TPR; total peripheral vascular resistance.

AVO_2 difference; arteriovenous oxygen difference

* Values are means with SEM in parentheses, and differences between means with 95% confidence intervals in brackets; adjusted for differences in age, height, body weight and sex.

** Parameters indexed for square meter of body surface area are adjusted for differences in age and sex only.

same in the offspring of two hypertensive parents as in the offspring of two normotensive parents. The three groups of offspring showed a similar circadian pattern for 24-hour SBP and DBP with a low level during 01:00 AM and 06:00 AM, a rise between 7:00 AM and 11:00 AM, a continuously high level between 11:00 AM and 05:00 PM, and a period of decline starting between 5:00 PM and 8:00 PM and ending at 01:00 AM.

The offspring of two hypertensive parents had a slightly lower heart rate throughout the 24-hour period compared to the offspring of two normotensive parents (table 2). Moreover, the offspring of one hypertensive and one normotensive parent showed the highest heart rates during the 24-hour period. The pattern of the 24-hour heart rate was similar for the three groups: biphasic with a similar pattern of rise and decline as for 24-hour SBP and DBP.

Calculated mean 24-hour ambulatory SBP and DBP were higher in the offspring of two hypertensive parents, than in the offspring of two normotensive parents (table 2). No difference in mean 24-hour heart rate was seen among the groups. Although the differences in SBP and DBP among the three groups changed in magnitude during the 24-hour period (figure 1), SBP and DBP did not show a difference in variability (table 2).

When the groups were compared for blood pressure and heart rate at an increasing physical activity level, at each level of activity the offspring of two hypertensive parents showed a higher SBP compared to the offspring of two normotensive parents. However, the difference was smallest and statistically nonsignificant at activity level 0 (difference, 2.7 mmHg; 95% CI -0.8 to 6.2) (figure 2). At activity score 0, the difference in DBP between the offspring of two hypertensive and two normotensive parents was slightly higher (3.9 mmHg, CI 1.0 - 6.8). Heart rate did not differ between the groups at any level of activity score.

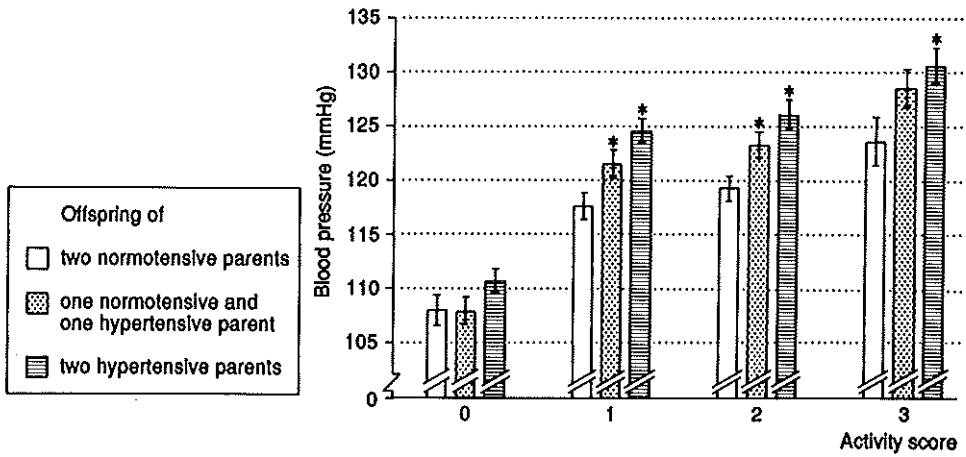
Cardiovascular Characteristics

The offspring of two hypertensive parents had a lower resting heart rate than the offspring of two normotensive parents which, however, just failed to reach statistical significance (table 3). Mean stroke volume, measured by area length method, was not significantly different in the offspring of hypertensive parents. Cardiac output and cardiac index were the same in the three groups. No differences in absolute oxygen consumption or in arteriovenous oxygen difference were present among the groups. However, cardiac dimensions showed differences between the offspring groups; the thickness of both the left

ventricular posterior wall and the intraventricular septum was increased, although not reaching statistical significance, and the end diastolic left ventricular diameter was significantly increased in the offspring of two hypertensive parents. The estimated left ventricular mass and mass index, calculated from these parameters, was respectively 12.3 g and 8.7 g/m^2 greater in the offspring of two hypertensive parents compared with offspring of two normotensive parents. To further assess whether the differences in left ventricular mass index were dependent on blood pressure, data were analyzed separately in children with a relatively low blood pressure (below or median). Low blood pressure offspring of two hypertensive parents had an index of 109.9 g/m^2 (SEM 3.2), and low blood pressure offspring of two normotensive parents had an index of 101.2 g/m^2 (SEM 2.6); the difference is 8.7 g/m^2, and the 95% CI is 0.5 - 17.0]. If additional adjustments were made for small and non significant differences in blood pressure between the groups in each stratum (SBP in lowest stratum was 114.9±1.7 mmHg for offspring of hypertensive parents and 110.7±1.5 mmHg for offspring of two normotensive parents), the left ventricular mass index between the groups remained 110.0±3.2 g/m^2 for low blood pressure offspring of two hypertensive parents and 101.1±2.6 g/m^2 for low blood pressure offspring of two normotensive parents (difference, 8.9 g/m^2; 95% CI 0.7 - 17.1). In the high blood pressure offspring separately, no significant difference in left ventricular mass was present between the offspring groups. This analysis indicates that the overall findings cannot be explained by elevated blood pressure in a subgroup of children because the difference in left ventricular mass index is most pronounced for the low blood pressure group. Similarly, data were analyzed according to two age groups based on median age, indicating that findings are most pronounced in the youngest group; left ventricular mass index adjusted for differences in proportion of males, SBP, and age was 112.2 g/m^2 (SEM, 2.9) in the younger offspring of two hypertensive parents and 100.5 g/m^2 (SEM, 3.0) in the younger offspring of two normotensive parents (difference, 11.7 g/m^2; 95% CI 3.4 - 20.0).

As both casual (table 1) and 24-hour ambulatory blood pressures (figure 1 and table 2) were increased in the offspring of hypertensive parents, this might be related to the differences in left ventricular mass index. If the differences in left ventricular mass index were adjusted for differences in mean 24-hour SBP, the difference became only slightly smaller (offspring of two normotensive parents, 106.8±2.5 g/m^2; offspring of two hypertensive parents 114.5±2.0 g/m^2; difference, 7.7 g/m^2, 95% CI, 1.3 - 14.1).

Figure 2 Mean systolic blood pressure at increasing levels of activity in the three groups, adjusted for differences in age, height, body weight and sex. Activity score 0 is bedrest; Activity score 1 is sitting, eating, reading, watching television; Activity score 2 is walking at normal pace, bicycling leisurely; and 3 is sports activities, running, bicycling fast.
* $p<0.05$ for the difference with the offspring of two normotensive parents.



The calculated total peripheral vascular resistance was slightly but not statistically significant higher in the offspring of hypertensive parents.

No relations between cardiac index or left ventricular mass index and mean ambulatory blood pressure, blood pressure variability, or blood pressure at different activity levels were seen.

Discussion

The findings of this study do not support the presence of a difference in cardiac index between the offspring of hypertensive parents and the offspring of normotensive parents. Moreover, no signs of a decreased arteriovenous oxygen difference in the offspring of

hypertensive parents was present. Although not confirmative of the existence of a hyperkinetic phase in prehypertensive subjects, the findings may be compatible with the presence of structural cardiac changes in the early phase of primary hypertension; dimensions of the left ventricular mass index were higher in the offspring of hypertensive parents. From the 24-hour blood pressure measurements, it appeared that the offspring of hypertensive parents had a higher SBP and DBP throughout the day compared with the offspring of two normotensive parents, with the differences most pronounced at a high activity level and lowest during rest. No differences in heart rate at rest or during 24-hour measurements were seen.

Many other investigators have studied the importance of changes in cardiac output in relation to blood pressure development. Studies in young borderline hypertensive subjects were not conclusive; findings in favor,[3-7] or against[8-10] a hyperkinetic circulatory phase have been reported. The prerequisite of a true hyperkinetic circulation, the existence of a similar or decreased oxygen consumption together with an increased cardiac output and thus a decreased arteriovenous oxygen difference, has not been described in borderline hypertension. There appears to be no "luxury" perfusion, that by whole body autoregulation, could secondarily increase peripheral resistance and decrease cardiac output. In young subjects with a wide range of blood pressure, no clear relation between cardiac index and blood pressure level[11,12] or blood pressure rise[13] has been reported.

In our study selection of participants was not based on individual blood pressure level but rather on parental blood pressure. The strategy was to select offspring at different risks for hypertension irrespective of actual blood pressure level. Haemodynamic factors reported to be predictive of future blood pressure rise in young normotensive subjects include an initially increased blood pressure reactivity to mental stress,[19] an increased SBP response during physical exercise,[13] an initially high SBP,[13,19] an initially large left ventricular mass,[13] and a family history of hypertension.[19] In our study, the haemodynamic profile of the offspring of two hypertensive parents compared with the offspring of two normotensive parents (i.e., higher casual and mean ambulatory blood pressure, greater increase in SBP for each activity level, and an increased left ventricular mass index but a similar cardiac index) resembles the haemodynamic profile observed in other studies[13,19] of children exhibiting the largest pressure rise in these studies. Both individual and familial risks for primary hypertension seem to be characterized by high initial blood pressure

levels, high blood pressure responses during stress, and increased left ventricular mass index, but not by a high cardiac output. This is supported by others comparing offspring of hypertensive and normotensive parents, who showed an increased left ventricular mass in males with hypertensive parents,[20-22] although in one small study among young sons and daughters of hypertensive parents, no clear increase of left ventricular mass was seen.[23] A study comparing male offspring groups at a mean age of 34 years did not show the increased haemodynamic response in offspring of hypertensive parents during mental or physical stress, but at rest the differences between the offspring groups in cardiac index, blood pressure and total peripheral resistance showed a similar pattern compared to our study at a mean age of 22 years.[24]

Our finding of an increased left ventricular mass index in offspring of hypertensive parents, which remained after adjustment for the differences in 24-hour ambulatory blood pressure, could indicate the presence of structural changes of the heart in the early phase of primary hypertension. This could suggest that cardiac hypertrophy occurs due to stimuli other than an increased after-load only. In a recent randomized trial, a similar reduction of blood pressure with either verapamil or atenolol was accompanied by a reduction of left ventricular mass in verapamil treated patients only, suggesting that reduction of left ventricular mass was not solely dependent on blood pressure decrease.[25] It has been suggested that an increase of intrinsic growth factors due to a genetic predisposition or due to an increased adrenergic stimulation or sensitivity might cause cardiac hypertrophy.[26,27] These different stimuli as well as an increased afterload would increase intracellular calcium, which triggers the induction of proto-oncogenes thereby stimulating protein synthesis.[28] These factors may be similar to the factors that give rise to vascular hypertrophy.

In conclusion, the absence of a difference in cardiac output and oxygen consumption at rest between offspring of hypertensive parents and offspring of normotensive parents does not support the existence of a hyperkinetic circulation in the early phase of primary hypertension. The difference in left ventricular mass appears to be independent of differences in blood pressure between the groups, suggesting that the finding is not the consequence of an increase in afterload only. Whether the rise in left ventricular mass is the result of functional or structural vascular and cardiac changes, or a combination of both, remains to be established.

Acknowledgements

This study was supported by grant 35.004 from the Netherlands Heart Foundation. The Dutch Hypertension and Offspring Study is a collaborative study supervised by a Steering Committee with members of four Dutch universities and major clinical research centers. The study is conducted in Zoetermeer, in the Netherlands. Prof. H.A. Valkenberg is acknowledged for his general support and organisation of the population study in Zoetermeer. Prof M.A.D.H. Schalekamp, Dr. P. van Brummelen, Prof H.J.A. Struyker Boudier, Prof K.H. Rahn, Prof P.W. de Leeuw, Prof Th. Thien, as members of the Steering Committee, are gratefully acknowledged for their support and contribution. We are greatly indebted to all participants in this study and their parents. Finally, we thank H. Kornman for assistance throughout the study and J.C.H.M. van Meurs for her invaluable contribution in the echocardiographic examination and analysis.

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4.1.4 CHARACTERISTICS OF THE COMMON CAROTID ARTERY IN YOUNG MALES WITH AND WITHOUT A FAMILY HISTORY OF HYPERTENSION: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

Functional and structural vascular changes may develop during the development of hypertension. Changes in functional characteristics of the common carotid artery have been found in young borderline hypertensive subjects. However, these findings might have been influenced by the elevated blood pressure level rather than predate its development. We investigated characteristics of the common carotid artery in two groups of young males with a different probability to develop primary hypertension, defined by parental blood pressure. Using an ultrasound-doppler technique similar characteristics were found in the two groups; no difference could be detected for arterial diameter, change in diameter or cross-sectional area relative to pressure changes during the cardiac cycle. It is concluded that either no functional changes in large arteries as the common carotid artery are present in youngsters at risk for hypertension, or vascular changes are too small to be detected by the method employed.

Introduction

Structural vascular changes are considered to be of importance for the early rise of peripheral resistance during the development of primary hypertension.[1] A theory of an increased wall to lumen ratio has been proposed based on findings of a diminished vasodilatation after arterial occlusion during isometric exercise and local heating in hypertensive compared to normotensive subjects.[2] This increased arterial wall to lumen ratio was observed in experimental[3] and human hypertension,[4] but not in isolated resistance vessels of offspring of hypertensive parents compared to offspring of normotensive parents.[5] However, the presence of a decreased maximal forearm blood flow after arterial occlusion and exercise in offspring of hypertensive parents compared to offspring of normotensive parents,[6] does suggest functional vascular changes in the early phase of primary hypertension. Dynamic vascular properties can be measured noninvasively

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* Submitted,
presented at the XIth congress of the European Society of Cardiology (1989, Nice, France)

in vivo using ultrasound and a multi-gate pulsed Doppler system to measure velocity profiles and calculate the arterial diameter and cross sectional area and their change relative to pulse pressure during the cardiac cycle.[7] Using this technique, borderline hypertensive subjects appeared to have a decreased arterial distensibility and cross sectional compliance compared to normotensive subjects.[8] However, it is unknown if changes in carotid vascular properties are present before hypertension develops. In the Bogalusa Heart Study, youngsters with a blood pressure and cholesterol in the upper tertile showed a decreased arterial distensibility compared to youngsters in the lower tertile.[9] In the Dutch Hypertension and Offspring Study, youngsters at different risk for hypertension were selected on bases of parental blood pressure.[10] In a subgroup of this study, in young males aged at least 18 years of age of selected from the offspring of two hypertensive parents and of the offspring of two normotensive parents, vascular properties of the common carotid artery using the ultrasound and multi-gate pulsed Doppler technique were measured. Males were chosen to increase precision in this relatively small sample, as carotid artery distensibility appeares to be influenced by gender with a decreased distensibility in women.[11] The age limit was pragmatic, as the offspring had to make a two-hour train-journey to visit the research centre at Maastricht, The Netherlands.

Methods

Population

The offspring were invited from two groups of youngsters with either two hypertensive parents or two normotensive parents. The families were selected from a population based survey between 1975-1978 on cardiovascular risk indicators, as described elswhere.[10, 13] The "hypertensive" and "normotensive" blood pressure status of the parents was defined according to their position in one-year-age and gender specific blood pressure distributions at respectively the upper quartile for systolic and diastolic blood pressure or the lower quartile. The blood pressure of the selected parents had to meet the same criteria during a remeasurement in 1986. After remeasurement 51 families with two hypertensive parents and 35 families with two normotensive parents were selected. For this study males aged at least 18 years of age were invited; 16 offspring of two hypertensive parents and 12 youngsters of two normotensive parents gave informed consent and participated. The number of participants was thought to be sufficient, as differences in vascular characteristics between

groups of this size had been observed for borderline hypertensive subjects compared to normotensive subjects.[8]

Table 1 General characteristics of the subgroup of male offspring of two normotensive parents and of two hypertensive parents, that participated in the study.*

	offspring of two normotensive parents	offspring of two hypertensive parents
Number of males	12	16
Age (years)	22.3 ± 4.8	24.4 ± 4.5
Height (cm)	186.1 ± 5.9	182.8 ± 6.3
Body weight (kg)	80.1 ± 11.0	74.5 ± 8.1
SBP (mmHg)**	122.9 ± 8.1	126.1 ± 7.3
DBP (mmHg)**	59.0 ± 5.8	63.9 ± 6.5
Pulse pressure (mmHg)	64.0 ± 7.2	62.1 ± 7.6

* Values are means ± SD.
** SBP = systolic blood pressue, DBP = diastolic blood pressure.

Measurements

Subjects were in supine position. After 15 minutes rest, brachial blood pressure was measured eight times with an automatic device (Dinamap) on both left and right arm. At the same time the common carotid artery was brought into view with 2-D ultrasound imaging. With a multi-gate pulsed Doppler system velocity profiles in the artery were recorded from which the arterial diameter during the systole was calculated.[7] The measurements were done both at the right and left common carotid artery.

Pulse pressure was calculated from measured systolic and diastolic brachial pressure. The average of the four last measurements were taken, and the mean left and right brachial pulse pressure were added and divided by two to give the estimated brachial pulse pressure. Carotid artery vessel wall properties were calculated relative to the brachial pulse pressure and expressed as change in diameter per unit of pressure for the distensibility coefficient

(formula 1 Table 2), and as change in cross sectional area per unit of pressure for the cross sectional compliance coefficient (formula 2, table 2).

Table 2 Vessel wall characteristics of the common carotid artery in male offspring of two normotensive parents and of two hypertensive parents.*

	offspring of two normotensive parents		offspring of two hypertensive parents		difference [95% confidence interval]
Diameter (D)(mm)	6.5	(0.15)	6.6	(0.13)	0.1 [-0.3, 0.5]
Change in diameter (δD/D)(%)	8.3	(0.5)	8.4	(0.4)	0.1 [-1.2, 1.4]
DC (10^{-3}/kPa)**	19.0	(1.1)	20.6	(0.9)	1.6 [-1.3, 4.5]
CC ($10^{-7}m^2$/kPa)**	6.4	(0.4)	6.9	(0.4)	0.5 [-0.5, 1.5]

* Values are means with SEM in parentheses, and differences between means with 95% confidence intervals in brackets; adjusted for differences between the groups in age, height and body weight.

** DC = distensibility coefficiënt, CC = cross sectional compliance.
Formula 1: DC = ((δD/D) / PP) * 15.037.
Formula 2: CC = ((δD/D) / PP) * D^2 * 118.1.

Results

In table 1 the general characteristics of the two groups of offspring are given. Although differences in age, body weight, height and blood pressure were present, none reached statistical significance. The brachial pulse pressure was similar for the two groups of offspring.

Table 2 shows the vessel properties of the common carotid artery in the two groups of offspring. No difference in any of the parameters was observed, neither for the static measure of the artery diameter, nor for the dynamic measure of relative change in diameter during the cardiac cycle. Relative to pulse pressure, no difference between the groups in change of diameter or cross sectional area was measured. In the two groups of youngsters at presumed different risk for hypertension, at a mean age of 23,5 years no differences in vessel wall stiffness or cross sectional compliance of the common carotid artery could be detected.

Discussion

No difference between normotensive youngsters of hypertensive parents and youngsters from normotensive parents were observed in vessel wall properties of the common carotid artery. Between groups of borderline hypertensive subjects and normotensive subjects of similar size as the groups presented here, a decreased vessel wall stifness and cross sectional compliance was reported using the same method.[8] Therefore, it appears unlikely that the method used or the numbers of subjects studied was insufficient to detect differences in dynamic vessel wall characteristics in the early phase of hypertension. These findings might indicate that either no functional or structural changes in the vessel wall of large arteries at rest are present in the pre-hypertensive period, or vascular changes in the pre-hypertensive period are too small to be detected with this method. Alternatively functional vascular changes of large arteries in the prehypertensive period may only become apparent after stimulation (i.e. maximal vasodilatation after local occlusion and exercise), as suggested by earlier studies.[6]

In conclusion unaltered dynamic vascular properties relative to pulse pressure during the cardiac cycle at rest in offspring of hypertensive parents compared to offspring of normotensive parents, do not favor a role for a decreased elasticity of the vessel wall in the prehypertensive phase. Limitations in size and methodology of the present study do, however, not permit definitive conclusions.

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4.2 THE KIDNEY

Renal haemodynamics

Renal blood flow, calculated from the clearance rate of para-amino-hippuric acid (methods see chapter 3.3.1 and 3.3.2), was lower in the offspring of two hypertensive parents compared to the offspring of normotensive parents (difference -198 ml/min/1.73m^2, [95% confidence interval -318 to -78]). Moreover, filtration fraction and renal vascular resistance were increased (difference 3.0% [0.9 to 5.1] and 2.7 mmHg/dl/min/1.73m^2 [1.2 to 4.2], respectively) (section 4.2.1).

Findings were similar, though less pronounced, using the rate of intravenous infusion of inuline and para-amino-hippuric acid (methods see chapter 3.3.1 and 3.3.2) for measurement of respectively glomerular filtration and renal blood flow (section 4.2.1).

To assess whether the difference in renal blood flow was confounded by the difference in blood pressure between the groups the data were adjusted for differences in systolic blood pressure. In this analysis the differences in renal blood flow between the groups remained (section 4.2.1).

To study whether the difference in renal blood flow between the three groups was already apparent at a young age, when blood pressure differences between the groups are smaller and have been present for a shorter period of time, subgroups were studied according to tertiles of age. In each age stratum similar differences in renal blood flow were present between the groups of offspring (section 4.2.1, figure 1).

No relation was found between renal haemodynamics as renal blood flow or glomerular filtration and 24-hour sodium excretion.

Renin-angiotensin-aldosterone system

The offspring of two hypertensive parents had a reduced plasma renin concentration (difference -3.3 mU/l, (95% confidence interval -6.4 to -0.2)), and a lower plasma aldosterone level (-111 pmol/l, (-182 to -40)) compared to the offspring of two normotensive parents (section 4.2.1). The difference in renin was not reflected in the angiotensin-II level, which showed no significant difference between the study groups. The ratio of angiotensin-II to PRA was higher in the offspring of two hypertensive parents (0.97%/hour) as compared to offspring of two normotensive parents (0.72%/hour), with a difference of 0.25 (95% CI 0.05 to 0.45). The ratio of aldosterone to angiotensin-II was

lower in the offspring of two hypertensive parents (36.7) as compared to offspring of two normotensive parents (51.7), with a difference of -15.0 (-23.2 to -6.8). The results were similar if plasma renin activity and plasma aldosterone were expressed per mmol of sodium excreted during a 24-hour period (section 4.2.1, figure 2). Both measures were lowest in the offspring of two hypertensive parents.

As reported, blood pressure was already higher in the offspring of two hypertensive parents at the time of measurement in the Dutch Hypertension and Offspring Study. To study if this might have influenced, for example through renal autoregulation, the difference in renin levels between the groups, plasma renin activity and active plasma renin concentration were compared between groups after adjustments for systolic blood pressure. The differences between the offspring of two normotensive parents and the offspring of two hypertensive parents remained; active plasma renin concentration -3.3 mU/l (95% CI -6.5 to -0.1), and plasma renin activity -0.41 pmol/ml/hour (-0.79 to -0.03). However, after correction of renin for the differences between the groups in renal vascular resistance, a measure more directly related to the physiology of renin regulation, the difference in renin was less marked and not statistically significant; difference in renin adjusted for renal vascular resistance calculated by the rate of urinary excretion of para-amino-hippuric acid: active plasma renin concentration -2.93 mU/l (-6.33, 0.47) and plasma renin activity -0.35 pmol/ml/hour (-0.70, 0.00).

No significant association was present for renal blood flow, renal vascular resistance or glomerular filtration rate with plasma renin activity, active plasma renin concentration, angiotensin-II or aldosterone, across or within groups.

For a more extensive presentation and discussion of these results see section 4.2.1.

4.2.1 RENAL HAEMODYNAMICS AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN NORMOTENSIVE SUBJECTS WITH HYPERTENSIVE AND NORMOTENSIVE PARENTS *

Abstract

Background and Methods. The kidney is important in blood-pressure regulation, but its role in the development of essential hypertension is still subject to debate. We compared renal haemodynamics, measured in terms of the clearance of para-aminohippuric acid and inulin, and the characteristics of the renin-angiotensin-aldosterone system in three groups of normotensive subjects at different degrees of risk for hypertension: 41 subjects with two normotensive parents, 52 with one normotensive and one hypertensive parent, and 61 with two hypertensive parents. The subjects ranged in age from 7 to 32 years.

Results. The mean renal blood flow was lower in the subjects with two hypertensive parents than in those with two normotensive parents (mean difference [±SE], 198±61 ml per minute per 1.73 m^2 of body-surface are; P = 0.002). Moreover, both the filtration fraction and renal vascular resistance were higher in the subjects with two hypertensive parents (filtration fraction: mean difference, 3.0±1.1 percentage points; P = 0.006; renal vascular resistance: mean difference, 2.7±0.8 mm Hg per deciliter per minute per 1.73 m^2; P = 0.006). The subjects with two hypertensive parents had lower plasma concentrations of renin (mean difference, 3.3±1.6 mU per liter; P = 0.03) and aldosterone (mean difference, 111±36 pmol per liter; P = 0.003) than those with two normotensive parents. The differences could not be explained by the small differences in blood pressure between the groups. The values in the subjects with one hypertensive and one normotensive parent fell between those for the other two groups.

Conclusions. Renal vasoconstriction is increased and renin and aldosterone secretion is decreased in young persons at risk for hypertension. These findings support the hypothesis that alterations in renal haemodynamics occur at an early stage in the development of familial hypertension.

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* New England Journal Medicine 1991; 324:1305-11

Introduction

The role of the kidney as a long-term regulator of blood volume and blood pressure is well established.[1] Any increase in blood pressure is compensated for by an increase in the renal excretion of water and salt. During the development of hypertension, the relation between blood pressure and natriuresis is set at a higher level of arterial pressure, but the mechanism responsible for the change in the setting is unknown.[2] On the basis of the results of renal-transplantation experiments in animals and humans, it appears that this mechanism may reside in the kidney itself.[3-5] However, renal-function tests in subjects with established[6-9] or borderline[10,11] hypertension or in young subjects with hypertension[12-14] have not had consistent results.

Before hypertension develops, the influence of the kidney can be studied by comparing renal function in subjects with a high risk and those with a low risk of future hypertension. The risk of hypertension may be estimated on the basis of the presence or absence of a family history of hypertension.[15] Bianchi et al. reported a higher mean level of renal perfusion in young people with two hypertensive parents than in those with two normotensive parents.[16,17] These findings, however, contrast with those of others, which suggest that renal vasoconstriction is increased in the prehypertensive state.[18-22]

We studied renal haemodynamics and the renin-angiotensin-aldosterone system in 154 normotensive young people (age range, 7 to 32 years) from 97 families in which both, one, or neither of the parents had hypertension. To avoid errors in classifying the parents as hypertensive or normotensive, and in order to include relatively young children, we recruited the parents from a group of 1642 couples who participated in a population-based study, conducted in Zoetermeer, the Netherlands. Selection was based on blood-pressure levels that remained hypertensive or normotensive over a 10-year follow-up period. In this way we were able to study renal haemodynamics and the renin-angiotensin-aldosterone system in a relatively large, relatively young group of normotensive children of three groups of parents who differed markedly in blood-pressure characteristics.

Methods

Subjects

From 1975 to 1978, all the residents of two districts of the town of Zoetermeer, the Netherlands, were invited to participate in a study of blood pressure and other

cardiovascular risk factors.[23] Blood pressure was measured in 10,532 of the 13,462 eligible residents (78 percent). This group included 1642 couples with children. A stringent selection procedure, described previously,[24] was applied to these couples to select groups whose children would have a maximal contrast in familial predisposition to hypertension. Individual parents with both systolic and diastolic blood pressure in the upper (hypertensive) or lower (normotensive) quartile of the age- and sex-specific blood-pressure distribution were selected, as part of the Dutch Hypertension and Offspring Study, a collaborative study supervised by a steering committee drawn from four Dutch universities and clinical research centers. Those who were receiving antihypertensive medication were included in the hypertensive group. Three groups of couples with children were invited for remeasurement of blood pressure for this study after a period of more than ten years: couples of which both members were normotensive, those with one normotensive and one hypertensive member, and those of which both members had hypertension. At the time of remeasurement, the same criteria for hypertension and normotension were applied as at the initial screening. Of 250 couples that were remeasured (80 percent of those invited), 121 were still in the blood-pressure category to which they had originally been assigned: 35 couples of which both members were normotensive, 35 with one normotensive and one hypertensive member, and 51 of which both members were hypertensive. These 121 couples had 291 healthy biological children, all of whom were invited to take part in this study. Of these children, who ranged from 7 to 32 years of age, 154 participated: 41 with two normotensive parents, 52 with one normotensive and one hypertensive parent, and 61 with two hypertensive parents. The blood-pressure values and other characteristics of the parents and their children (subjects) at the time of enrollment are shown in Table 1. The study protocol was approved by the ethics committee of the University Hospital Dijkzigt, and informed consent was obtained from the subjects and their parents.

Protocol

All the subjects collected a 24-hour urine sample during the day before the study. Their usual diet was not altered, but they were asked to refrain from smoking and from drinking coffee. At the examination centre, the blood pressure in the left arm was measured with a random-zero sphygmomanometer by a trained paramedical assistant. Two readings were made with the subject seated, and the mean of these readings was used in the analysis. The

blood pressure of the parents had been measured in the same way at base line (from 1975 to 1978) and during the selection process for the current study (1986). Weight and height were measured with the subject wearing indoor clothes but no shoes. Before the beginning of the renal-function tests, an intravenous cannula was inserted: the subject then remained supine in a quiet room for 30 minutes, after which fasting venous blood samples were collected: 6 ml in a chilled tube containing disodium EDTA (final concentration, 5 mmol per liter) and *o*-phenanthroline (1.25 mmol per liter) for measurement of immunoreactive angiotensin II and 16 ml in a tube containing disodium EDTA (5 mmol per liter) for measurement of plasma renin activity and renin, prorenin, and aldosterone concentrations. For the renal-function tests, a second intravenous cannula was inserted in the opposite arm. The effective renal plasma flow and glomerular filtration rate were calculated on the basis of measurements of the clearance of para-aminohippuric acid and inulin (Inutest, Boehringer-Mannheim, Mannheim, Germany) with use of a constant-infusion technique and timed collections of urine.[25] After a bolus injection, a continuous infusion was given for 2½ hours to achieve levels of 200 mg of inulin per liter and 20 mg of para-aminohippuric acid per liter in extracellular fluid and plasma. During this period the subjects were not allowed to eat, but they were asked to drink at least 10 ml of fluid per kilogram of body weight per hour. To determine the starting dose of inulin and para-aminohippuric acid, the volume of extracellular fluid was estimated to be 20 percent of body weight. The doses for the subsequent infusion of inulin were calculated from the clearance of inulin according to the formula of Cockcroft and Gault, which estimates the creatinine clearance on the basis of age, weight, sex, and serum creatinine level.[26] To determine the doses of para-aminohippuric acid to be infused, the clearance of para-aminohippuric acid was assumed to be five times the calculated creatinine clearance. The amount of inulin and para-aminohippuric acid excreted by the kidneys was intended to be in equilibrium with the amount infused after 1½ hour. Urine samples were collected by active voiding before and 1½ and 2½ hours after the beginning of the infusion. Blood samples were collected just after voiding. Clearance rates were calculated from both the rate of intravenous infusion and the rate of urinary excretion for the 1-hour period between 1½ and 2½ hours after the beginning of the infusion.

Measurements

Inulin and para-aminohippuric acid were measured in the infused solution and in the plasma and urine samples drawn before and 1½ and 2½ hours after the beginning of the infusion. Inulin was measured indirectly. After deproteinization with 0.6 *N* hydrochloric tetroxide, endogenous fructose and glucose were measured (designated A_1). After a subsequent incubation period of 15 minutes at 70°C, inulin was converted into fructose, and the fructose was measured (A_2). The difference between the values A_2 and A_1 was the amount of fructose originating from the acid hydrolysis of inulin (coefficient of variation for the assay: in plasma, 2.2 percent; in urine, 2.1 percent). Fructose was measured by an enzymatic method (716260, Boehringer-Mannheim) adapted to an automatic analyzer (Cobas Bio, Hoffman-La Roche, Basel, Switzerland) in which the conversion of NADP to NADPH is measured. Para-aminohippuric acid was measured by the method of Bratton and Marshall as modified by Smith et al.,[27] after hydrolysis of conjugated para-aminohippuric acid by heating the sample with 4 *N* hydrochloric acid for one hour in a boiling-water bath (coefficient of variation: in plasma, 4.7 percent; in urine, 1.7 percent). In this way the rate of intravenous infusion could also be used to calculate the effective renal plasma flow from the total para-aminohippuric acid clearance.[25]

The glomerular filtration rate and the effective renal plasma flow were calculated from the rates of clearance of inulin and para-aminohippuric acid; these rates were derived, in turn, from the rate of intravenous infusion if the plasma levels in individual blood samples were within 5 percent of the mean inulin or para-aminohippuric acid levels (plasma method) and from the rate of urinary excretion if urine samples could be obtained at the three sampling times (urinary method). The renal-function tests were completed for 135 of the 154 subjects: 32 with two normotensive parents, 44 with one normotensive and one hypertensive parent, and 59 with two hypertensive parents. Results were unavailable for the remaining 19 subjects because they declined to participate in the infusion studies (n = 13), because the test was not completed (n = 3), or because blood or urine samples were missing (n = 3) so that neither the rate of urinary excretion nor the rate of intravenous infusion could be estimated. Of the results from the 135 completed renal-function tests, the rates of clearance of inulin or para-aminohippuric acid calculated by the urinary method could be used for 131 subjects (30 with two normotensive parents, 42 with one normotensive and one hypertensive parent, and 59 with two hypertensive parents); complete urine samples were

not available for the other 4 subjects. The plasma method could be used to calculate the glomerular filtration rate for 118 subjects (n = 27, 39, and 52, respectively). Calculations could not be made because of instability of the plasma inulin level in 17 subjects. The effective renal plasma flow could be calculated by the plasma method for 109 subjects with stable plasma levels of para-aminohippuric acid (n = 24, 38, and 47, respectively).

Renal blood flow was estimated by dividing the effective renal plasma flow by 1 minus the hematocrit. There was no reason to suspect differences in para-aminohippuric acid extraction among the groups, and no correction was made for incomplete extraction.[8] The filtration fraction was calculated by dividing the glomerular filtration rate by the effective renal plasma flow. All calculations were standardized for body-surface area.[28] Renal vascular resistance was estimated by dividing the calculated mean arterial pressure by the renal blood flow.

Immunoreactive angiotensin II and aldosterone were measured in plasma as described previously by Lijnen et al.[29] and Malvano et al.[30] Plasma renin was measured in two ways - as plasma renin activity and as the plasma renin concentration. Plasma renin activity was determined by a radioimmunoassay for angiotensin I generated from endogenous renin substrate and expressed in femtomoles per liter per second (coefficient of variation, 10 percent). Plasma renin concentrations were determined by measuring the capacity of renin to generate angiotensin from saturating amounts of purified sheep renin substrate; the angiotensin formed was measured by radioimmunoassay (coefficient of variation, 11 percent).[31] The conversion of prorenin to renin in plasma was activated by adding Sepharose-bound trypsin (coefficient of variation, 11 percent).[31] Renin and prorenin levels were expressed in terms of milliunits per liter of the Medical Research Council human kidney renin standard (MRC 68/356, WHO International Laboratory for Biological Standards, Holly Hill, Hampstead, London). One milliunit of this standard equals about 1.4 mg of renin.[32]

Statistical Analysis

Descriptive data for the three groups are presented as means and standard deviations. For comparisons between groups, means and standard errors of the means are given, with the two-sided P value for the difference. Adjustments for differences in age, height, weight, and proportion of males among the three groups were made with use of a multiple linear

regression model. Associations between variables were adjusted for differences in group characteristics (with use of indicator variables for group) and for age, height, weight, and sex (by multiple regression analysis).

Results

General Characteristics

Table 1 shows the general characteristics of the three groups of subjects. The systolic and diastolic blood pressure was higher in the subjects with two hypertensive parents than in the other two groups. These differences persisted after adjustments for differences in age, height, weight, and proportion of males among the groups. The serum levels and 24-hour urinary excretion of sodium and potassium were similar in the three groups, as was the hematocrit (data not shown).

Renal Haemodynamics

Renal-function characteristics calculated by the urinary method are shown in Table 2. All values have been standardized for body-surface area and adjusted for differences in age and proportion of males among the groups. Compared with the value in the group of subjects with two normotensive parents, renal blood flow was significantly lower in the group with two hypertensive parents (mean difference [±SE], 198±61 ml per minute per 1.73 m^2 of body-surface are; P = 0.002), but the glomerular filtration rate was not significantly different. The filtration fraction and renal vascular resistance were higher in the subjects with two hypertensive parents (filtration fraction: mean difference, 3.0±1.1 percentage points; P = 0.006; renal vascular resistance: mean difference, 2.7±0.8 mm Hg per deciliter per minute per 1.73 m^2; P = 0.006). The pattern of differences was similar when the renal-function characteristics were calculated by the plasma method (Table 2). To assess whether the difference in renal blood flow was confounded by the difference in blood pressure between the groups, the results were adjusted for systolic blood pressure. After adjustment, the mean renal blood flow as estimated by the urinary method was 1167 ml per minute per 1.73 m^2 in the subjects with two normotensive parents, 928 ml per minute per 1.73 m^2 in those with one normotensive and one hypertensive parent (mean difference, 239±65 ml per minute per 1.73 m^2; P<0.001), and 921 ml per minute per 1.73 m^2 in those with two hypertensive parents (mean difference, 246±62 ml per minute per 1.73 m^2; P<0.001).

Table 1 Blood Pressure and Related Characteristics of Subjects and Their Parents, According to Study Group.*

Characteristic	Two Normotensive Parents	One Normotensive and One Hypertensive Parent	Two Hypertensive Parents
Parents			
No. of couples	29	30	38
Age (yr)	46.7 ± 8.8	50.9 ± 8.9	52.1 ± 8.3
Blood pressure (mm Hg)			
Systolic	11.3 ± 6.3	133.0 ± 11.4	151.8 ± 14.1
Diastolic	68.5 ± 5.4	80.6 ± 6.9	89.8 ± 6.4
Drug treatment for hypertension (%)	0	23.3	38.2
Subjects			
Sex (M/F)	25/16	27/25	37/24
Age (yr)	21.3 ± 7.0	22.1 ± 6.1	23.4 ± 5.9
Height (cm)	170.0 ± 17.8	172.8 ± 13.3	175.2 ± 11.4
Weight (kg)	62.5 ± 19.2	63.6 ± 13.5	68.2 ± 13.5
Blood pressure (mm Hg)			
Systolic	117.7 ± 11.2	124.8 ± 13.1	128.0 ± 10.6
Diastolic	71.1 ± 8.7	75.8 ± 9.7	78.8 ± 7.8
Adjusted blood pressure (mm Hg)**			
Systolic	119.3 ± 9.3	125.4 ± 9.4	126.5 ± 9.4
Diastolic	71.6 ± 8.6	75.8 ± 8.6	78.4 ± 8.6
Serum sodium (mmol/liter)	141 ± 2.0	141 ± 2.3	141 ± 2.3
Urinary sodium (mmol/24 hr)	126 ± 51	136 ± 65	135 ± 49
Serum potassium (mmol/liter)	4.1 ± 0.3	4.2 ± 0.3	4.2 ± 0.3
Urinary potassium (mmol/24 hr)	67.7 ± 27.1	68.3 ± 25.5	63.8 ± 22.3

* Plus-minus values are means ±SD.

** Adjusted for differences among the groups in age, height, weight, and proportion of males.

To determine whether the difference in renal blood flow among the three groups was already apparent at a young age, when the blood-pressure differences between the groups were smaller and of shorter duration,[33] the three groups were divided into thirds according to age. For each stratum, the differences in renal blood flow among the three groups of subjects were similar (Fig. 1). For the youngest third of subjects (mean age, 11 years), the mean (±SE) differences between the subjects with two normotensive parents and the other two groups were as follows: subjects with one normotensive and one hypertensive parent, 55±94 ml per minute per 1.73 m^2 of body-surface area ($P = 0.50$); and subjects with two hypertensive parents, 346±102 ml per minute per 1.73 m^2 ($P = 0.004$). For the middle third (mean age, 19 years), the differences were as follows: subjects with one normotensive and

one hypertensive parent, 300±124 ml per minute per 1.73 m^2 (P = 0.02); and subjects with two hypertensive parents, 245±119 ml per minute per 1.73 m^2 (P = 0.04). For the oldest third (mean age, 27 years), the differences were as follows: subjects with one normotensive and one hypertensive parent, 175±90 ml per minute per 1.73 m^2 (P = 0.05); and subjects with two hypertensive parents, 181±82 ml per minute per 1.73 m^2 (P = 0.03). No relation was found between the renal blood flow or the glomerular filtration rate and 24-hour sodium excretion. Moreover, we found no significant association between the renal blood flow, renal vascular resistance, or the glomerular filtration rate on the one hand, and plasma renin activity or the plasma renin, angiotensin II, or aldosterone concentration on the other, either among or within groups.

Table 2 Renal-Function Characteristics in the Subjects, According to Study Group.+

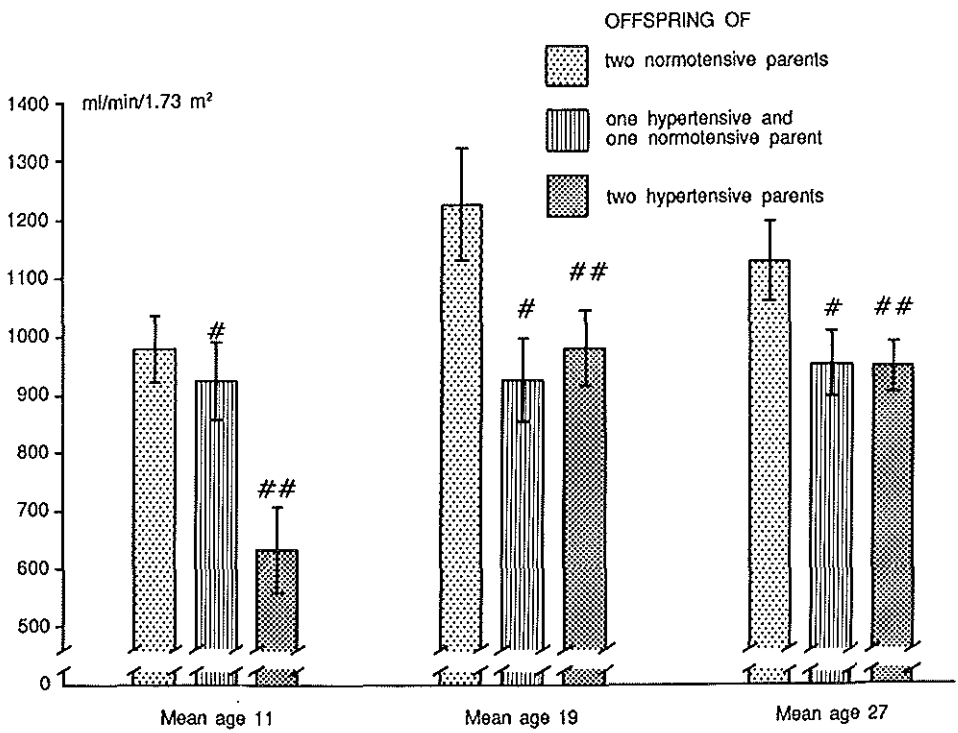
Characteristic	Two Normotensive Parents	One Normotensive and One Hypertensive Parent	Two Hypertensive Parents
Urinary method			
Effective renal plasma flow (ml/min/1.73 m^2)	709 ± 30	584 ± 25*	591 ± 21*
Renal blood flow (ml/min/1.73 m^2)	1132 ± 50	934 ± 41*	934 ± 35*
Glomerular filtration rate (ml/min/1.73 m^2)	147 ± 6	127 ± 5**	137 ± 4
Filtration fraction (%)	20.9 ± 0.9	22.0 ± 0.7	23.9 ± 0.6*
Renal vascular resistance (mm Hg/dl/min/1.73 m^2)	7.6 ± 0.6	9.5 ± 0.5**	10.3 ± 0.4*
Plasma method			
Effective renal plasma flow (ml/min/1.73 m^2)	566 ± 18	516 ± 14**	531 ± 13
Renal blood flow (ml/min/1.73 m^2)	903 ± 29	828 ± 23**	847 ± 20
Glomerular filtration rate (ml/min/1.73 m^2)	119 ± 3	114 ± 2	121 ± 2
Filtration fraction (%)	21.8 ± 0.7	22.3 ± 0.6	23.1 ± 0.5
Renal vascular resistance (mm Hg/dl/min/1.73 m^2)	9.1 ± 0.4	10.5 ± 0.3*	10.4 ± 0.3*

\+ Effective renal plasma flow, renal blood flow, glomerular filtration rate, filtration fraction, and renal vascular resistance were calculated from the clearance of para-aminohippuric acid or inulin both on the basis of the rate of urinary excretion (urinary method) and on the basis of the rate of intravenous infusion (plasma method). Values are means ±SE, adjusted for differences in age and sex.

* P<0.01 for the comparison with the subjects with two normotensive parents.

** P<0.05 for the comparison with the subjects with two normotensive parents.

Figure 1 Renal Blood Flow, as Measured by the Urinary Method, in Subjects with Two Normotensive Parents, One Normotensive and One Hypertensive Parent, or Two Hypertensive Parents, According to Age.
The values are means ± SE (indicated by I bars), adjusted for differences in age and sex. The group of subjects was divided into thirds according to age for this analysis.
Differences are given in comparison to subjects with two normotensive parents:



Difference, SE of the difference and P value, compared to the offspring of two normotensive parents:

Youngest third (mean age 11):
-55 ± 94 ml/min/1.73m², p=0.5
-346 ± 102 ml/min/1.73m², p= 0.004

Middle Third (mean age 19):
-300 ± 124 ml/min/1.73m², p=0.02
-245 ± 119 ml/min/1.73m², p= 0.04

Oldest Third (mean age 27):
-175 ± 90 ml/min/1.73m², p=0.05
-181 ± 82 ml/min/1.73m², p=0.03

Hormones

The results of the measurements of plasma renin activity and of the plasma renin, angiotensin, and aldosterone concentrations are shown in table 3. All values were adjusted for differences in age, height, weight, and proportion of males among the groups. The level of renin, measured as either plasma renin activity or the plasma renin concentration, was significantly lower in the subjects with two hypertensive parents (mean difference in the plasma renin concentration, 3.3±1.6 mU per liter; P = 0.03). The values for prorenin were similar among the three groups. The plasma aldosterone level was lower in the groups of subjects with one or two hypertensive parents than in the group with two normotensive parents (mean difference, 111±36 pmol per liter; P = 0.003). The difference in renin values was not reflected in the angiotensin II level, which did not differ significantly among the three groups. The ratio of the plasma angiotensin II level to plasma renin activity was higher among the subjects with two hypertensive parents than among the offspring of two normotensive parents (35.0 vs. 26.1, P = 0.02). The ratio of the plasma aldosterone level to the plasma angiotensin level was lower among subjects with two hypertensive parents than among those with two normotensive parents (36.1 vs. 50.7, P = 0.001).

Figure 2 shows the ratios of plasma renin activity and the plasma aldosterone concentration to urinary sodium excretion during a 24-hour period for each of the three study groups. Both ratios were lower in the subjects with two hypertensive parents. The differences in the ratios (±SE) between the subjects with one normotensive and one hypertensive parent and those with two normotensive parents were as follows: plasma renin activity, 0.64±0.64 fmol per liter per second per millimole of sodium per day (P = 0.3); and plasma aldosterone concentration, 1.08±0.52 pmol per liter per millimole of sodium per day (P = 0.04). The differences in the ratios (±SE) between the subjects with two hypertensive parents and those with two normotensive parents were as follows: plasma renin activity, 1.28±0.64 fmol per liter per second per millimole of sodium per day (P = 0.04); and plasma aldosterone concentration, 1.11±0.50 pmol per liter per millimole of sodium per day (P = 0.03).

As shown in table 1, the blood pressure was higher in the subjects with two hypertensive parents. To determine whether this factor might have influenced the difference in renin levels among the groups - for example, through renal autoregulation - we compared plasma renin activity and plasma renin concentrations among the groups after adjusting for

systolic blood pressure. The differences between the subjects with two normotensive parents and those with two hypertensive parents remained; the plasma renin concentration was lower by 3.3±1.6 mU per liter (P = 0.05) and plasma renin activity was lower by 117.1±53.1 fmol per liter per second (P = 0.04) in the subjects with two hypertensive parents. After we corrected the plasma renin concentration and renin activity for the differences between the groups in renal vascular resistance, a measure more directly related to the physiology of renin regulation, however, the differences were smaller and no longer statistically significant; the adjusted difference between the subjects with two normotensive parents and those with two hypertensive parents was 2.9±1.7 mU per liter for the plasma renin concentration (P = 0.10) and 97.7±49.4 fmol per liter per second for plasma renin activity (P = 0.06).

Table 3 Characteristics of the Renin-Angiotensin-Aldosterone System in the Subjects, According to Study Group.+

Characteristic	Two Normotensive Parents	One Normotensive and One Hypertensive Parent	Two Hypertensive Parents
Plasma renin activity (fmol/liter/sec)	464.8 ± 39.8	454.8 ± 35.3	362.0 ± 32.7*
Plasma renin (mU/liter)	18.3 ± 1.2	16.5 ± 1.1	15.0 ± 1.0*
Plasma prorenin (mU/liter)	124 ± 15	109 ± 13	107 ± 12
Plasma aldosterone (pmol/liter)	423 ± 28	333 ± 25*	317 ± 23**
Plasma angiotensin II (pmol/liter)	9.42 ± 0.89	8.84 ± 0.79	9.95 ± 0.73

\+ Values are means ±SE, adjusted for differences in age, height, weight, and sex.
* P<0.05 for the comparison with the subjects with two normotensive parents.
** P<0.01 for the comparison with the subjects with two normotensive parents.

Discussion

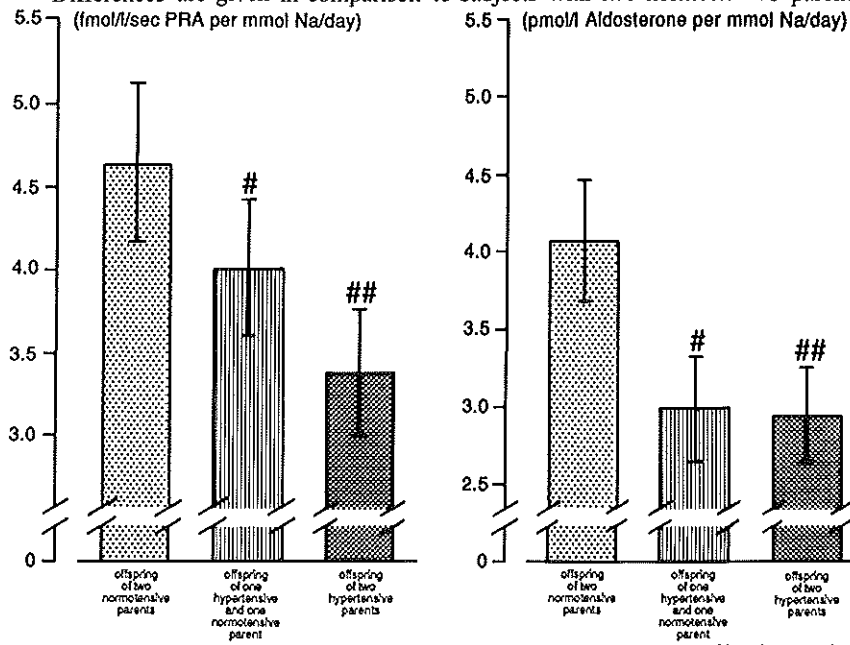
The findings of this study indicate that renal blood flow is lower and plasma renin and aldosterone concentrations are suppressed in the children of hypertensive parents, as compared with the children of normotensive parents. The reduction in renal blood flow was greater when renal haemodynamics were measured by the urinary method than when measured by the plasma method. The difference in the results obtained with the two methods may have resulted from the smaller number of test results that could be calculated by the plasma method. The absolute mean levels of renal blood flow were lower when calculated by the plasma method than when calculated by the urinary method. The opposite - i.e., a higher renal blood flow when calculated by the plasma method as compared with the urinary method - has been reported to occur as a result of extrarenal clearance of conjugates of para-aminohippuric acid.[25] In our study, however, this problem was eliminated by hydrolysis of the conjugated para-aminohippuric acid in the plasma samples. In addition, the temporary accumulation of para-aminohippuric acid and inulin in the kidney at the end of the equilibrium period and the beginning of the clearance period, which results from a relatively limited diureses during the equilibrium period, may further raise the estimates obtained by the urinary method. The direction of the differences among the three groups calculated by the two methods was similar, however. Interestingly, the mean renal blood flow was already lower at a mean age of 11 years in the subjects with one or two hypertensive parents.

We included a group of subjects with one normotensive and one hypertensive parent in our study because of the possibility of expanding the study with genetic analyses. In the comparisons, this group was not consistently similar to either of the other two groups, although their mean values in general fell between those for the other two groups.

The mechanisms responsible for the decrease in renal blood flow, the increase in renal vascular resistance and the filtration fraction, and the decrease in the plasma renin and aldosterone levels in the early phase of essential hypertension are not known. Increased renal vasoconstriction may reduce renal blood flow if the blood pressure and cardiac output remain normal. The combination of reduced renal blood flow with an increased filtration fraction and a reduced plasma renin concentration might point to an increase in resistance in renal efferent arterioles.[34] Such abnormalities, in a more pronounced form, have been found in adults with established hypertension.[35] Recently, Dluhy and coworkers proposed the

presence of "nonmodulators" in subjects with a family history of hypertension,[36] who are characterized by an inability to modulate normally the responsiveness of the renal vasculator and adrenal gland to angiotensin II at different levels of sodium intake.

Figure 2 Ratios of Plasma Renin Activity and of the Plasma Aldosterone Concentration to 24-Hour Sodium Excretion in Subjects with Two Normotensive Parents, One Normotensive and One Hypertensive Parent, or Two Hypertensive Parents.
The values are means ±SE (indicated by I bars), adjusted for differences in age, height, weight, and sex. Plasma renin activity was measured in femtomoles per liter per second, and the plasma aldosterone concentration in picomoles per liter; the ratios for each measure are expressed per millimole of sodium excreted per day.
Differences are given in comparison to subjects with two normotensive parents:



Difference, SE of the difference and P value, compared to the offspring of two normotensive parents:

Plasma renin activity:
\# -0.64 ± 0.64 fmol/l/sec per mmol Na/day (p=0.3)
\## -1.28 ± 0.64 fmol/l/sec per mmol Na/day (p=0.0.4)

Plasma aldosterone:
\# -1.08 ± 0.52 pmol/l per mmol Na/day (p=0.04),
\## -1.11 ± 0.50 pmol/l per mmol Na/day (p=0.03).

Such nonmodulation could reflect increased renal vasoconstriction. In our study several characteristics of the normotensive subjects with hypertensive parents were compatible with nonmodulation. In particular, the reduced renal blood flow in subjects with hypertensive parents resembled the reduced renal blood flow measured in nonmodulators with a high salt intake,[37,38] and the reduced ratio of plasma aldosterone to plasma angiotensin II is analogous to the diminished responsiveness of aldosterone to infused angiotensin II.[37] However, we found differences between the groups of subjects in our study without the infusion of vasoactive substances, perhaps because of the large contrast in familial predisposition to hypertension among the groups, which resulted from the strict selection criteria.

In apparent contrast with our findings are those of Bianchi and coworkers,[16,17] who reported that young people with a family history of hypertension had increased renal blood flow, normal cardiac output, and slightly increased glomerular filtration rates. The age range and blood-pressure level of these subjects were similar to those in our study group. Only measurements obtained by the plasma method were reported, however, and the conjugation of para-aminohippuric acid was not taken into account. If the extrarenal clearance of para-aminohippuric acid had been more pronounced in subjects with hypertensive parents, this difference could have artificially created the differences in renal blood flow observed between the groups.[25] Another problem with the calculation of renal blood flow and glomerular filtration rates by the plasma method may be the selective withdrawal of those with unstable plasma levels of para-aminohippuric acid and inulin.[25]

Differences in sodium balance might explain the different results of studies comparing renin profiles in various groups. We found that plasma renin activity and renin levels were lower in subjects with hypertensive parents, both with and without adjustment for sodium intake. Bianchi et al. reported both similar and decreased levels of renin in the children of hypertensive parents, as compared with those of normotensive parents.[16,17] In both these studies, the sodium balance was the same in both groups, but the mean intake was higher than in our study.

In conclusion, we found that renal blood flow, plasma renin activity, and plasma renin and aldosterone concentrations were lower and renal vascular resistance was higher in subjects with hypertensive parents than in those with normotensive parents. Our results support the hypothesis that changes in renal haemodynamics take place at an early stage in the development of familial hypertension. These changes may set the stage for a more rapid

and pronounced increase in blood pressure with age in the children of hypertensive parents.[2] The mechanisms responsible for the differences in renal haemodynamics remain to be established.

Acknowledgements

We are indebted to Professor H.A. Valkenburg for his general support and organization of the population study in Zoetermeer; to Professor P. van Brummelen, Professor K.H. Rahn, and Dr. Th. Thien for their support and contributions; to all the study subjects and their parents; to H. Kornman for assistance throughout the study; and to J. van 't Hof, J. Ambagtsheer, P. Tchang, R. de Bos, and R. de Bruin for their laboratory work.

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4.3 THE SYMPATHETIC NERVOUS SYSTEM

The activity of the sympathetic nervous system in the three groups of offspring was assessed in several ways, by measurement of catecholamines and of cellular adrenoreceptor densities.

Catecholamines were measured in plasma taken at rest after half an hour in supine position, and in an acidified 24-hour timed urine portion (see 3.3.2). Moreover, during the third phase of the Dutch Hypertension and Offspring Study (see 3.3.1), catecholamine excretion was measured in three timed urine-portions at rest during a two hour period, and after physical- and mental-stress testing.

All results are presented and discussed in section 4.3.1. Plasma adrenaline was slightly, but not significantly, higher in the offspring of two normotensive parents compared to the offspring of two hypertensive parents. No difference between the groups could be found in 24 hour urinary excretion of noradrenaline and adrenaline. Although a tendency towards higher levels of excretion in the children of two hypertensive parents was seen, trends across the three groups were not statistically significant. The urinary excretion of noradrenaline at rest, in a two hour urine sample, was somewhat increased in the offspring of hypertensive parents (2429±195 ng/hour) compared to the offspring of two normotensive parents (1908±219 ng/hour, difference 521±293 ng/hour, 95% confidence interval [-61, 1103], p=0.08). No difference was observed for urinary excretion of adrenaline in this two hour sample at rest.

The alpha$_2$-adrenoreceptor density on platelets was approximately 20% higher in the offspring of two hypertensive parents compared to offspring of two normotensive parents (difference: 48 fmol/mg protein, [18 to 78]). A positive linear trend of 22.8 fmol/mg protein (SEM=7.30, p<0.001) across the three groups was present, indicating a gradual increase in α_2-adrenoreceptor density between groups of offspring of two normotensive parents, one hypertensive and one normotensive parent and two hypertensive parents. No differences were found in the density of beta$_2$-adrenoreceptors between the groups of offspring. Consequently, the ratio of alpha$_2$-/beta$_2$- adrenoreceptor density was higher in the offspring of two hypertensive parents; 21.5 [0.9 to 42.1]. No differences between the groups in platelet α_2-adrenoreceptor or and lymphocytic $ß_2$-adrenoreceptor affinity was seen.

To assess the importance of the blood pressure differences between the groups in explaining the differences in α_2-adrenoreceptor density, the data were analysed taken blood pressure into account. The difference in α_2-adrenoreceptor density remained when

adjustments were made for SBP in a multivariate model (difference in α_2-adrenoreceptor density between offspring of two hypertensive parents and offspring of two normotensive parents 51.2 fmol/mg protein, [20.4 to 82.0]).

No relation was observed between adrenoreceptor density or affinity and age, height, body weight or gender. Also, α_2- or $ß_2$-adrenoreceptor density and systolic or diastolic blood pressure were not associated.

Across the three groups, no relation between the α_2- or $ß_2$-adrenoreceptor density and plasma catecholamine level or 24-hour urinary excretion of catecholamines was observed. However, the α_2-/$ß_2$-adrenoreceptor density ratio appeared to modify the relation between 24-hour urinary catecholamine excretion and the total peripheral resitance (TPR) in the groups of children of either one or two hypertensive parents; at levels above the median for α_2-/$ß_2$-adrenoreceptor density ratio a nonsignificant positive relation was found between 24-hour urinary excretion of adrenaline and TPR (b=0.23 (mmHg/l/min/1.73m ^{2})/(µg/24-hour), SEM=0.30); at or below the median for α_2-/$ß_2$-adrenoreceptor density ratio an inverse association was seen (b=-1.36 (mmHg/l/min/1.73m ^{2})/(µg/24-hour), SEM=0.65). The difference in slope was 1.59 with a 95% confidence interval of 0.18 to 3.00 (F test for interaction 2.74, p=0.02). See for presentation and discussion of these results section 4.3.1.

4.3.1 SYMPATHETIC NERVOUS SYSTEM ACTIVITY AND CELLULAR ADRENORECEPTORS IN OFFSPRING OF HYPERTENSIVE AND NORMOTENSIVE PARENTS *

Abstract

Enhanced sympathetic nervous system activity has been implicated in the etiology of primary hypertension. The objective of the present study was to assess the activity of the sympathetic nervous system and the role played by adrenoreceptors in the development of high blood pressure in humans. Young normotensive subjects with two, one or no hypertensive parents, and therefore at a different risk for hypertension, were studied.

Methods Three groups of normotensive youngsters participated in the study; 53 children of two normotensive parents, 58 children of one hypertensive and one normotensive parent and 69 children of two hypertensive parents. In these offspring the density of adrenoreceptors on blood cells, catecholamine levels in venous blood and in a 24-hour urine sample, and selected cardiovascular characteristics were measured (24-hour pulse rate and pulse rate variability, cardiac output, and calculated total peripheral vascular resistance).

Results The alpha$_2$-adrenoreceptor density on platelets was approximately 20% higher in the offspring of two hypertensive parents (285±10.0 fmol/mg protein) compared to the offspring of two normotensive parents (237±11.2 fmol/mg), with a difference of 48 fmol/mg, and a 95% confidence interval of [18 to 78], ($p<0.01$). The ratio of alpha$_2$-/beta$_2$- adrenoreceptor density was increased in the offspring of two hypertensive parents (154.3±6.57) compared to the offspring of two normotensive parents (132.8±8.01, difference 21.5 [0.9, 42.1], $p<0.05$). Plasma levels and 24 hour urinary excretion of catecholamines did not differ significantly between the groups. The urinary excretion of noradrenaline at rest, in a two hour urine sample, was somewhat increased in the offspring of hypertensive parents (2429±195 ng/hour) compared to the offspring of two normotensive parents (1908±219 ng/hour, difference 521±293 ng/hour, [-61, 1103], $p=0.08$).

Conclusion The results might suggest a modest increase in activity of the sympathetic nervous system at rest in youngsters at risk for hypertension. More clearly, the findings are compatible with a role of an increased density of cellular alpha$_2$-adrenoreceptors, and an

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* Submitted

Presented in part at the Second International Conference on Preventive Cardiology, 1989, Washington DC, USA

increased ratio of cellular alpha$_2$-/beta$_2$-adrenoreceptor density, that might represent an increased sympathetic resistance vessel tone and promote vasoconstriction, in the development of primary hypertension.

Introduction

A contribution of the sympathetic nervous system to the pathophysiology of primary hypertension has long been suspected.[1] The focus has been primarily on venous catecholamine levels as an estimate of sympathetic nervous activity.[2] In some studies, where hypertensive and normotensive subjects were compared, raised levels of plasma noradrenaline and adrenaline in hypertensive individuals have been detected.[2,3,4] This was most prominent in relatively young subjects or during stressful manoeuvres.[3,4] Between groups of normotensive youngsters with and without a family history of hypertension, no clear differences in venous plasma catecholamine levels were observed.[5,6,7] However, an exaggerated response to exogenous noradrenaline was reported in offspring of hypertensive families.[5,6,7,8] Recently, arterial noradrenaline levels were found to be increased in offspring of hypertensive parents compared to offspring of normotensive parents.[9]

Whether circulating catecholamines give rise to an exaggerated response not only depends on the level of catecholamines, but also on the number and affinity of adrenoreceptors on cardiac and vascular cells.[10] In view of the increased response to exogenous noradrenaline in offspring of hypertensive parents (see above), changes in characteristics of adrenoreceptors therefore might be important in mediating the effect of the sympathetic nervous system in youngsters at risk for hypertension. Properties of receptors, such as density or affinity, can be measured by radioligand binding studies.[10,11,12] As cardiovascular tissue is relatively inaccessible, studies of adrenoreceptors have been developed using blood cells; platelets for α_2-adrenoreceptors[11] and lymphocytes for β_2-adrenoreceptors.[12]

We studied the activity of the sympathetic nervous system and adrenoreceptor characteristics in groups of youngsters selected from families based on parental blood pressure levels from a well defined population based study.[13]

Subjects and methods

Subjects

The procedures used to select the study population have been described previously.[10, 13] In brief: From 1975 to 1979 all residents of two districts of the town of Zoetermeer in the Netherlands, were invited to participate in a study of blood pressure and other cardiovascular risk indicators.[14] Blood pressure was measured in 10,532 (78%) of 13,462 eligible subjects. This group included 1,642 parental couples. A stringent selection procedure was applied to these couples to select groups of offspring with a maximal contrast in familial predisposition for hypertension. Individual parents with both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the upper ("hypertensive") or lower ("normotensive") quartile of the age- and sex-specific blood pressure distribution were selected. Those on anti-hypertensive medication were included in the hypertensive group. In 1986, three groups of parental couples selected from this initial screening were invited for remeasurement of blood pressure: couples of two hypertensive parents, of one hypertensive and one normotensive parent and couples of two normotensive parents. At this occasion, the same criteria for hypertension and normotension were applied as for the initial examination. Of 250 parental couples that were remeasured (80% of those invited), 35 couples remained in the group with *two normotensive parents*, 35 in the group with *one hypertensive and one normotensive parent* and 51 in the group with *two hypertensive parents*. These parents together had 291 healthy biological children, aged 5 to 30 years, who were invited to take part in this study. Of these, 180 (62%) participated: 53 children with two normotensive parents, 58 children with one hypertensive and one normotensive parent and 69 children with two hypertensive parents.[15]

Protocol

Blood pressure was measured on the left arm with a random-zero sphygmomanometer, by a trained paramedical assistant. Two readings were made with the subject sitting and the mean of these readings was used in the analysis. The procedure was repeated with the subject in supine position. Body weight and height were measured with the subject wearing indoor clothes without shoes. Fasting venous blood samples were obtained, by an intravenous cannula inserted 30 minutes before sampling. During these 30 minutes the subject had been lying supine in a quiet room. After the blood samples were taken, a 4-chamber

echocardiogram using 2D-echocardiography was made to measure cardiac output and cardiac dimensions. The participants collected one 24-hour urine sample the day before visiting the examination centre and refrained from smoking and coffee use during that day until after the examination. On a day of their own choice, participants were invited to carry an ambulatory blood pressure measurement device (Spacelabs 90202 monitor, Redmond, Washington, USA) during a 24-hour period for measurement of blood pressure and heart rate. The ambulatory blood pressure monitor was applied to the non-dominant arm to take measurements at a frequency of one reading an hour between midnight and 6.00 a.m., three times an hour between 6.00 a.m. and 6.00 p.m. and twice an hour between 6.00 p.m. and midnight. The results of the readings were not disclosed to the participant. On a separate occasion, during the third phase of the Dutch Hypertension and Offspring Study in the offspring of two hypertensive parents and the offspring of two normotensive parents, a timed two-hour urine sample was collected during a two hour stay at rest in the research centre, for measurements of fasting urinary catecholamine excretion at rest, without the possible confounding of differences in diet and activities that might have occurred between the groups during the 24-hour period of urine sampling.

Measurements

Platelets were isolated from platelet-rich plasma, prepared from 20 ml of blood containing EDTA, immediately frozen and stored at -80°C. Platelet α_2-adrenoreceptors were measured by Scatchard analysis of ^{3}H-Yohimbine binding on cell membranes.[11] From 20 ml of heparinized blood, lymphocytes were isolated and kept frozen for a binding assay of ^{125}I-cyanopindolol to lymphocytic membranes, after counting cell numbers.[12]

Plasma from 10 ml of immediately chilled, heparinized blood, containing 12 mg reduced glutathione, was frozen and kept at -80°C for analysis of catecholamines using high-performance liquid chromatography with electrochemical detection.[16] Aliquots of 100 ml of the timed 24-hour urine sample, and of the timed 2-hour sample, were acidified and frozen for analysis of sodium and catecholamine excretion.

Cardiac output was calculated as the mean from six 4-chamber echocardiograms by area-length method. Cardiac index (CI) was estimated from cardiac output and calculated body surface area.[15, 16] Mean arterial pressure (MAP) was calculated from SBP and DBP measured in supine position, and was used to calculate total peripheral vascular resistance

by dividing MAP by CI (both measured in supine position). To obtain the mean 24-hour heart rate, heart rate was calculated per period of the day (midnight - 6.00 a.m., 6.00 a.m. - 6.00 p.m., 6.00 p.m. - midnight) if at least 75% of the measurements per period were successful. Readings with less than 75% successful measurements per period were left out. Mean 24-hour heart rate was averaged from the three periods, weighted for number of hours per period. As a measure of 24-hour heart rate variability the standard deviation of the mean 24-hour heart rate was divided by the mean 24-hour heart rate.

Statistics

Descriptive data for the three groups are presented as means and standard deviations. For comparisons between groups, means and standard errors are given with the difference and 95% confidence intervals of the difference between groups. Adjustments for differences in age, height, weight and proportion of males between the three groups were made using a model for multiple linear regression. Trend analyses were performed with a model for linear regression, after checking for non-linear trends and weighted for differences in variance between groups and adjusted for differences in age, height, weight and proportion of males. Associations between study variables were studied across groups adjusted for group characteristics (using indicator variables), and age, height, weight and gender by multiple regression analysis.

Results

General characteristics

Table 1 provides a general description of the three groups. At a mean age of 20 years, a difference in sitting SBP and DBP and supine MAP between the offspring of two hypertensive parents and the offspring of two normotensive parents was already present. The blood pressure difference remained after adjustment for differences in age, height, body weight and proportion of males between the groups.

Sympathetic nervous system

Venous plasma noradrenaline levels were similar in the groups (table 2). Venous plasma adrenaline was slightly, but not significantly, higher in the offspring of two normotensive parents compared to the offspring of two hypertensive parents. No difference between the

Table 1 Blood pressure and related characteristics in offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents.*

	offspring of two normotensive parents	offspring of one hypertensive parent	offspring of two hypertensive parents
Males/Females (number)	34 / 19	29 / 29	40 / 29
Age (years)	18.7 ± 7.3	20.4 ± 6.4	22.1 ± 6.4
Height (cm)	166.0 ± 21.8	169.8 ± 15.9	172.7 ± 14.9
Body weight (kg)	57.7 ± 20.6	60.0 ± 14.9	66.1 ± 15.1
SBP** sitting (mmHg)	116.1 ± 11.7	123.6 ± 13.1	127.2 ± 11.4
DBP** sitting (mmHg)	70.3 ± 8.9	74.9 ± 9.8	78.9 ± 8.1
MAP** supine (mmHg)	78.3 ± 7.8	81.8 ± 8.7	87.2 ± 8.4
SBP*** sitting (mmHg)	117.9 ± 9.2	124.2 ± 9.1	125.3 ± 9.2
DBP*** sitting (mmHg)	70.3 ± 8.8	74.9 ± 8.8	78.4 ± 8.9
MAP*** supine (mmHg)	79.3 ± 7.5	82.3 ± 7.5	86.1 ± 7.5
Urinary sodium excretion (mmol/24 hour)	128 ± 56	134 ± 70	134 ± 60

* Values are means ± SD.
** SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial blood pressure.
*** Adjusted for differences between the groups in age, height, body weight and proportion of males.

groups could be found in 24 hour urinary excretion of noradrenaline and adrenaline (table 2). Although a tendency towards higher levels of excretion in the children of two hypertensive parents was seen, trends across the three groups were not statistically significant. In the two-hour urine collection, sampled during protocolized rest at the research centre, an increase in urinary excretion of noradrenaline of borderline significance was observed in the offspring of two hypertensive parents compared to the offspring of two normotensive parents (adjusted mean difference 521 ± 293 ng/hour, 95% confidence interval [-61, 1103], p=0.08). No difference between the two groups was found for the urinary adrenaline excretion in the two-hour urine sample at rest.

Findings on density and affinity of platelet α_2- and lymphocytic β_2-adrenoreceptors are given in table 2. Mean platelet α_2-adrenoreceptor density was higher in the offspring of two hypertensive parents (285±10.0 fmol/mg protein) compared to the offspring of two normotensive parents (237±11.2 fmol/mg, difference 48 [18 to 78], $p<0.01$), with the offspring of one hypertensive parent in between (249±10.9 fmol/mg protein). For the platelet α_2-adrenoreceptor density a positive linear trend of 23 fmol/mg protein (SEM=7.30, $p<0.001$) across the three groups was present, indicating a gradual increase in α_2-adrenoreceptor density from the offspring of two normotensive parents, to the offspring of one hypertensive and one normotensive parent to the offspring of two hypertensive parents. No difference in α_2-adrenoreceptor affinity was seen between the groups. The groups of offspring had similar lymphocytic β_2-adrenoreceptor density and affinity (table 2). The ratio of α_2-/β_2-adrenoreceptor density was relatively increased in the children of two hypertensive parents (154.3±6.57) compared to the offspring of two normotensive parents (132.8±8.01, difference 21.5 [0.9, 42.1], $p<0.05$), (table 2).

To assess the importance of the blood pressure differences between the groups for the differences in α_2-adrenoreceptor density, the data were analysed taking blood pressure into account. The difference in α_2-adrenoreceptor density remained essentially unchanged when adjustments were made for SBP in a multivariate model (difference in α_2-adrenoreceptor density between offspring of two hypertensive parents and offspring of two normotensive parents 51.2 fmol/mg protein, [20.4 to 82.0], $p<0.01$). No relation was observed between adrenoreceptor density or affinity with age, height, body weight or gender. Also, α_2- or β_2-adrenoreceptor density and systolic or diastolic blood pressure were not associated.

Mean heart rate per 24 hour period, heart rate variability and cardiac index were similar in the groups (Table 2). Total vascular peripheral resistance was slightly higher in the children of two hypertensive parents compared to children of two normotensive parents (table 2), and showed a positive trend across the groups (2.13 mmHg/l/min/1.73m^2, SEM=1.09, p=0.05).

Across the three groups, no relation between the α_2- or β_2-adrenoreceptor density and plasma catecholamine level or 24-hour urinary excretion of catecholamines was observed. Therefore, results of the α_2- or β_2-adrenoreceptor density for the three groups of

Table 2 Parameters of sympathetic nervous system activity and cardiovascular characteristics in the offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents. Cellular α_2- and β_2-adrenoreceptor densities (Bmax) and binding coefficients (Kd), plasma catecholamine level, 24-hour and 2-hour urinary excretion of catecholamines, mean 24-hour pulse rate and 24-hour pulse rate variability, cardiac index and total peripheral vascular resistance (TPR), adjusted for differences in age, height, body weight and gender.+

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A		
Sympathetic nervous system											
Noradrenaline (pg/ml plasma)	246	(14.0)	250	(11.7)	235	(12.0)	4	[-33, 41]	-11	[-48, 26]	
Adrenaline (pg/ml plasma)	27.4	(2.52)	22.1	(2.10)	23.3	(2.15)	-5.3	[-11.8, 1.2]	-4.1	[-10.6, 2.4]	
Noradrenaline (µg/24-hour urine)	26.7	(2.59)	27.3	(2.41)	31.0	(2.16)	0.6	[-6.5, 7.7]	4.3	[-2.4, 11.0]	
Adrenaline (µg/24-hour urine)	5.5	(0.67)	5.5	(0.62)	6.3	(0.56)	0.0	[-1.8, 1.8]	0.8	[-1.0, 2.6]	
Noradrenaline (ng/hour urine at rest)	1908	(219)	not measured		2429	(195)	not measured		521	[-61, 1103]	
Adrenaline (ng/hour urine at rest)	470	(66)	not measured		567	(59)	not measured		97	[-80, 274]	
α_2 Bmax (fmol/mg protein)	237	(11.2)	249	(10.9)	285	(10.0)	12	[-19, 43]	48	[18, 78]	**
β_2 Bmax (fmol/10^6 cells)	1.81	(0.030)	1.88	(0.028)	1.83	(0.025)	0.07	[-0.02, 0.16]	0.02	[-0.06, 0.10]	
α_2-β_2 Bmax ratio	132.8	(8.01)	128.5	(7.53)	154.3	(6.57)	-4.3	[-26.2, 17.6]	21.5	[0.9, 42.1]	*
α_2 Kd (nM)	2.26	(0.087)	2.30	(0.084)	2.26	(0.078)	0.04	[-0.21, 0.29]	0.00	[-0.24, 0.24]	
β_2 Kd (pM)	41.2	(1.17)	40.2	(1.08)	41.9	(0.96)	-1.0	[-4.2, 2.2]	0.7	[-2.4, 3.8]	

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	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
Cardiovascular system										
Mean 24-hour pulse rate (beats/min)	75.1	(1.51)	76.5	(1.36)	73.7	(1.19)	1.4	[-2.6, 5.4]	-1.4	[-5.2, 2.4]
24-hour pulse rate variability (%)	17	(0.9)	18	(0.8)	18	(0.7)	1	[-2, 4]	1	[-2, 4]
Cardiac index (l/min/1.73 m^2)	1.85	(0.062)	1.80	(0.066)	1.80	(0.062)	-0.05	[-0.23, 0.13]	-0.05	[-0.23, 0.13]
TPR (mmHg/l/min/1.73m^2)	44.4	(1.73)	47.9	(1.84)	48.7	(1.73)	3.5	[-1.6, 8.6]	4.3	[-0.7, 9.3]

\+ Values are means with SEM in parentheses, and differences between offspring of two hypertensive parents and offspring of two normotensive parents with 95% confidence intervals in brackets.

* $p < 0.05$ for the difference between offspring of two hypertensive parents and offspring of two normotensive parents.

** $p < 0.01$ for the difference between offspring of two hypertensive parents and offspring of two normotensive parents.

offspring remained unaltered if an additional adjustment was done for 24-hour urinary excretion of either noradrenaline or adrenaline, or for plasma level of noradrenaline and adrenaline. However, the α_2-/$ß_2$-adrenoreceptor density ratio appeared to modify the relation between 24-hour urinary catecholamine excretion and the total peripheral resistance in the groups of children of either one or two hypertensive parents; at levels above the median for α_2-/$ß_2$-adrenoreceptor density ratio a nonsignificant positive relation was found between 24-hour urinary excretion of adrenaline and total peripheral resistance (b=0.23 (mmHg/l/min/1.73m^2)/(µg/24-hour), SEM=0.30); at or below the median for α_2-/$ß_2$-adrenoreceptor density ratio an inverse association was seen (b=-1.36 (mmHg/l/min/1.73m^2)/(µg/24-hour), SEM=0.65). The difference in slope was 1.59 with a 95% confidence interval of 0.18 to 3.00 (F test for interaction 2.74, p=0.02).

Discussion

The findings of this study suggest a slightly higher urinary noradrenaline excretion in the offspring of two hypertensive parents, as assessed in a two hour resting urine sample. No increase was found using venous plasma samples or 24 hour urinary excretion. A higher density of α_2-adrenoreceptors was observed in the offspring of two hypertensive parents, with the offspring of one hypertensive parent in an intermediate position. No difference between the groups was found for the $ß_2$-adrenoreceptor density. However, the α_2-/$ß_2$-adrenoreceptor density ratio was higher in the offspring of two hypertensive parents. In offspring of one or two hypertensive parents with an α_2-/$ß_2$-adrenoreceptor density ratio above the median a positive association was observed for 24-hour urinary excretion of adrenaline and total peripheral resistance.

Our findings of similar venous plasma and 24-hour urinary levels of catecholamines in three groups of children at a different probability for hypertension, could indicate that the level of sympathetic nervous system activity is not increased at rest in the early phase of primary hypertension. However, venous plasma levels of catecholamines may not adequately represent sympathetic nervous system activity at rest.[17] During rest, arterial plasma levels or total body spillover rate of noradrenaline might be more informative on the activity level of the sympathetic nervous system.[9] Arterial sampling was not feasible in this study. Therefore, our indices of autonomic function are relatively crude and one must be cautious not to overemphasize the negative results. As the precision of catecholamine

measurement in 24-hour urine samples during daily life might easily be affected by sampling errors and by differences in activity or diets during a 24-hour period, a shorter sampling time under controlled circumstances might reveal differences between the youngsters at different risk for hypertension. Therefore, during a third phase of The Dutch Hypertension and Offspring Study, a fasting, timed two hour urine sample collected during a protocolized leisure stay at the research centre was used to measure catecholamines. Using this sample, a more marked difference in noradrenaline between the offspring of two hypertensive parents and the offspring of two normotensive parents was observed compared to the 24-hour urine sample. This was not shown for adrenaline. The finding of an elevated noradrenaline at rest, and not of adrenaline, in the offspring of two hypertensive parents, compared to the offspring of two normotensive parents, is coherent with results reported recently by Ferrier et al. using a more sophisticated technique for measurement of sympathetic nervous sytem activity.[9]

Our finding of an increased platelet α_2-adrenoreceptor density in youngsters with a raised probability to develop hypertension later in life is supported in part by findings in other studies using smaller or less well defined groups with a contrasting family history of hypertension, in either hypertensive subjects,[18] or in normotensive subjects.[19] However, the presence and role of changes in adrenoreceptor characteristics in established hypertension is controversial. Studies comparing platelet α_2- and lymphocytic $ß_2$-adrenoreceptor densities in hypertensive and normotensive subjects have yielded conflicting results.[18,20,21,22,23,24,25] This may be due to differences in selection of study groups, and to the methods used to measure the adrenoreceptor densities. Moreover, it is not clear if adrenoreceptor densities are influenced by sympathetic nerve activity or circulating catecholamines[26,27] and whether changes in adrenoreceptors develop secondary to hypertension. Studies taking the family history of hypertension into account, have reported a higher density of α_2-adrenoreceptors in the group of hypertensive subjects with a positive family history compared to hypertensive subjects without a family history for hypertension.[18] A similar pattern was found if normotensive offspring with and without a family history of hypertension were compared,[19] however, this was not reported by others.[28,29] Michel et al.[19] reported an increased density of platelet α_2-adrenoreceptors in the normotensive offspring of one hypertensive parent compared to offspring of normotensive parents, without differences in circulating catecholamine levels. We could not

detect a difference in density of platelet α_2-adrenoreceptors between the offspring of one hypertensive parent and the offspring of two normotensive parents, but the offspring of one hypertensive parent had an intermediate density of platelet α_2-adrenoreceptors with a level between the offspring of two hypertensive and the offspring of two normotensive parents. Skrabal et al.[28] did not observe an increased platelet α_2-adrenoreceptor density in normotensive youngsters with a family history of hypertension, and Umemura et al.[29] only on a low sodium diet. Skrabal[28] however, did report an increased platelet α_2-adrenoreceptor density in salt sensitive normotensive youngsters. From these findings,[19,28,29] and our results of a gradual increase in α_2-adrenoreceptor density in offspring of two normotensive, one hypertensive and one normotensive, and two hypertensive parents, we suggest that the number of platelet α_2-adrenoreceptors might be a marker for familial risk for future hypertension, possibly even in relation to salt sensitivity.

The ratio of α_2-/$ß_2$-adrenoreceptor density was calculated, as this ratio had been shown to be of relevance as a marker in other diseases such as atopic illnesses and cystic fibrosis.[10] With regard to vasoconstriction the cellular α_2-/$ß_2$-adrenoreceptor ratio may also be of importance; the platelet α_2-adrenoreceptor is inhibitorily coupled to adenylate cyclase, giving vasoconstriction if located in vascular smooth muscle cells, and the lymphocytic $ß_2$-adrenoreceptor is excitatorily coupled to adenylate cyclase, giving vasodilatation if located in vascular smooth muscle cells.[10] The possible importance of the cellular α_2-/$ß_2$-adrenoreceptor density ratio in primary hypertension was suggested before by Skrabal et al.[30] They observed a relation between the rise in α_2-/$ß_2$-adrenoreceptor density ratio with the blood pressure increase seen in normotensive subjects on change from a low to high sodium intake.[30] In view of these results and the known opposing effects of the α_2- versus $ß_2$-adrenoreceptors on resistance vessels, and on renal tubules, it was suggested that the α_2-$ß_2$-adrenoreceptor density ratio might represent sympathetic resistance vessel tone, especially as a function of variations in salt intake.[30]

The vascular response to activity of the sympathetic nervous system may be enhanced by an increased cellular α_2-/$ß_2$-adrenoreceptor density ratio, as present in the group with two hypertensive parents. As the cellular α_2-/$ß_2$-adrenoreceptor density ratio might represent sympathetic resistance vessel tone,[30] the association between catecholamines and calculated total peripheral resistance was stratified for the cellular α_2-/$ß_2$-adrenoreceptor density ratio. In this way, at a high level of the cellular α_2-/$ß_2$-adrenoreceptor density ratio

(above the median) a positive association was seen between 24-hour urinary adrenaline excretion and total peripheral resistance. At a low level of cellular α_2-/β_2-adrenoreceptor density ratio (below the median) an inverse association was seen. As α_2-adrenoreceptors located post-synaptically promote vasoconstriction in competition with the post-synaptically located vasodilating β_2-adrenoreceptor, the opposite association between catecholamines and total peripheral resistance observed in our study at a high or low level of cellular α_2-/β_2-adrenoreceptor density ratio can be understood.

To conclude that an increased cellular α_2-adrenoreceptor density or α_2-/β_2-adrenoreceptor density ratio in prehypertensive subjects may play a role in the etiology of primary hypertension some issues need to be addressed. First the extent to which cellular α_2- and β_2-adrenoreceptors are a qualitative and quantitative measure for adrenoreceptors in the cardiovascular system at large should be clear. Second, the function and location of the cardiovascular adrenoreceptors needs to be related to blood pressure regulation.

From binding studies in different tissue-types and from infusion studies using selective agonists and antagonists, evidence has been obtained that α_2-adrenoreceptors are functionally present in cardiovascular, cerebral and renal tissues.[10,31,32,33] Moreover, platelet α_2-adrenoreceptors and lymphocytic β_2-adrenoreceptors have similar binding characteristics and appear to reflect changes in number of these adrenoreceptors in solid tissues as the human heart, the human saphenous vein (β_2-adrenoreceptor density and affinity),[34] human myometrium (α_2-adrenoreceptor density and affinity),[35] and the human kidney (α_2-adrenoreceptor binding characteristics).[36]

Animal studies have shown that α_2-adrenoreceptors are present on large arterioles and venules in skeletal muscle and in precapillary arterioles, and that their stimulation leads to vasoconstriction.[32,37] In the kidney of the rat α_2-adrenoreceptors are found in the renal cortex at different sites and are involved in renal tubular sodium and water reabsorption, renin secretion and renal vasoconstriction.[32,38] Infusion in humans of adrenaline or selective α_2-adrenoreceptor agonists, combined with ß-blockade or selective α_1- and/or α_2-adrenoreceptor antagonists, showed a vasoconstricting effect of peripheral α_2-adrenoreceptor stimulation that appears to be enhanced in hypertensive subjects.[39] Intra-renal infusions of α_1- and α_2-antagonists in humans at rest and during isometric exercise showed that constriction of the renal vessels occurs predominantly through stimulation of α_2-adrenoreceptors.[33]

We conclude that our findings of an increased platelet α_2-adrenoreceptor density and cellular α_2-/β_2-adrenoreceptor density ratio in the offspring of hypertensive parents might represent an increased sympathetic resistance vessel tone, and might be a marker of their future risk for hypertension. If we speculate with our findings in relation to functional characteristics of cardiovascular and renal adrenoreceptors in blood pressure regulating mechanisms known from other reports, an increase of the vascular α_2-/β_2- adrenoreceptor density ratio in young subjects at risk for hypertension is likely to promote vasoconstriction in the years to come. This would lead to an increase in vascular resistance and an elevation of blood pressure. In addition, if the increased ratio of α_2-/β_2-adrenoreceptor density is also present in the kidney, changes in renal haemodynamics and sodium excreting mechanisms might further contribute to a rise in blood pressure.[40,41]

Acknowledgements

This study was supported by grant 35.004 from the Netherlands Heart Foundation. The Dutch Hypertension and Offspring Study is a collaborative study supervised by a Steering Committee with members of four Dutch universities and major clinical research centers. The study is conducted in Zoetermeer, in the Netherlands. Prof. H.A. Valkenburg is acknowledged for his general support and organisation of the population study in Zoetermeer. Prof. M.A.D.H. Schalekamp, Prof. P. van Brummelen, Prof. P.W. de Leeuw, Prof. K.H. Rahn and Prof. Th. Thien, as members of the Steering Committee, are gratefully acknowledged for their support and contribution. We are greatly indebted to all participants in this study and their parents. Finally we like to thank H. Kornman for assistance throughout the study and, with G. Jansen, for preparation of the blood samples.

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4.4 ELECTROLYTE HOMOEOSTASIS

Factors related to sodium homoeostasis

Atrial natriuretic factor

The level of atrial natriuretic peptide (ANP) was studied in relation to sodium excretion and blood pressure. Mean plasma ANP levels were similar among the there groups of offspring (section 4.4.1), and no overall relation with sodium excretion was seen. However, if the offspring of two hypertensive parents and two normotensive parents was stratified by either high or low 24-hour sodium excretion a difference in mean level of plasma ANP could be observed on a high sodium intake with a decrease in the offspring of two hypertensive parents compared to the offspring of two normotensive parents (section 4.4.2).

Both height and body weight showed an inverse association with ANP (section 4.4.1). Moreover in the total group, ANP was negatively associated with DBP. This overall association resulted from an association in the offspring of two normotensive parents (b=-0.13 mmHg/(pg/ml), (se=0.05), p=0.02), but not in the offspring of two hypertensive parents (-0.06 mmHg/(pg/ml) (0.04), ns).

Sodium intake

24-hour Urinary electrolyte excretion, (sodium and potassium) was not different among the groups (section 4.4.3). 24-Hour sodium excretion was associated with SBP in the three groups of offspring combined and in the offspring of two hypertensive parents or of one hypertensive parent separately, but not in the offspring of two normotensive parents (section 4.4.1).

Sodium-potassium ATP-ase activity in the erythrocytic membrane

The mean level of sodium-potassium ATP-ase activity was slightly, but not significantly, higher in the offspring of two hypertensive parents and one hypertensive parent, compared to offspring of two normotensive parents (section 4.4.3). No association of Na-K-ATPase activity blood pressure or with 24-hour urinary sodium excretion was observed.

Circulating Inhibitor

No difference in total nonesterified fatty acids in plasma, as a measure of a possible circulating inhibiting factor, (section 1.2.4, ref. 188 - 189), was found between the group of offspring (section 4.4.3). However, fractions of nonesterified fatty acids appeared to be different between the groups. Compared to the offspring of two normotensive parents a high fraction of palmitoleic acid was present both in the offspring of two hypertensive parents and in the offspring of one hypertensive parent. Also the fractions of myristic and linoleic acid were increased, and the fraction of stearic acid was somewhat decreased in the offspring of one hypertensive parent (section 4.3.3). To study whether differences in total fat intake influenced the pattern of nonsaturated fatty acids among the groups of offspring, both the level of total non-esterified fatty acids and fractions of the 6 types of fatty acids were studied after additional adjustment for total fat intake. However, this adjustment did not change the results of total non-esterified fatty acids and fraction of myristic, palmitoleic, linoleic acid and stearic acid. Across the groups no association was seen for the level of non-esterified fatty acids and blood pressure or 24-hour urinary sodium excretion.

Intra-cellular electrolytes

Total intra-erythrocytic sodium and potassium did not differ between the groups (section 4.4.3) The intra-erythrocytic sodium-potassium ratio showed a weak positive association with systolic blood pressure across the groups. No association between 24-hour urinary sodium excretion and intra-erythrocytic electrolytes was found. No clear relation was seen between Na-K-ATPase activity and intra-erythrocytic sodium, but a significant inverse relation was present for Na-K-ATPasé with both intra-erythrocytic potassium, and intra-erythrocytic sodium/potassium ratio (section 4.4.3).

Sodium excretion; salivary gland and kidney

Excretion of sodium in saliva, sampled in a fasting period, was somewhat increased in the offspring of two hypertensive parents, although not statistically significant. Potassium excretion in saliva was similar for the three groups. No relation of salivary electrolyte excretion with blood pressure or 24-hour urinary sodium excretion was found. Moreover, there was no association between Na-K-ATPase activity and salivary sodium, but a positive relation with salivary potassium was present (section 4.4.3).

Factors related to sodium homoeostasis

Findings on renal sodium, lithium and uric acid excretion, calculated in a three hour fasting clearance period, are presented in section 4.4.3. Fractional lithium excretion appeared to be somewhat lower in the offspring of two hypertensive parents, and fractional uric acid excretion was significantly decreased in the offspring of two hypertensive parents. Although no clear association with 24-hour sodium excretion was shown, the difference in fractional excretion of uric acid between the offspring of two hypertensive parents and the offspring of two normotensive parents was attenuated when adjusted for 24-hour urinary sodium excretion. Renal fractional excretion of sodium, lithium or uric acid was not associated with blood pressure.

Summary

Although the findings in the Dutch Hypertension and Offspring Study are not conclusive, it appears that offspring of hypertensive parents are different from offspring of normotensive patents with regard to sodium homoeostasis and blood pressure regulation. An increased 24-hour urinary sodium excretion was associated with an elevated SBP in offspring of hypertensive parents, but not in offspring of normotensive parents. Moreover, on a relatively high 24-hour sodium excretion the level of atrial natriuretic peptide was lower in offspring of hypertensive parents compared to offspring of normotensive parents. In the offspring of normotensive parents an inverse association of atrial natriuretic peptide with blood pressure was seen. It cannot be excluded that the positive assocation of blood pressure with sodium intake in the offspring of hypertensive parents is a consequence of an insufficient response of atrial natriuretic peptide to an increase in sodium intake. A tendency to sodium retention in offspring of hypertensive parents relative to offspring of normotensive parents could be reflected in a lower fractional excretion of lithium and uric acid in the former. However, if true, this sodium retention has no effect on the level of intracellular sodium nor the sodium-potassium-ATP-ase activity.

4.4.1 SIMILAR PLASMA ATRIAL NATRIURETIC FACTOR LEVELS IN CHILDREN AND YOUNG ADULTS WITH A HIGH AND LOW PROBABILITY OF DEVELOPING HYPERTENSION: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

The role of atrial natriuretic factor (ANF) in the genesis of primary hypertension is not clear. However, a natriuretic and blood pressure lowering effect has been observed after infusion of ANF. Therefore, subjects at risk of future hypertension might be deficient in ANF or less responsive to it. To address this question we studied ANF, sodium excretion and blood pressure in 180 young normotensive people with different probabilities of developing hypertension later in life. The 180 subjects had either two, one or no hypertensive parents. Sixty-nine offspring had a high, 58 an intermediate and 53 a small probability of developing hypertension ('high', 'mixed' and 'low' groups). Mean plasma levels of ANF did not differ among the three groups of offspring. A negative association was found between ANF and diastolic blood pressure, being most pronounced in the low-risk group. The similar levels of plasma ANF in these groups suggest that ANF is not directly related to the development of high blood pressure.

Introduction

The role of plasma α-human ANF in blood pressure control is not yet clear.[1] Both elevated and normal values have been described in established hypertensive compared with normotensive subjects.[2-7] However, natriuretic effects and reductions in blood pressure after infusion of ANF in normotensive and hypertensive subjects suggest that a deficiency in ANF might account for the higher blood pressure in hypertensive subjects.[8,9] To determine whether ANF is related to the development of high blood pressure, plasma ANF levels were measured in young normotensive subjects with genetically different probabilities of developing hypertension. The study was conducted in three groups of offspring defined according to the hypertensive status of their parents, on the assumption that these three groups of offspring had different probabilities of developing hypertension later in life.

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* Journal of Hypertension 1989;7(Suppl 1):S43-44.

Methods

In the Dutch Hypertension and Offspring Study three groups of children have been defined on the basis of parental blood pressure levels.[10] The families were selected from a population-based study of cardiovascular risk factors conducted between 1975 and 1979 in two districts of the Dutch town of Zoetermeer. Blood pressure was measured in 1642 families, comprising 6864 subjects. When both parents had a systolic and diastolic blood pressure in the upper 25% of the age- and sex-specific blood pressure distribution or were taking antihypertensive medication, the family was considered a high-risk family. If both parents had both systolic and diastolic blood pressure in the lowest quartile without medication the family was considered a low-risk family. A mixed-risk family comprised one parent with both systolic and diastolic blood pressure in the upper 25% or on antihypertensive medication, and the other parent with both systolic and diastolic blood pressure in the lower 25% without medication. Parents meeting these criteria and having natural children between 5 and 30 years of age were invited for re-measurement in 1986. Of the 312 invited couples, 250 (80%) responded. Offspring of those parents that met the criteria again after the 10-year period were eligible for the present study. This procedure gave 51 high, 35 mixed and 35 low parental couples with 291 healthy children between 5 and 30 years of age, of which 180 (62%) took part (69 high, 58 mixed, 53 low).

Blood pressure was measured on the left arm with a random zero sphygmomanometer. Diastolic blood pressure was taken as Korotoff phase V. The mean of two blood pressure readings was used in the analysis. Following the blood pressure measurements, an intravenous cannula was inserted and after half an hour in the supine position a 10-ml blood sample was taken. Atrial natriuretic factor was measured by radio-immunoassay after extraction.[11] Differences between the three groups were compared using a t-test for unpaired observations, and adjustments for confounding variables were made using multiple linear regression analysis.

Results

Blood pressure and associated characteristics, ANF levels and 24-h urinary sodium excretion for the three groups are given in Table 1. A more elaborate description of blood pressure in the three groups is given elsewhere.[12] No significant differences in plasma ANF level or 24-h sodium excretion were found among the three groups. Both height and body

weight showed a negative association with ANF. The linear regression coefficient for height was -0.38 pg/ml per cm (s.e.m. 0.13), $P = 0.004$, and for weight -0.32 pg/ml per kg (0.13), $P = 0.020$. Moreover, in the total group ANF was negatively associated with diastolic blood pressure [b = -0.11 mmHg per pg/ml (0.02), adjusted for differences in age, height and body weight]. The negative association of diastolic blood pressure with ANF was more pronounced in the low-risk offspring than in the high-risk offspring [low, b = -0.13 mmHg per pg/ml (0.05), $P = 0.02$; high, -0.06 mmHg per pg/ml (0.04), NS].

Table 1 Blood pressure and related characteristics, plasma levels of atrial natriuretic factor (ANF) and 24-h sodium excretion in three groups of offspring.

Offspring	Low	Mixed	High	*P*
Males/females (no.)	34/19	29/29	40/29	NS
Age (years)	18.7 (1.0)	20.4 (0.9)	22.1 (0.8)	0.008
SBP (mmHg)	116.1 (1.6)	123.6 (1.7)	127.2 (1.4)	< 0.001
DBP (mmHg)	70.3 (1.0)	74.9 (1.3)	78.9 (1.0)	< 0.001
Height (cm)	166 (3.0)	170 (2.1)	173 (1.8)	0.046
Body weight (kg)	57.7 (2.8)	60.0 (2.0)	66.1 (1.8)	0.011
ANF (pg/ml)	57.7 (3.4)	60.1 (4.4)	51.0 (3.2)	NS
Urinary sodium (mmol/24 h)	128.4 (7.8)	134.2 (9.3)	133.6 (7.3)	NS

Means (s.e.). *P* values are given for difference between high- and low-risk groups of offspring; NS, not significant; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Conclusion

Our observations that similar levels of plasma ANF are found in young people with different genetic predispositions to develop primary hypertension suggest that ANF is not directly related to the development of high blood pressure. Raised levels of circulating ANF

observed in established hypertensive subjects may therefore reflect a homeostatic response, secondary to the hypertensive process.

The more pronounced inverse association of ANF with diastolic blood pressure in the low-risk offspring, however, may suggest a different ANF effect on blood pressure regulation in the three groups. Further experimental studies should help to clarify this relationship.

Acknowledgements

We thank the 180 youngsters and their parents for their enthusiastic participation in this study. We gratefully acknowledge the contributions from other members of the steering committee of the Dutch Hypertension and Offspring Study: Dr P. van Brummelen, Dr P.W. de Leeuw, Prof M.A.D.H. Schalekamp, Dr T. Thien.

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4.4.2 ATRIAL NATRIURETIC FACTOR AND SODIUM INTAKE IN OFFSPRING OF HYPERTENSIVE AND NORMOTENSIVE PARENTS *

The importance of atrial natriuretic factor (ANF) in volume and sodium homoeostasis is well recognized.[1] Yet the part played by this hormone in the pathogenesis of primary hypertension is still subject to debate.[2] Ferrier et al. (Nov. 3 issue)[3] recently reported an impaired response of ANF to high salt intake in sons of hypertensive parents, as compared with sons of parents with normal blood pressure. They observed no differences in plasma ANF levels between the two groups of offspring when their sodium intake was low. Ferrier et al. indicated a role for a circulating inhibitor of sodium-potassium-ATPase in the relation between renal sodium retention and hypertension.

We recently measured plasma ANF levels, renal sodium excretion, and erythrocytic-membrane sodium-potassium-ATPase activity in sons and daughters of normotensive parents and those of hypertensive parents as part of the Dutch Hypertension and Offspring Study.[4] In this study the offspring of two hypertensive parents (FH+) and of two normotensive parents (FH-) were selected as described elsewhere.[4] The blood pressure of 1642 sets of parents was measured over a 10-year period, and their children were invited to take part in the study when the blood pressure of both parents remained in either the upper or the lower quartile of the blood-pressure distribution for their age and sex.

Forty-one sons and 28 daughters in the FH+ group and 34 sons and 19 daughters in the FH- group participated. Their mean age was 20.4 years (range, 5 to 30). The average systolic blood pressure was 125 mm Hg (95 percent confidence interval, 123 to 127) in the FH+ group and 118 mm Hg (116 to 120) in the FH- group. The difference in blood pressure between these two normotensive groups of youngsters reflects the difference in the probability of future hypertension as a result of the large contrast in parental blood pressure.[5]

Plasma ANF levels were similar in the two groups (FH+ group, 51.0 pg per milliliter [95 percent confidence interval, 44.5 to 57.5]; FH- group, 57.7 pg per milliliter [51.0 to 64.4]). A significant difference in ANF levels was observed between men and women (49.1 pg per milliliter [43.1 to 55.1] and 61.7 pg per milliliter [53.9 to 69.5], respectively), independently of age, body-mass index, and sodium intake. The mean sodium intake, as

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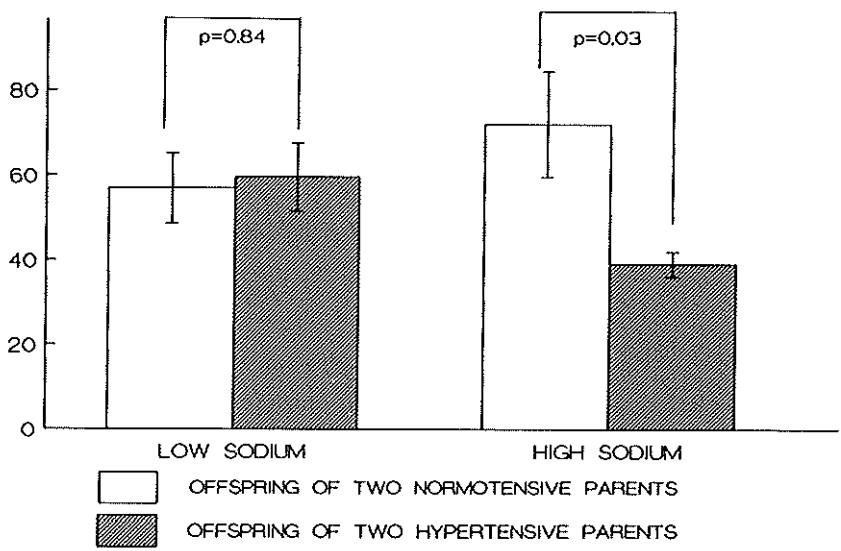
* New England Journal of Medicine (letter) 1989;320:867

assessed in a 24-hour urine sample, was 134 mmol over a 24-hour period (95 percent confidence interval, 120 to 148) in the FH+ group and 128 mmol in 24 hours (112 to 144) in the FH- group. The range of sodium intake in both groups was wide enough to permit study of the level of plasma ANF in each group in the subjects with low sodium intake (below 80 mmol in 24 hours) and in those with high sodium intake (above 200 mmol in 24 hours). In the offspring with low sodium intake, no difference in ANF levels could be detected between the groups (Fig. 1). However, among offspring with high sodium intake, the level of ANF in the FH- group was higher by 33.0 pg per milliliter (95 percent confidence interval, 11.7 to 54.4) than that in the FH+ group. The mean level of sodium-potassium-ATPase activity was, if anything, higher in the FH+ offspring (FH+ group, 0.30 μmol of phosphorus per hour per milligram of protein [95 percent confidence interval, 0.27 to 0.33]; FH- group, 0.26 μmol of phosphorus per hour per milligram of protein [0.21 to 0.31]). Furthermore, no relation could be observed between sodium intake and sodium-potassium-ATPase activity in either group.

Figure 1 Plasma Concentrations of Atrial Natriuretic Factor at Low and High Levels of Sodium Intake, in Offspring with Two Normotensive Parents and Those with Two Hypertensive Parents.



Data are derived from 24 subjects with low sodium intake (12 in each group) and 21 subjects with high sodium intake (9 children of normotensive parents and 12 children of hypertensive parents). Means ±SEM are shown.

These findings support the hypothesis that the response of ANF to relatively high levels of sodium intake is diminished in the offspring of parents with hypertension. However, we question whether this diminished response leads to the inhibition of cellular sodium-potassium-ATPase activity, as suggested by Ferrier et al.[3]

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4.4.3 RENAL SODIUM HANDLING, INTRA-CELLULAR SODIUM, SODIUM-POTASSIUM ATP-ase ACTIVITY, AND UNSATURATED FATTY ACIDS IN OFFSPRING OF HYPERTENSIVE AND NORMOTENSIVE PARENTS: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

Background

Sodium intake and homoeostasis are thought to be related to the level of blood pressure in salt-sensitive hypertensive subjects. Not all hypertensive subjects are salt sensitive. Moreover, it is not known if changes in sodium homoeostasis precede the development of hypertension.

Methods

Therefore we studied characteristics of cellular sodium transport in relation to 24-hour sodium excretion and blood pressure in three groups of offspring at different risk for primary hypertension, defined according to parental blood pressure in the Dutch hypertension and Offspring Study.

Results

No difference in 24-hour urinary sodium excretion were observed, but a positive association of 24-hour sodium excretion and blood pressure was observed in the offspring of hypertensive parents (0.03 mmHg/(mmol Na), SEM 0.01, P=0.01 adjusted for age, height, body weight and gender), and not in the offspring of normotensive parents (0.01 mmHg/(mmol Na), SEM 0.025, P=0.7). Intra-erythrocytic sodium or potassium level and sodium-potassium ATP-ase activity were similar among the groups. Non-esterified fatty acids in blood and the percentual division of fractions of fatty acids, taken as a measure for circulating sodium transport inhibitor, were similar among the groups. Fractional sodium excretion, distal delivery of sodium and absolute or relative distal reabsorption of sodium did not differ between the groups. However, proximal tubular sodium reabsorption, estimated from the inverse of fractional excretion of uric acid, was increased in the offspring of two hypertensive parents; 8.5% (SEM 0.4) in the offspring of two hypertensive parents, compared to 9.7% (0.5) in the offspring of two normotensive parents, difference 1.2 % [95% confidence interval of -2.4, 0.0].

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* Submitted

Conclusions

From these data, together with earlier reported findings on a lower renin and aldosterone level, and low atrial natriuretic peptide on a high sodium intake, it is concluded that in the offspring of two hypertensive parents compared to offspring of two normotensive parents, a relative sodium retention (increased proximal sodium reabsorption and similar fractional distal reabsorption of sodium on a relatively low aldosterone level) together with a diminished increase of atrial natriuretic peptide on a high sodium intake might influence the blood pressure increase in youngsters at risk for hypertension on a liberal sodium intake.

Introduction

Supported by clinical observations in primary and secondary forms of hypertension, several hypotheses on the pathophysiology of primary hypertension have linked an increased vascular peripheral resistance to sodium retention.[1] Changes in sodium intake in different individuals do not lead to consistent changes in either sodium retention or blood pressure, and a difference in response of sodium retention and blood pressure increase to a high sodium diet can be observed between groups of hypertensive and normotensive subjects,[2] and within groups of hypertensive subjects.[3] In offspring of hypertensive parents a significant rise of blood pressure was observed on a high sodium diet compared to a low sodium/high potassium diet, that was not seen in offspring of normotensive parents.[4] Salt sensitive subjects more often have a positive family history of hypertension compared to salt resistant subjects.[5] However, Watt et al., in a randomized trial, could not observe a blood pressure reduction on a low sodium diet in offspring of hypertensive parents.[6] Much speculation remains on the mechanisms involved in salt sensitivity. The search for these mechanisms might be biased by an arbitrary division in salt sensitive and salt resistant subjects following a positive or negative blood pressure response to salt-loading.[7]

A hypothesis that links sodium and volume retention to an increase of blood pressure, assumes an increase of a circulating factor, or hormone, secondary to volume retention, that inhibits sodium-potassium-ATP-ase and thereby increases natriuresis.[8,9,10] However, this circulating sodium transport inhibitor also raises intracellular sodium and thereby tone and vascular reactivity of smooth muscle of arteries and veins. Before, we did not report a difference in erythrocytic sodium-potassium-ATP-ase activity between hypertensive and normotensive subjects.[11] However, we have observed an inhibitory effect

of unsaturated fatty acids on sodium-potassium-ATP-ase activity[12] and a positive association of the amount of unsaturated fatty acids in plasma of normotensive and hypertensive subjects with mean arterial pressure.[13]

Tobian et al., starting with a different hypothesis as described above, were the first to draw attention to an increased sodium content of the arterial wall in hypertensive subjects compared to normotensive subjects, using postmortem tissue measurements.[14] While this assumes that electrolyte changes are present in all cells, most studies have only considered blood cells.[15] Sodium transport characteristics of leucocytes were shown to correlate well with those of resistance vessels measured in vitro.[16] Normotensive subjects with a family history of hypertension compared to normotensive subjects without parental hypertension, did not show an elevated intracellular sodium content in either leucocytes,[17,18,19] or lymphocytes,[20] while results in erythrocytes were negative,[19,21,22,23] or positive.[24,25,26] If anything, results of these studies never documented a decreased intracellular sodium concentration in subjects at risk for hypertension, rather a tendency towards an increased level could be found. Moreover, according to Blaustein,[27] a concentration increase of 5% only could be enough to modify vascular contractility.

Therefore, we studied sodium transport characteristics in youngsters at different risk for hypertension in the Dutch Hypertension and Offspring Study, that were not selected on salt-sensitivity;[28] i.e. sodium-potassium-ATP-ase activity in erythrocytic membranes, unsaturated fatty acids as a measure of circulating sodium transport inhibitor, intra-erythrocytic sodium and potassium concentration, fractional lithium and uric acid excretion and the relation of the parameters with blood pressure. Moreover, findings of the Dutch Hypertension and Offspring Study on the renin-angiotensin-aldosterone system[29] and atrial natriuretic peptide[30,31] reported before, will be discussed together with the cellular sodium transport characteristics.

Methods

Population

Families were selected from a cohort of 10.532 subjects that participated in a study on cardiovascular risk indicators in the Netherlands between 1975-1978.[32] The cohort included 1,642 families. Using age and gender specific blood pressure percentiles, parental couples were selected if both parents had a systolic blood pressure (SBP) and a diastolic blood

pressure (DBP) in the upper quartile, or used anti-hypertensive medication (two hypertensive parents), or had one parent in the upper and one parent in the lower quartile (one hypertensive and one normotensive parent), or both parents in the lower quartile for SBP and DBP (two normotensive parents). In 1986 selected parents were invited for re-examination to assess whether their blood pressure had remained in the same age- and gender specific category as at the initial examination. Finally, after re-examination 121 families were selected, with 291 healthy children aged 5 - 30 years of age in 1987. For the study presented here, 154 of the offspring gave informed consent and participated; 61 offspring of two hypertensive parents, 52 offspring of one hypertensive and one normotensive parent, and 42 offspring of two normotensive parents. The protocol was approved by the ethics committee of the Universtity Hospital Dijkzigt.

Protocol

Participants were visited at home to explain full details of the protocol and to give the 24-hour urine sampling device and lithiumcarbonate tablets. Next the offspring visited the research centre for a session of 4 to 5 hours duration scheduled between 07.45 a.m. and 01.00 p.m. They were asked to refrain from smoking and coffee drinking during the day before visiting the research centre until after the examination. Subjects were asked to take the lithiumcarbonate tablets (8 - 10 mg/kg body weight) at bedtime, but no later than midnight, with water, and to drink only water afterwards. One hour before their visit to the research centre participants were asked to void, and register the time, and to collect all urine that might be voided after that time separately before arriving at the research centre. At the research centre deviations from the protocol were registered. After anthropometric measurements an intravenous cannula was inserted. Blood was sampled for measurement of sodium-potassium-ATPase activity in the erythrocyte membrane and unsaturated fatty acids in plasma. After 30 minutes of supine rest, in a quiet room, blood was sampled for measurement of renin, aldosterone, angiotensin-II, plasma lithium, electrolytes and creatinine, at 1½ hour after voiding at home. Subjects were asked to collect some saliva for measurement of electrolytes, and subsequently were asked to drink at least 10 ml per kg body weight per hour during the renal function test, described and presented elsewhere.[29] After another 1½ hour, urine samples were collected by active voiding. The total volume and exact duration of the approximate 3-hour clearance period were noted. Together with

the blood sample taken during the second series of blood sampling in the middle of the 3-hour period, clearances and fractional excretion of lithium, sodium, potassium, could be calculated.

During the stay in the research centre, an extensive dietary assessment was made by a supervised dietician in training to register dietary intake in the preceding month using a dietary recall with a cross check list to estimate daily intake of various nutrients (carbohydrates, protein, saturated and unsaturated fatty acids, calcium, phosphate, fiber, alcohol). With younger children, the dietician started interviewing the child but frequently had to turn to the mother also. If necessary, preparation of food was checked with the subject in the household that attended cooking, i.e. either a parent or partner of the participant. Estimates of the amount of food and beverages used were facilitated by presence of numerous numbered cups, glasses and spoons, of which the dietician knew the volume. As use of butter on bread may account for a large part of fat intake in subjects eating many slices of bread each day, as in youngsters, participants were asked to butter a piece of bread that was weighted before and after buttering, without notice of the participant, to estimate fat intake per slice of bread.

Measurements

Methods for blood pressure and anthropometric measurements have been described previously.[29] Serum sodium, potassium, and uric acid were measured by standard laboratory methods using an autoanalyzer (SMAIII). Urinary electrolytes as sodium and potassium in 24 hour urine and 3 hour urine portions were measured by flame-photometry. Creatinine and uric acid in urine was measured by autoanalyzer. Fractional excretion of electrolytes was calculated from the concentration in the three-hour fasting urine portion (U_x), the concentration of plasma creatinine (P_{Cr}) sampled after 1½ hour, the urinary concentration of creatinine (U_{Cr}) and the plasma concentration of the electrolyte (P_x) according to the following formula: $((U_x * P_{Cr}) / (U_{Cr} * P_x))$. Lithium in serum and urine was measured by atomic photometric absorption.

On the assumption that lithium is reabsorbed in the proximal tubulus and neither reabsorbed nor secreted beyond the proximal tubulus, estimates of distal tubular sodium handling could be derived from lithium clearance.[33] Distal delivery of sodium was estimated from the clearance of lithium multiplied by the plasma concentration of sodium.

The absolute distal reabsorption of sodium was calculated from the distal delivery of sodium minus the urinary sodium excretion (urinary volume times urinary concentration) during the sampling period. The fractional distal reabsorption of sodium was calculated from the ratio of absolute distal reabsorbed sodium to the distal delivered sodium.

Intra-erythrocytic sodium and potassium were measured in erythrocytes isolated from blood containing 5 mmol/l di-sodium-EDTA, and washed three times with ice-cold 140 mM choline chloride. Isolated cells were kept at 4°C until lysed with Aqua-dest and Triton X100. Subsequently, sodium and potassium concentration in the lysate were measured by flame-photometry. Haemoglobin was measured both in the cell suspension and in the lysate and the haematocrit in the cell suspension, to express sodium and potassium concentration in mmol/l cells.

For measurement of sodium-potassium ATP-ase activity and unsaturated fatty acids 20 ml blood was sampled in a chilled tube containing sodium-heparin and centrifuged for 10 minutes at 2000g at 4° C. The supernatant was discarded, and kept in a tube under gaseous nitrogen, immediately frozen at -180° C until assayed for unsaturated fatty acids. As a measure for the endogenous ouabain-like inhibitor,[34] plasma total free fatty acids and fatty acids composition were measured by extraction with thin-layer chromatography and gas-liquid-chromatography respectively.[35] After removal of the buffycoat from the erythrocyte pellet, cells were washed three times with ice-cold 0.3 M sucrose, 2mM EDTA buffer (pH 7.4) and subsequently suspended in buffer and frozen by -180°C. For isolation of the erythrocyte membrane, cells were lysed with 35 ml 20 mM Tris-HCl, 2mM EDTA (pH 7.4), centrifuged and washed three times with Tris-EDTA. A fluffy-pink sediment was suspended in a small volume of water and freeze dried, and stored at -20°C until assay. Na-K ATPase activity was calculated from the difference between Mg-ATPase activity and Na-K ouabain insensitive ATPase activity, measured by spectrophotometry at 700 nm absorbency, as described.[11] Activity was expressed in µmole of phosphate released per mg of protein per hour (i.e. µmole Pi/hr.mg protein). Protein in the membrane preparations was measured as proposed by the method of Lowry.[36]

Results

A general description of the three offspring groups is given in table 1. As discussed in more detail elsewhere, the blood pressure in the offspring of hypertensive parents is higher

Table 1 Blood pressure and related characteristics in offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents.*

	offspring of two normotensive parents	offspring of one hypertensive parent	offspring of two hypertensive parents
Males/Females (number)	25 / 16	27 / 25	37 / 24
Age (years)	21.3 ± 7.0	22.1 ± 6.1	23.4 ± 5.9
Height (cm)	170.0 ± 17.8	172.8 ± 13.3	175.2 ± 11.4
Body weight (kg)	62.5 ± 19.2	63.6 ± 13.5	68.2 ± 13.5
SBP** sitting (mmHg)	117.7 ± 11.2	124.8 ± 13.1	128.0 ± 10.6
DBP** sitting (mmHg)	71.1 ± 8.7	75.8 ± 9.7	78.8 ± 7.8
SBP*** sitting (mmHg)	119.3 ± 9.3	125.4 ± 9.4	126.5 ± 9.4
DBP*** sitting (mmHg)	71.6 ± 8.6	75.8 ± 8.6	78.4 ± 8.6

* Values are means ± SD.
** SBP=systolic blood pressure, DBP=diastolic blood pressure.
*** Adjusted for differences between the groups in age, height, body weight and proportion of males.

compared to offspring of normotensive parents, with and without adjustments for differences in age, height, body weight and proportion of males.[29]

Sodium intake, measured as 24-hour urinary excretion, was similar in the three groups of offspring (table 2). For the offspring groups combined, a positive association of 24-hour sodium excretion with SBP was observed; crude (b=0.07 mmHg/[mmol Na], [SEM=0.01], p<0.0001) and adjusted for age, height, body weight and gender (b=0.03 mmHg/[mmol Na], [SEM=0.01], p=0.01). For the groups separate, this association was seen both in the offspring of two hypertensive parents (crude b=0.06 mmHg/[mmol Na], [SEM=0.02], p=0.004; adjusted b=0.034 mmHg/[mmol Na], [SEM=0.020], p=0.08) and in the offspring of one hypertensive parent (crude b=0.07 mmHg/[mmol Na], SEM=0.02, p=0.002; adjusted b=0.035 mmHg/[mmol Na], [SEM=0.020], p=0.09), but less clear in the offspring of two normotensive parents (crude b=0.08 mmHg/[mmol Na], [SEM=0.03], p=0.004; adjusted 0.010 mmHg/[mmol Na], [SEM=0.025], p=0.7).

The mean level of sodium-potassium ATP-ase activity was slightly, but not significantly, higher in the offspring of two hypertensive parents and one hypertensive

Table 2 Aspects of sodium homoeostasis in the offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents; 24-hour urinary sodium and potassium excretion, plasma sodium and potassium, Intra-erythrocytic sodium (Intra-ery-Na) and potassium (Intra-Ery-K), Na-K-ATPase activity on the erythrocytic membrane, total non esterified fatty acids (NEFA) and % of non-esterified fatty acids) and sodium and potassium excretion in saliva.*

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
24-Hour urinary excretion										
Sodium (mmol/24 hour)	127.0	(6.9)	139.8	(6.4)	131.7	(5.8)	12.8	[-6.1, 31.7]	4.7	[-13.4, 22.8]
Potassium (mmol/24 hour)	64.5	(2.6)	67.9	(2.4)	59.5	(2.2)	3.4	[-3.8, 10.6]	-5.0	[-11.9, 1.9]
Creatinine (mmol/24 hour)	13.7	(0.4)	13.2	(0.4)	13.4	(0.4)	-0.5	[-1.6, 0.6]	-0.3	[-1.4, 0.8]
Blood values of										
Sodium (mmol/l)	141.4	(0.3)	140.9	(0.3)	141.0	(0.3)	-0.5	[-1.4, 0.4]	-0.4	[-1.3, 0.5]
Potassium (mmol/l)	4.10	(0.05)	4.16	(0.04)	4.15	(0.04)	-0.06	[-0.19, 0.07]	-0.05	[-0.17, 0.07]
Intra-Ery-Na (mmol/l cells)	6.8	(0.4)	6.8	(0.1)	6.9	(0.2)	0.0	[-0.7, 0.7]	0.1	[-0.7, 0.9]
Intra-Ery-K (mmol/ l cells)	84.5	(3.2)	88.0	(2.8)	81.9	(3.3)	3.5	[-4.9, 11.9]	-2.6	[-11.7, 6.5]
Na-K-ATPase (μmP/hour/mg pr.)	0.26	(0.02)	0.29	(0.02)	0.29	(0.01)	0.03	[-0.02, 0.08]	0.03	[-0.01, 0.07]
Unsaturated fatty acids										
- NEFA (μmol/l)	386	(30)	453	(28)	392	(26)	67	[-15, 149]	6	[-72, 84]
- Myristic acid (14:0) (%)	2.0	(0.1)	2.5	(0.1)	2.2	(0.1)	0.5	[0.1, 0.9] §	0.2	[-0.2, 0.6]
- Palmitic acid (16:0) (%)	25.4	(0.5)	25.2	(0.4)	25.0	(0.4)	-0.2	[-1.4, 1.0]	-0.4	[-1.6, 0.8]
- Palmitoleic acid (16:1) (%)	3.9	(0.3)	4.7	(0.2)	4.6	(0.2)	0.8	[0.1, 1.5] §	0.7	[0.0, 1.4] §
- Stearic acid (18:0) (%)	9.6	(0.5)	7.9	(0.4)	9.2	(0.4)	-1.7	[-3.0, -0.4]	-0.4	[-1.6, 0.8]
- Oleic acid (18:1) (%)	43.9	(0.7)	42.5	(0.6)	43.9	(0.6)	-1.5	[-3.3, 0.3]	-0.0	[-1.8, 1.8]
- Linoleic Acid (18:2) (%)	15.2	(0.6)	17.2	(0.6)	15.2	(0.5)	2.0	[0.3, 3.7] §	0.0	[-1.6, 1.6]
Saliva										
Sodium excretion (mmol/l)	8.9	(0.8)	8.3	(0.8)	10.6	(0.7)	-0.6	[-2.9, 1.7]	1.7	[-0.4, 3.8]
Potassium excretion (mmol/l)	23.0	(0.9)	24.1	(0.9)	23.0	(0.8)	1.1	[-1.5, 3.7]	0.0	[-2.4, 2.4]

* Values are means with SEM in parentheses, and differences between means with 95% confidence intervals in brackets, adjusted for differences between the groups in age, height, body weight and proportion of males. § $p < 0.05$.

Table 3 Estimated dietary intake of total fat, unsaturated and mono- and polysaturated fat intake, crude and adjusted for energy intake in the offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents.*

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
Saturated fat (gram/day)	58.0	(2.6)	51.1	(2.4)	49.1	(2.2)	-7.9	[-13.9, 0.1]	-8.9	[-15.5, -2.3]
- energy adjusted (gram/day)	53.7	(1.1)	52.6	(1.1)	51.0	(1.0)	-1.1	[-4.2, 2.0]	-2.7	[-5.7, 0.3]
Mono-unsaturated fat (gram/day)	51.6	(2.3)	46.7	(2.2)	44.6	(2.0)	-4.9	[-11.4, 1.6]	-7.0	[-13.1, -0.9]
- energy adjusted (gram/day)	47.7	(1.1)	48.1	(1.0)	46.4	(0.9)	0.4	[-2.5, 3.3]	-1.3	[-4.1, 1.5]
Poly-unsaturated fat (gram/day)	22.6	(1.1)	21.7	(1.4)	20.9	(1.2)	-0.9	[-4.5, 2.7]	-1.7	[-5.1, 1.7]
- energy adjusted (gram/day)	20.8	(1.0)	22.2	(0.9)	21.8	(0.8)	1.4	[-1.3, 4.1]	1.0	[-1.6, 3.6]
Total fat intake (gram/day)	137.7	(6.1)	122.3	(5.9)	117.6	(5.3)	-15.4	[-32.2, 1.4]	-20.1	[-36.1, -4.1] §
- energy adjusted (gram/day)	127.3	(2.5)	126.0	(2.4)	122.4	(2.2)	-1.3	[-8.2, 5.6]	-4.9	[-12.7, 2.9]
Linoleic Acid (gram/day)	18.9	(1.3)	18.1	(1.2)	18.1	(1.1)	-0.8	[-4.3, 2.7]	-0.8	[-4.2, 2.6]
- energy adjusted (gram/day)	17.4	(1.0)	18.7	(0.9)	18.8	(0.8)	-1.3	[-3.9, 1.3]	-1.4	[-3.9, 1.1]
Cholesterol (mgram/day)	394	(21)	346	(20)	335	(18)	-48	[-106, 10]	-59	[-114, -5] §
- energy adjusted (gram/day)	366	(15)	356	(14)	348	(13)	-10	[-51, 31]	-18	[-57, 21]

* Values are means with SEM in parentheses, and differences between means with 95% confidence intervals in brackets; adjusted for differences in age, height, body weight and gender. § $p < 0.05$

parent, compared to offspring of two normotensive parents (table 2). Across groups, no relation of Na-K-ATPase activity with blood pressure or with 24-hour urinary sodium excretion was seen. No difference in endogenous circulating sodium-potassium-ATPase inhibitor, expressed as total nonesterified fatty acids in plasma, was found between the groups (table 2). However, the fraction of nonesterified fatty acids appeared to be different between the groups. Compared to the offspring of two normotensive parents the fraction of palmitoleic acid was elevated both in the offspring of two hypertensive parents and in the offspring of one hypertensive parent. Also, the fractions of myristic and linoleic acid were increased, and the fraction of stearic acid was decreased in the offspring of one hypertensive parent compared to the offspring of two normotensive parents. No association was observed with either blood pressure or 24-hour urinary sodium excretion across the groups.

Dietary intake of total, saturated and mono- and poly-unsaturated fat was slightly higher in the offspring of two normotensive parents (table 3). However, when adjusted for total energy intake, no difference between the groups in fat intake remained. To study if differences in total fat intake, unadjusted for energy intake, could explain the pattern of the plasma unsaturated fatty acids, both the level of total non-esterified fatty acids and fraction of the 6 types of fatty acids were calculated adjusted for total fat intake. However, results did not change; total non-esterified fatty acids remained similar for the groups, and the fraction of myristic, palmitoleic and linoleic acid remained somewhat higher in the offspring of one and/or two hypertensive parents compared to offspring of two normotensive parents, and the fraction of stearic acid remained somewhat decreased in offspring of one hypertensive parent.

Intra-erythrocytic sodium and potassium was the same in the groups (table 2). The intra-erythrocytic sodium-potassium ratio showed a weak positive association with systolic blood pressure (1.14 mmHg/ratio/l cells, SEM=0.66, p=0.09). No association between 24-hour urinary sodium excretion and intra-erythrocytic electrolytes were found.

Excretion of sodium in saliva, sampled in a fasting period, was somewhat higher in the offspring of two hypertensive parents, but not significantly (table 2). Potassium excretion in saliva was similar in the three groups. No relation of salivary electrolyte excretion with either blood pressure or 24-hour urinary sodium excretion was found.

Na-K-ATPase activity was only weakly related to intra-erythrocytic sodium (-2.56 (mmol/l cells)/(μmP/hour/mg protein), SEM=1.46, p=0.08) but a significant relation was

Table 4 Renal sodium homoeostasis in the offspring of two normotensive parents, of one hypertensive and one normotensive parent, and of two hypertensive parents; Excretion of sodium, creatinine and lithium in a fasting clearance period, and derived factors as fractional excretion of sodium and lithium (Fe Na, Fe Lithium), distal delivery of sodium (DD Na), absolute distal resorption of sodium (ADR Na), fractional distal resorption of sodium (FDR Na).*

	offspring of two normotensive parents (A)		offspring of one hypertensive parent (B)		offspring of two hypertensive parents (C)		difference between B and A		difference between C and A	
Fasting Clearance period										
Sodium (ml/min/1.73 m^2)	1.19	(0.08)	1.14	(0.07)	1.15	(0.06)	-0.05	[-0.25, 0.15]	-0.04	[-0.23, 0.15]
Lithium (ml/min/1.73 m^2)	25.3	(1.3)	26.7	(1.1)	26.2	(1.0)	1.4	[-2.0, 4.8]	0.9	[-2.4, 4.2]
Uric Acid (ml/min/1.73 m^2)	11.7	(0.6)	10.2	(0.5)	10.9	(0.5)	-1.5	[-3.1, 0.1]	-0.8	[-2.4, 0.8]
Creatinine (ml/min/1.73 m^2)	123.6	(4.6)	128.3	(4.1)	127.0	(3.7)	4.7	[-7.6, 17.0]	3.4	[-8.4, 15.2]
Tubular function										
Fractional sodium excr. (%)	0.99	(0.07)	0.92	(0.06)	0.91	(0.05)	-0.07	[-0.24, 0.10]	-0.08	[-0.25, 0.09]
Fractional uric acid excr. (%)	9.7	(0.5)	8.2	(0.4)	8.5	(0.4)	-1.5	[-2.7, -0.3]	-1.2	[-2.4, 0.0] §
Fractional lithium excr. (%)	21.0	(0.8)	20.8	(0.7)	20.6	(0.6)	-0.2	[-2.3, 1.9]	-0.4	[-2.4, 1.6]
DD of sodium (mmol/min)	3.74	(0.18)	3.82	(0.16)	3.74	(0.15)	0.08	[-0.40, 0.56]	0.00	[-0.46, 0.46]
ADR of sodium (mmol/min)	3.56	(0.18)	3.66	(0.16)	3.57	(0.14)	0.10	[-0.37, 0.57]	0.01	[-0.44, 0.46]
FDR of sodium (%)	95.1	(0.3)	95.6	(0.3)	95.4	(0.2)	-0.5	[-1.3, 0.3]	-0.3	[-1.1, 0.5]

* Values are means with SEM in parentheses, and differences between means with 95% confidence intervals in brackets, adjusted for differences between the groups in age, height, body weight and proportion of males.

§ $p < 0.05$

seen for Na-K-ATPase with both intra-erythrocytic potassium (-49.0 (mmol/l/cells) per (μmP/hour/mg protein), SEM=20.1, p=0.02), and intra-erythrocytic sodium/potassium ratio (1.98 (ratio/l/cells)/μmP/hour/mg protein), SEM=0.86, p=0.02). Na-K-ATPase activity was not related to salivary sodium, but a positive relation with salivary potassium (15.1 (mmol/l)/(μmP/hour/mg protein), SEM=5.0, p<0.01) was seen.

Renal sodium, lithium and uric acid excretion, calculated for a three hour fasting clearance period, are shown in table 4. Fractional lithium excretion appeared to be somewhat lower in the offspring of two hypertensive parents, and fractional uric acid excretion was significantly decreased in the offspring of two hypertensive parents compared to offspring of two normotensive parents. Renal fractional excretion of sodium, lithium or uric acid were not associated with blood pressure. The distal tubular renal handling of sodium, expressed as absolute and fractional distal reabsorbtion of sodium, was similar in the groups of offspring (table 4).

Discussion

In our study, across the three groups of youngsters at different risk for primary hypertension, no difference in circulating sodium transport inhibitor was found, estimated from total non-esterified fatty acids and subfractions of fatty acids. In other reports, unsaturated fatty acids showed an inhibitory effect on sodium-potassium-ATP-ase activity.[12,34] We did not study other candidates for a circulating sodium transport inhibitor, as endogenous Ouabain,[37] that was recently purified and identified from human plasma as a sodium potassium pump inhibitor.[38]

A slightly increased erythrocytic membrane sodium-potassium-ATPase activity was observed in the offspring of two and one hypertensive parents compared to offspring of two normotensive parents. Others have shown an increased,[39,40] a decreased,[17,24,41,42] or a similar[18,19,21,25,43,44] cellular sodium-potassium pump activity in subjects at different risk for hypertension. Although in our study the elevated erythrocytic sodium-potassium pump activity does not reach statistical significance, these differences may not be without meaning as an association of sodium-potassium-ATPase activity with salivary potassium concentration (positive) and intra-erythrocytic potassium concentration (negative association) was also present. These findings may reflect the possible mechanism of the slightly increased sodium-potassium-ATPase activity.

We tentatively suggest that the sodium-potassium-ATPase activity is not primarily increased in offspring of hypertensive parents, because intra-erythrocytic potassium appeared to be decreased rather than increased. Rather, we feel that sodium-potassium-ATP-ase activity is slightly increased in offspring of hypertensive parents secondary to a tendency to a decreased intracellular potassium and increased intracellular sodium. No clear differences in intra-erythrocytic sodium or potassium were seen between the groups of offspring, but these differences may have been compensated by an increase in sodium-potassium-ATPase activity. The primary phenomenon that could lead to the increased intracellular sodium and decreased intracellular potassium, might be a membrane defect, leading to increased sodium reabsorption.

Sodium reabsorption was estimated from fractional uric acid and lithium clearence. Fractional lithium clearance or uric acid clearance are thought to provide an inverse measure of proximal tubular sodium reabsorbtion. Weder[45] observed a decreased renal fractional lithium clearance in hypertensive subjects and in offspring of hypertensive parents compared to appropriate controls. This was confirmed by others for fractional uric acid clearance,[46] but not for fractional lithium clearance.[47,48,49]

In our study, an increased sodium reabsorption is supported by two observations. First, the lower fractional excretion of uric acid in the offspring of two and one hypertensive parents suggests an increased proximal sodium reabsorption in the offspring of hypertensive parents. Second, the similar fractional distal reabsorption of sodium in the three groups of offspring, with a lower plasma aldosterone level in the offspring of two hypertensive parents (as previously reported[29]) is compatible with an increased sensitivity for aldosterone in the offspring of two hypertensive parents, possibly through membrane alterations in favour of sodium reabsorption.

Our findings do not support a major role of a circulating sodium transport inhibitor, measured as unsaturated fatty acids, and suggest that a primary membrane alteration leads to an increase of sodium reabsorption. Due to a subsequent increased sodium-potassium-ATPase activity no deviations remain in intracellular electrolyte levels. An increased sodium reabsorption might lead to a blood pressure increase, to correct sodium excretion through pressure-natriuresis. In agreement with this a positive association between 24-hour urinary sodium excretion and systolic blood pressure in the offspring of two or one hypertensive parents was present; an association that was not apparent in the offspring of two

normotensive parents. Other potential explanations include a diminished response of atrial natriuretic peptide to high sodium intake in the offspring of hypertensive parents, as reported previously.[31]

In conclusion, our findings do suggest that a high sodium intake and retention, through increased sodium reabsorption and / or a diminished sodium excretion, might be important in the early phase of familial hypertension.

Acknowledgements

We gratefully acknowledge the contributions from other members of the steering committee of the Dutch Hypertension and Offspring Study: Dr P. van Brummelen, Prof. P.W. de Leeuw, Prof M.A.D.H. Schalekamp, dr. L.J.P van Doornen, dr. H.A.P. Pols, Prof. K.H. Rahn, Prof. H.A.J. Struijker-Boudier, Prof. R.R.P. de Vries. Moreover, we are gratefull to H. Kornman, E. Roose for their invaluable contribution in preparation and executing the laboratory assessments. K. Hulshof is acknowledged for assistance in evaluation of the dietary history. Last, but not least, we acknowledge the youngsters and their parents for their enthusiastic participation in this study.

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Factors related to calcium homoeostasis

Urinary and plasma levels of calcium, magnesium, phosphate and parathyroid hormone

Baseline characteristics of calcium metabolism were studied in the three groups of offspring during phase one and two of the study, and are presented in section 4.4.4. Mean serum calcium levels were slightly lower in the offspring of two hypertensive parents (difference -0.02 mmol/l, 95% confidence interval -0.05 to 0.01) and, similarly, magnesium and phosphate were reduced in the offspring of hypertensive parents; -0.03 mmol/l (-0.06 to 0.00) and -0.05 mmol/l (-0.10 to 0.00), respectively. Plasma intact parathyroid hormone [1-84] was higher in the offspring of hypertensive parents compared to offspring of normotensive parents (0.58 pmol/l, (0.09 to 1.07)). In contrast mean 1,25-dihydroxyvitamin D_3 levels were similar among the groups (section 4.4.4 table 2) and no differences in dietary intake of calcium, phosphate or fiber were found (section 4.4.4 table 3). Urinary calcium excretion per 24 hour and the ratio of 24-hour urinary calcium excretion to daily calcium intake were somewhat higher in the offspring of hypertensive parents,but not statisticaaly significant. Renal fractional excretion of calcium during a three-hour period in the morning, was similar in the offspring of two hypertensive parents, and renal fractional excretion of phosphate was lower in the offspring of two hypertensive parents compared to offspring of two normotensive parents (-1.50%, (-2.98 to -0.02)) (section 4.4.4, table 4).

To assess whether the differences in indices of calcium metabolism were confounded by differences in blood pressure between the groups, values for serum calcium, phosphate, magnesium and plasma intact PTH [1-84], adjusted for blood pressure, were calculated. A similar pattern was seen, with a significantly lower calcium and magnesium, a borderline significantly decreased serum phosphate and a clearly elevated plasma intact PTH [1-84] in the offspring of two hypertensive parents compared to offspring of two normotensive parents (section 4.4.4 table 5).

Several relations between variables were studied across the groups. Plasma intact PTH [1-84] was inversely associated with serum calcium; -2.30 pmol/mmol (-4.59 to -0.01). 1,25-dihydroxyvitamin D_3 tended to be positively associated with PTH [1-84], but this association failed to reach statistical significance; 3.16 pmol/pmol (-0.57 to 6.89). No relation between either plasma phosphate or plasma calcium with 1,25-dihydroxyvitamin D_3 was seen. A positive association for fractional excretion of phosphate and PTH [1-84] was observed (0.56 %/(pmol/l), (0.09 to 1.03)). An inverse relation of fractional excretion of calcium with PTH [1-84] was found (-0.065 %/(pmol/l), (-0.110 to -0.020)). No relations

were seen for plasma intact PTH [1-84] with either dietary intake of calcium or phosphate, or with the ratio of 24-hour excretion to dietary intake of calcium or phosphate. 24-hour sodium excretion was related to 24-hour calcium excretion (0.014 mmol/mmol, (0.008 to 0.020)), but not to plasma intact PTH [1-84].

In summary, from the above data it appears that calcium metabolism in offspring of two hypertensive parents is maintained at a higher level of circulating parathyroid hormone, with a small decrease in serum calcium, magnesium and phosphate and a decrease in fractional phosphate excretion. In addition there are no clear differences in fractional calcium excretion, but a tendency to an increased 24 hour urinary calcium excretion. this may suggest that either the kidney in the offspring of hypertensive parents is less sensitive to parathyroid hormone for retention of calcium, or that parathyroid hormone level was increased secondary to a relative increased urinary calcium excretion ("calcium leak") in the offspring of two hypertensive parents.

Therefore, in phase 3 of the Dutch Hypertension and Offspring Study in 1989, urinary calcium excretion was studied during a two hour clerance period before and a four hour period after a calcium load of 1000 mgr (for description of methods chapter 3, section 3.4.). This was studied in the offspring of two hypertensive parents and in the offspring of two normotensive parents. In table 1 the general charcateristics of the two groups of offspring in 1989 are given. Although the offspring of two hypertensive parents was older, heavier and taller, the difference in blood pressure observed between the two groups of offspring remained after adjustment for these differences. The pattern of 24-hour urinary electrolyte excretion sampled the day before the visit to the research centre was similar in the two groups.

The results of the calcium-challenge test are presented in table 2. Urinary excretion of calcium, phosphate, creatinine and plasma levels of intact PTH [1-84], calcium and phosphate are given for the two hour clearance period before the oral calcium load (T0), and during the first (T1) and second (T2) two-hour clearance period after the calcium load, and the total four hour period (T3) after the calcium load. Results on phosphate excretion are given only for T0 and T1, as determinations of phosphate in urine of T2 were unsuccessfull in most of the participants. Fractional excretion of calcium is given for T0, T1, T2. During the third phase no differences in fasting levels of plasma calcium and plasma intact PTH [1-84] were observed between the two groups.

Table 1 General characteristics of the offspring of two normotensive parents and of two hypertensive parents, during phase 3 in 1989-1990.*

	offspring of two normotensive parents		offspring of two hypertensive parents		
Male/female (number)	28/15		35/24		
Age (years)	21.6 ±	7.6	24.3 ±	6.7	
Height (cm)	170.3 ±	17.9	175.1 ±	11.5	
Body Weight (kg)	63.4 ±	20.1	69.6 ±	14.1	
SBP (mmHg)	113.1 ±	13.2	124.4 ±	13.2	#
DBP (mmHg)	68.3 ±	9.1	75.0 ±	9.3	#
Heart freq (beats/30 sec)	36.5 ±	6.5	35.1 ±	6.1	
SBP adj.** (mmHg)	114.6 ±	9.7	122.7 ±	9.7	#
DBP adj.** (mmHg)	69.3 ±	8.4	74.2 ±	8.4	#
24 Hour urinary excretion of					
- sodium (mmol/24 hour)	140.5 ±	51.8	140.8 ±	58.2	
- potassium (mmol/24 hour)	74.6 ±	27.8	68.8 ±	25.9	
- calcium (mmol/24 hour)	3.37 ±	2.14	3.28 ±	1.50	
- creatinine (mmol/24 hour)	15.15 ±	6.40	15.96 ±	4.89	
- phosphate (mmol/24 hour)	30.39 ±	11.14	28.87 ±	9.28	
- calcium/creatinine ratio	0.226 ±	0.114	0.214 ±	0.088	

* Values are means with SD.
** Adjustments were made for age, height, body weight and proportion of males.
\# Difference between the two groups with $p < 0.05$.

The fasting calcium excretion before the calcium loading was twice as high in the offspring of two normotensive parents, compared to the offspring of two hypertensive parents, although not statistically significant. These results were obtained under fasting conditions. After the calcium load, the excretion of calcium became similar for the two groups, due to a rise in urinary calcium excretion after calcium loading in the offspring of two hypertensive parents (4 times), in excess of the rise in calcium excretion seen in the offspring of two normotensive parents (2.0 times). An increase in fractional excretion of calcium after the calcium load was observed in both groups to achieve similar absolute levels of 21 to 22%. However, the relative increase was more in the offspring of two hypertensive parents (36 times baseline) compared to the offspring of normotensive parents (10 times baseline). The exagerated rise in absolute and fractional urinary calcium excretion after a calcium load was observed despite a similar decrease in plasma intact PTH [1-84] in the two groups of offspring. Plasma intact PTH [1-84] was 3.3 pmol/l lower in the offspring of hypertensive parents at baseline (not statistically significant) and after the calcium load a similar, but

Table 2 Results of the fasting calcium challenge test of 1 gram on plasma calcium, phosphate, intact PTH[1-84] and urinary excretion and fractional excretion of calcium and phosphate, in a two hour period before the challenge (T0), and after the calcium load; first two hours (T1), second two hours (T2), total four hours (T3), for the offspring of two normotensive parents and of two hypertensive parents, during phase 3 in 1989-1990.*

	offspring of two normotensive parents		offspring of two hypertensive parents		
Plasma levels of					
- calcium (mmol/l) T0	2.35	(0.01)	2.37	(0.01)	
- calcium (mmol/l) T1	2.48	(0.02)	2.52	(0.02)	
- calcium (mmol/l) T2	2.48	(0.02)	2.48	(0.02)	
- intact PTH [1-84] (pmol/l) T0	22.9	(1.35)	19.6	(1.19)	
- intact PTH [1-84] (pmol/l) T1	7.3	(0.61)	5.0	(0.54)	#
- intact PTH [1-84] (pmol/l) T2	12.5	(0.9)	9.3	(0.79)	#
Urinary excretion of					
- calcium (mmol/2 hour) T0	0.51	(0.16)	0.26	(0.14)	
- calcium (mmol/2 hour) T1	0.50	(0.04)	0.46	(0.03)	
- calcium (mmol/2 hour) T2	0.56	(0.06)	0.53	(0.05)	
- calcium (mmol/4 hour) T3	1.06	(0.08)	0.99	(0.07)	
- phosphate (mmol 2 hour) T0	2.58	(0.21)	1.95	(0.18)	#
- phosphate (mmol 2 hour) T1	1.14	(0.10)	0.98	(0.09)	
- creatinine (mmol/2 hour) T0	1.49	(0.08)	1.47	(0.07)	
- creatinine (mmol/2 hour) T1	1.19	(0.05)	1.17	(0.04)	
- creatinine (mmol/2 hour) T2	1.15	(0.08)	1.15	(0.07)	
Fractional Urinary excretion of					
- calcium (%) T0	2.07	(0.91)	0.60	(0.74)	
- calcium (%) T1	1.49	(0.11)	1.26	(0.09)	
- calcium (%) T2	21.45	(3.03)	21.98	(2.46)	
- phosphate (%) T0	11.04	(0.95)	8.09	(0.77)	#
- phosphate (%) T1	8.64	(2.09)	8.53	(1.69)	

* Values are means with standard errors in parentheses, adjusted for age, height, body weight and proportion of males.

\# Difference between the two groups with $p < 0.05$.

statistically significant difference was present. The finding of a relative increased urinary calcium excretion after an oral calcium load in the offspring of two hypertensive parents, with a similar decrease in plasma intact PTH [1-84], does not exclude an attenuated action of parathyroid hormone on renal calcium retention. However this may not be deduced from

the absolute levels of urinary calcium excretion after the calcium load, as these were similar between the two groups.

Cellular calcium characteristics

Intracellular calcium and magnesium was measured in lymphocytes during phase 2. With a rather large variability in each group (standard deviation 70% of the mean), no differences could be found between the groups of offspring (table 3). Similar negative results were found for calcium binding to the erythrocytic membrane.

Table 3 Characteristics of cellular calcium metabolism; intralymfocytair calcium and magnesium and erythrocyte-membrane calcium binding in the offspring of two normotensive parents and of two hypertensive parents.*

	offspring of two normotensive parents		offspring of two hypertensive parents	
Intralymphocytic concentration of				
- calcium (µmol/kg dry weight)	61.40	(7.35)	56.3	(5.76)
- magnesium (µmol/kg dry weight)	49.64	(5.60)	46.67	(4.70)
Erythrocytic membraneous binding of				
- calcium (µmol/gr protein)	5.76	(0.35)	6.21	(0.31)
- calcium (µmol/gr protein)**	5.76	(0.37)	6.13	(0.31)

* Values are means with standard error in parentheses.
** Adjusted for age, height, body weight and proportion of males.

Summary

It is concluded from the above findings that offspring of two hypertensive parents compared to offspring of two normotensive parents

- have a slighlty decreased plasma magnesium and phosphate plasma level.
- have an increased unchallenged plasma intact PTH[1-84].
- have similar plasma intact PTH[1-84] in a situation of similar plasma calcium levels.
- have a similar dietary intake of calcium, phosphate and fibers.
- have a tendency to an increased urinary excretion of calcium.
- have a similar response of plasma intact PTH [1-84] to an oral calcium load.
- show no differences in intracellular (intralymphocytic) calcium and cellular (erythrocytic) calcium membrane binding.

We propose that small differences in calcium metabolism between the offspring groups are related to a decreased renal sensitivity to plasma parathyroid hormone, which might lead to a relatively increased urinary calcium loss and a slighltly decreased plasma calcium level that influences parathyroid-hormone by feedback control. There are no indications of a primary membrane alteration that leads to an increased urinary calcium loss and secondarily to increased parathyroid hormone levels.

4.4.4 ALTERATIONS IN CALCIUM METABOLISM IN YOUNG PEOPLE AT RISK FOR PRIMARY HYPERTENSION: THE DUTCH HYPERTENSION AND OFFSPRING STUDY *

Abstract

Several disturbances in calcium metabolism have been reported in primary hypertensive subjects. It is, however, not clear whether these alterations predate the development of hypertension or occur as a consequence of high blood pressure. We studied indexes of calcium metabolism in three groups of normotensive children with different familial predispositions for hypertension, based on parental blood pressure levels, with two, one, or no hypertensive parents. Plasma intact parathyroid hormone [1-84] was higher in the offspring of hypertensive parents compared with offspring of normotensive parents (difference, 0.58 pmol/L; standard error of the difference [SED], 0.24; p=0.02). Mean serum calcium levels were slightly reduced in the offspring of two hypertensive parents (-0.019 mmol/L, SED=0.013, p=0.17). Plasma magnesium and phosphate levels were lower in the offspring of hypertensive parents (-0.032 mmol/L [SED=0.016, p=0.05] and -0.045 mmol/L [SED=0.024, p=0.05], respectively). Mean 1,25-dihydroxyvitamin D_3 levels were similar among the groups. No differences in dietary intake of calcium, phosphate, or fiber were found. Urinary calcium excretion per 24 hours and the ratio of 24-hour urinary calcium excretion to daily calcium intake were somewhat higher in the offspring of hypertensive parents. Renal fractional excretion of calcium was similar in the offspring of two hypertensive parents, and renal fractional excretion of phosphate was lower in the offspring of two hypertensive parents compared with offspring of two normotensive parents (-1.50%, SED=0.74, p=0.05). These findings indicate that changes in calcium metabolism may be implicated in the early pathogenesis of primary hypertension and suggest a reduced renal sensitivity to parathyroid hormone in those at risk for hypertension.

Introduction

Several disturbances of calcium metabolism have been associated with hypertension.[1-6] It has been suggested that a low dietary calcium intake increases the risk for high blood pressure.[7-11] In addition, some hypertensive subjects appear to have lower serum ionized

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* Hypertension 1993;21:267-272.

calcium levels,[12] increased urinary excretion of calcium,[13] raised intracellular calcium levels,[14] and reduced cellular membrane calcium binding.[15] Changes in magnesium and phosphate metabolism may be implicated in the relation between calcium and blood pressure regulation. In some hypertensive subjects, urinary excretion of magnesium may be decreased and show an inverse relation with blood pressure.[16] Serum phosphate concentration is reported to be lower in hypertension and negatively related to blood pressure.[17] Besides changes in serum and urinary electrolytes, raised circulating parathyroid hormone (PTH) levels have been demonstrated in hypertensive individuals.[13,18] We reported previously that plasma intact PTH is raised in young hypertensive subjects compared with normotensive subjects of similar age, suggesting that alterations in calcium metabolism may be important in the early phase of primary hypertension.[19,20]

Discussion remains as to whether changes in calcium metabolism are causally related to the development of high blood pressure.[21] In genetically hypertensive rats, calcium supplementation during the developmental phase of hypertension diminished the blood pressure increase.[22] In another study, however, genetically hypertensive rats fed a calcium-deficient diet did not show an increased blood pressure rise compared with rats fed a normal diet.[23] Parathyroidectomy in young genetically hypertensive rats, while keeping a normal serum calcium level, delayed the rise in systolic blood pressure for 42 weeks.[24] In humans, information on the part played by changes in calcium metabolism in the development of high blood pressure may be gained from comparison of parameters of calcium homoeostasis between offspring of hypertensive parents and offspring of normotensive parents.[25] We studied indexes of calcium metabolism in 180 normotensive offspring from 121 families with two, one, or no hypertensive parents, who were recruited out of 1,642 couples participating in a Dutch population study of risk factors for cardiovascular disease.

Methods

Population

The Dutch Hypertension and Offspring Study is a collaborative study of four Dutch Universities and is conducted in the town of Zoetermeer, a suburban residential area near The Hague in The Netherlands.[26] From 1975 to 1979, all residents of two districts of Zoetermeer were invited to participate in a study of blood pressure and other cardiovascular

risk indicators (EPOZ study).[27] Blood pressure was measured in 10,532 (78%) of 13,462 eligible subjects. This group included 1,642 parental couples. A stringent selection procedure was applied to these couples to select groups of offspring with a maximal contrast in familial predisposition for hypertension. The procedures for selection have been described elsewhere.[26] In brief, individual parents with both systolic blood pressure and diastolic blood pressure in the upper ("hypertensive") or lower ("normotensive") quartile of the age- and sex-specific blood pressure distribution were selected. Those taking anti-hypertensive medication were included in the hypertensive group. Couples of two hypertensive parents, of one hypertensive and one normotensive parent, and of two normotensive parents were invited for remeasurement of blood pressure in 1986. On this occasion, the same criteria for hypertension and normotension were applied as for the initial screening. Of 250 parental couples that were remeasured (80% of those invited), 51 couples of 74 remained in the group of two hypertensive parents, 35 of 106 in the group of one hypertensive and one normotensive parent, and 35 of 70 in the group of two normotensive parents. Together, these 121 selected parental couples had 291 healthy biological children, aged between 5 and 30 years, who were invited to take part in this study. Of these, 180 offspring (62%) of 97 families gave informed consent and participated in the study: 69 children with two hypertensive parents, 58 children with one hypertensive and one normotensive parent, and 53 children with two normotensive parents.

Measurements

The groups of offspring visited the research centre twice. In 1987, a short protocol was followed for anthropometric and blood pressure measurements, a blood sample was obtained for measurement of parameters of calcium metabolism, and a 24-hour urine sample was collected. In 1988, the measurements in the groups of offspring were repeated, this time including a 3-hour fasting period between 7 and 10 AM during which urine was sampled; a blood sample was taken after 90 minutes, at 8:30 AM. The participants had been asked to refrain from intake of food and beverages starting from midnight.

Blood pressure was measured on the left arm with a random-zero sphygmomanometer by a trained paramedical assistant. A series of two readings was made with the subject sitting, and the mean of these readings was used in the analysis. A fasting venous blood sample was obtained, and serum and plasma samples were stored at -70°C.

All participants collected two 24-hour urine samples. Levels of serum total calcium, inorganic phosphate, and magnesium as well as urinary calcium, sodium, potassium and creatinine were measured by standard laboratory methods. All variables were measured twice at each of the two visits, with the exception of plasma magnesium (first examination only) and 1,25-dihydroxyvitamin D_3 and clearance parameters (second examination only). Fractional excretion of electrolytes was calculated from the concentration in the 3-hour fasting urine portion (U_x), with the concentration of plasma creatinine (P_{Cr}), the urinary concentration of creatinine (U_{Cr}), and the plasma concentration of the electrolyte (P_x) according to the following formula: $(U_x \cdot P_{Cr})/(U_{Cr} \cdot P_x)$. At the second visit, dietary intake of calcium, phosphate, fiber, and total energy was assessed by a 1-month dietary recall with a cross check.[28] Plasma intact PTH [1-84] was determined by a two-step immunochemical method.[19,29] 1,25-Dihydroxyvitamin D_3 was measured by radioimmunoassay (Incstar Corp., Stillwater, Minn.). None of the participants used any medication or other substance known to influence calcium metabolism.

Data analysis

Descriptive data for the three groups are presented as means and standard deviations. For comparison between groups, means and SEM are given, and the difference (with standard error of the difference [SED]) compared with the offspring of two normotensive parents is presented. Adjustments for differences in age, height, weight, and proportion of males between the three groups were made using a model for multiple linear regression. Adjusted values were used for comparisons between groups, and a two-sided *t* test was applied to assess statistical significance. If variables were measured twice, the mean of two measurements is given.

Associations between study variables (blood, urinary, and dietary variables related to calcium metabolism) were studied across groups and adjusted for group status (using indicator variables) and age, height, weight, and gender by multiple regression analysis. Regression coefficients are given with SEM.

Results

General characteristics

Blood pressure, age, and anthropometric characteristics of the participating parental couples and their offspring in 1987 are given in Table 1. The selection based on age- and gender-specific blood pressure percentiles resulted in marked differences in parental blood pressure between the groups even though a small proportion of parents were taking drug therapy for hypertension. None of the children and young adults had clear hypertension, but blood pressure levels were higher in offspring of two hypertensive parents compared with offspring of two normotensive parents. No differences in 24-hour sodium and potassium urinary excretion were present between the groups of offspring.

Table 1 Blood Pressure and Related Characteristics of Participating Parental Couples and Offspring

	Families of two normotensive parents	Families of one hypertensive parent	Families of two hypertensive parents
Parents			
No. of couples	29	30	38
Age (years)	46.6 ± 8.8	50.9 ± 8.9	52.1 ± 8.3
SBP (mm Hg)	113.3 ± 6.3	133.0 ± 11.4	151.8 ± 14.1
DBP (mm Hg)	68.5 ± 5.4	80.6 ± 6.9	89.8 ± 6.4
Subjects treated for hypertension (%)	0	23.3	38.2
Offspring			
Males/females (No.)	34 / 19	29 / 29	40 / 29
Age (years)	18.7 ± 7.3	20.4 ± 6.4	22.1 ± 6.4
Height (cm)	166.0 ± 21.8	169.8 ± 15.9	172.7 ± 14.9
Body weight (kg)	57.7 ± 20.6	60.0 ± 14.9	66.1 ± 15.1
SBP, sitting (mm Hg)	116.1 ± 11.7	123.6 ± 13.1	127.2 ± 11.4
DBP, sitting (mm Hg)	70.3 ± 8.9	74.9 ± 9.8	78.9 ± 8.1
Adjusted SBP, sitting (mm Hg)*	117.9 ± 9.2	124.2 ± 9.1	125.3 ± 9.2
Adjusted DBP, sitting (mm Hg)*	70.3 ± 8.8	74.9 ± 8.8	78.4 ± 8.9
Plasma sodium (mmol/L)	141 ± 1.7	141 ± 2.4	141 ± 1.9
Urinary sodium excretion (mmol/24 hour)	128 ± 56	134 ± 70	134 ± 60
Plasma potassium (mmol/L)	4.3 ± 0.4	4.3 ± 0.3	4.3 ± 0.2
Urinary potassium excretion(mmol/24 hour)	58 ± 20	66 ± 24	61 ± 22

SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are mean±SD.
* Adjusted for differences between groups in age, height, body weight, and proportion of males.

Parameters of Calcium Metabolism

The mean levels of serum electrolytes, plasma intact PTH [1-84], and 1,25-dihydroxyvitamin D_3 are given in Table 2. Serum total calcium was slightly but not significantly lower in the offspring of two hypertensive parents compared with offspring of two normotensive parents. Serum magnesium and serum phosphate levels were reduced in the offspring of hypertensive parents: -0.032 mmol/L (SED=0.016, p=0.05) and -0.045 mmol/L (SED=0.024, p=0.05), respectively. Plasma intact PTH [1-84] was significantly higher both in the offspring of two hypertensive parents and in the offspring of one hypertensive parent compared with offspring of two normotensive parents. No differences were seen for the plasma level of 1,25-dihydroxyvitamin D_3 between the groups.

Daily dietary intake of nutrients important for calcium metabolism are given in Table 3. No statistically significant differences between the groups were seen for both total or energy-adjusted dietary intake of calcium, phosphate, or fiber.

The 24-hour urinary calcium excretion was somewhat higher, but not significantly so, in the offspring of hypertensive parents (Table 4). Twenty-four-hour urinary excretion of phosphate and creatinine were similar among the groups. During the 3-hour fasting period, the absolute excretion of calcium and phosphate per hour was similar among the three groups. Also, fractional excretion was the same for calcium, but fractional phosphate excretion was significantly lower in the offspring of two hypertensive parents (Table 4). The ratio of 24-hour urinary calcium excretion to daily dietary calcium intake was somewhat higher in the offspring of hypertensive parents. Twenty-four-hour sodium excretion was similar in the groups (Table 1). The ratio of the 24-hour urinary excretion of calcium to sodium appeared to be somewhat higher in the offspring of two hypertensive parents compared with the offspring of two normotensive parents, with a difference of 0.16% (SED=0.23, NS).

Table 2 Blood Levels of Indexes of Calcium Metabolism in Three Groups of Offspring.**

	Offspring of two normotensive parents	Offspring of one hypertensive parent	Offspring of two hypertensive parents
Calcium (mmol/L)*	2.36 (0.011)	2.37 (0.011)	2.34 (0.008)
Phosphate (mmol/L)*	1.18 (0.018)	1.14 (0.020)	1.13 (0.015) #
Magnesium (mmol/L)	0.87 (0.013)	0.85 (0.011)	0.84 (0.011) #
Parathyroid hormone [1-84] (pmol/L)*	3.12 (0.187)	3.77 (0.169) #	3.70 (0.155) #
1,25-Dihydroxyvitamin D3 (pmol/L)***	68.1 (3.47)	...	67.4 (2.87)

** Values are mean (±SEM), adjusted for differences in age, height, body weight, and gender.
* Values were calculated as the average of two examinations for each participant.
# $p<0.05$, for the difference with offspring of two normotensive parents.
*** Measured in offspring of two normotensive parents and of two hypertensive parents only.

Table 3 Total and Energy-Adjusted Dietary Intake of Calcium, Phosphate, and Fiber in Three Groups of Offspring.*

	Offspring of two normotensive parents	Offspring of one hypertensive parent	Offspring of two hypertensive parents
Calcium (mg/day)			
Total	1,644 (92.1)	1,461 (86.1)	1,434 (76.9)
Energy adjusted	1,580 (75.2)	1,483 (71.1)	1,479 (63.9)
Phosphate (mg/day)			
Total	2,194 (94.9)	2,040 (88.7)	2,023 (79.2)
Energy adjusted	2,088 (61.1)	2,103 (57.8)	2,081 (51.9)
Fiber (g/day)			
Total	31.6 (1.40)	31.5 (1.34)	30.6 (1.20)
Energy adjusted	30.3 (1.23)	31.9 (1.16)	31.2 (1.04)

* Values are mean (±SEM), adjusted for differences in age, height, body weight, and gender. Values in offspring of two hypertensive parents and offspring of one hypertensive parent did not show statistically significant differences with values in offspring of two normotensive parents.

Several relations between variables were studied across the groups. Plasma intact PTH [1-84] was inversely associated with serum calcium (-2.30 pmol/mmol, SEM=1.15, p=0.05). 1,25-Dihydroxyvitamin D_3 tended to be positively associated with PTH [1-84], but this association failed to reach statistical significance (3.16 pmol/pmol, SEM=1.88, NS). No

relation between either plasma phosphate or plasma calcium with 1,25-dihydroxyvitamin D_3 was seen. A positive association for fractional excretion of phosphate and PTH [1-84] was observed of 0.56 %/(pmol/L) (SEM=0.24, p=0.02). An inverse relation of fractional excretion of calcium with PTH [1-84] was found (-0.065 %/(pmol/L), SEM=0.023, p=0.01). No relations were seen for plasma intact PTH [1-84] either with dietary intake of calcium or phosphate or with the ratio of 24-hour excretion to dietary intake of calcium or phosphate. Twenty-four-hour sodium excretion was related to 24-hour calcium excretion (0.014 mmol/mmol, SEM=0.003, p=0.001) but not to plasma intact PTH [1-84].

To assess whether the differences in indexes of calcium metabolism had been confounded by the differences in blood pressure between the groups, we obtained values for levels of serum calcium, phosphate, magnesium, and plasma intact PTH [1-84] adjusted for blood pressure (Table 5).

Table 4 Twenty-four-Hour Urinary Excretion, Fasting Clearances, and Ratio of 24-Hour Urinary Excretion to Dietary Intake in Three Groups of Offspring.#

	Offspring of two normotensive parents	Offspring of one hypertensive parent	Offspring of two hypertensive parents	
24-Hour urinary excretion*				
Calcium (mmol/24h)	3.10 (0.242)	3.48 (0.225)	3.48 (0.205)	
Phosphate (mmol/24h)	32.5 (1.63)	32.3 (1.53)	31.2 (1.34)	
Creatinine (mmol/24h)	13.7 (0.41)	13.2 (0.39)	13.4 (0.35)	
Fasting clearance				
Calcium (mmol/hr)	0.16 (0.011)	0.15 (0.010)	0.16 (0.009)	
Phosphate (mmol/hr)	0.67 (0.011)	0.60 (0.051)	0.54 (0.047)	
Fractional calcium excretion (%)	0.88 (0.057)	0.81 (0.052)	0.87 (0.047)	
Fractional phosphate excretion (%)	7.84 (0.570)	7.04 (0.520)	6.34 (0.476)	**
Ratio of 24-hour urinary excretion to dietary intake				
Calcium (%)	8.8 (1.01)	10.7 (0.95)	10.9 (0.84)	
Phosphate (%)	48.3 (3.08)	52.0 (2.87)	49.2 (2.57)	

\# Values are mean (±SEM), adjusted for differences in age, height, body weight, and gender.
* Values were calculated as the average of two examinations for each participant.
** p<0.05, for the difference with offspring of two normotensive parents.

Table 5 Blood Levels of Indexes of Calcium Metabolism in Three Groups of Offspring After Adjustment for Differences in Diastolic Blood Pressure.**

	Offspring of two normotensive parents	Offspring of one hypertensive parent	Offspring of two hypertensive parents
Calcium (mmol/L)*	2.36 (0.011)	2.36 (0.011)	2.33 (0.008) #
Phosphate (mmol/L)*	1.18 (0.019)	1.14 (0.020)	1.14 (0.015)
Magnesium (mmol/L)	0.88 (0.013)	0.85 (0.011)	0.84 (0.011) #
Parathyroid hormone [1-84] (pmol/L)*	3.18 (0.173)	3.76 (0.174) #	3.66 (0.148) #

** Values are mean (±SEM), adjusted for differences in age, height, body weight, gender, and diastolic blood pressure.

* Values were calculated as the average of two examinations for each participant.

# $p<0.05$, for the difference with offspring of two normotensive parents.

Discussion

Our observation of slightly reduced serum calcium levels and significantly raised plasma intact PTH [1-84], combined with decreased serum magnesium and phosphate levels, in prehypertensive young subjects genetically at risk for hypertension supports the view that disturbances in calcium metabolism are present in the early phase of primary hypertension and may precede the development of high blood pressure.

Before this conclusion can be accepted, some issues need to be addressed. The offspring of hypertensive parents participating in the present study already had higher average blood pressure levels than the offspring of normotensive parents (Table 1). This is in agreement with observations in previous studies in young subjects with and without a family history of hypertension,[25] and it probably reflects the inevitable expression of the large difference in genetic susceptibility.[30] Although none of the children had clear hypertension, it is conceivable that the differences in blood pressure between the groups may have caused differences in calcium metabolism rather than the reverse. Although this possibility is difficult to exclude, one approach may be to adjust the observed differences for the difference in blood pressure between the groups (Table 5). Adjustment for differences in diastolic blood pressure did not clearly affect the level of and small differences in serum calcium between the groups, although the difference between the offspring of two hypertensive parents and the offspring of two normotensive parents just reached statistical significance. The difference for serum magnesium between the groups remained similar and statistically significant. After adjustments for diastolic blood pressure,

the differences in plasma intact PTH [1-84] became smaller but remained statistically significant between the offspring of two hypertensive parents and the offspring of two normotensive parents. The difference in plasma phosphate became smaller and failed to reach statistical significance. It should be noted, however, that adjusting for blood pressure level may also obscure true differences in characteristics related to the development of high blood pressure, because offspring with the highest blood pressures may be those with the highest risk of future hypertension.

Differences in diet between the groups may be important in explaining our findings. Of particular interest are calcium intake and nutrients that may interfere with the intestinal absorption of calcium, notably, dietary fiber and phosphate.[31] However, the levels of dietary calcium, phosphate, and fiber intake were not significantly different between the groups, and the small differences that were observed are unlikely to be fully responsible for the differences found in blood levels of calcium and intact PTH [1-84]. In agreement with this, when dietary factors were included in a multiple regression analysis, the differences between the groups for the various blood values of calcium metabolism remained similar.

1,25-Dihydroxyvitamin D_3, necessary for intestinal calcium absorption and bone resorption of calcium, may also affect calcium balance. The plasma level of 1,25-dihydroxyvitamin D_3 was not different between the groups and did not show a relation with either plasma phosphate, plasma calcium, or plasma intact PTH [1-84] levels.

Our findings of a relatively reduced serum calcium and increased plasma PTH [1-84] level in the offspring of hypertensive parents suggest a relative calcium deficiency in the offspring of hypertensive parents that is partly compensated by an increased plasma PTH. This profile of an increased plasma intact PTH [1-84] level and a somewhat lower serum calcium level in the offspring of hypertensive parents might be related to their risk for primary hypertension. PTH is known to increase intracellular calcium concentration and might thereby directly affect vascular smooth muscle tension.[2] We previously observed that the decrease in diastolic blood pressure to calcium supplementation in offspring with relatively high blood pressure was most clear in subjects with a relatively high plasma PTH [1-84] or low serum calcium.[32] In one other study, hypertensive subjects with a relatively large urinary calcium excretion showed more pronounced decreases in blood pressure to calcium supplementation compared with those with normal urinary calcium excretion.[33] Calcium supplementation was reported to diminish the blood pressure increase and weight

gain that accompany a high salt intake and is known to increase the urinary excretion of sodium.[34]

Although differences in intestinal absorption and bone resorption of calcium between the groups of offspring at different risk for hypertension cannot be excluded, several of our findings suggest that the kidney may play a part in the calcium deficiency. An increased renal calcium excretion at a given level of calcium intake might result in a lower plasma calcium level. The relatively higher ratio of urinary calcium excretion to dietary calcium intake in the offspring of two hypertensive parents, together with the lower plasma calcium in this group, supports this possibility. However, the similar absolute and fractional calcium excretion in the 3-hour fasting morning period in the three groups is at variance. In a recent study by Young and coworkers (unpublished results), a difference in 24-hour urinary calcium excretion between hypertensive and normotensive subjects could be detected only with subjects on a controlled diet of either 400 or 1,400 mg calcium, and not with subjects on a liberal diet. Moreover, no differences in fasting urinary calcium excretion with subjects on either of the two diets could be shown between the hypertensive and normotensive subjects (Young et al, unpublished results). We suggest, therefore, that the small differences in our study in 24-hour urinary calcium excretion are obscured by the liberal calcium intake of the participants. The differences in urinary calcium excretion might be best studied relative to the estimated daily calcium intake, as shown in Table 4. Plasma intact PTH [1-84] was inversely related to plasma calcium and was higher in the offspring of two hypertensive parents. Moreover, plasma intact PTH [1-84] was related to renal fractional excretion of both calcium and phosphate. Given these relations, one would expect to find a higher fractional excretion of phosphate and a lower fractional excretion of calcium in the offspring of hypertensive parents due to the higher plasma intact PTH [1-84] in this group, but the opposite was found. Apparently, the kidney in the offspring of hypertensive parents is less sensitive to PTH. Alternatively, the higher level of plasma intact PTH [1-84] in the offspring of hypertensive parents may be viewed as secondary to a relative calcium deficiency, which could arise from a renal calcium leak as proposed by McCarron et al[13] and Strazullo et al.[18] In the context of a decreased renal sensitivity to intact PTH [1-84], the level of 1,25-dihydroxyvitamin D_3, activated by PTH in the kidney, can be regarded as inappropriately low in the offspring of hypertensive parents. More research on calcium metabolism in these groups of offspring is needed. In particular, oral and intravenous

calcium challenges, to study possible differences in intestinal calcium absorption, and PTH stimulation tests of renal cyclic AMP production and urinary calcium and phosphate excretion are of interest.

In summary, it appears that calcium balance is maintained at a higher level of circulating PTH in offspring of hypertensive parents compared with offspring of normotensive parents, and this may be due to a decreased sensitivity of the kidney to PTH. Our findings suggest that certain alterations in calcium metabolism may play a part in the development of high blood pressure in young subjects genetically at risk for hypertension.

Acknowledgements

We acknowledge K. Hulshof for assistance in evaluation of the dietary history and H. Kornman for her indispensable work throughout the study. Finally, we gratefully acknowledge the parents and children for their enthusiastic participation.

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4.5 IMMUNOGENETIC FACTORS

The histocompatibility-leucocyte-antigen (HLA) complex was determined in 144 fathers and mothers (84 hypertensive and 60 normotensive parents) of the 121 parental couples. In table 1 data on the frequency of various HLA-locus antigens in the hypertensive and normotensive parents are given. The selection of the presented HLA-locus antigens in part A of table 1, is based on HLA-locus antigens that showed a higher frequency in hypertensive subjects in other studies (HLA-B27,[1] HLA-B15,[1,2] HLA-B12,[3,4] HLA-B18,[5,6] BW22,[5,4] and A28[1]). In part B of table 1, the results on the frequency of HLA-locus antigens in the hypertensive and normotensive parents are presented for those HLA-locus antigens that in other studies showed a lower frequency in hypertensive subjects (HLA-A2,[6] HLA-B5,[6] and HLA-Dr2[5]). Differences in frequencies of HLA-locus antigens for hypertensive and normotensive parents were determined by chi-square analysis with Yates' correction.[7] Odds ratios were calculated by the method of Woolf as estimate for relative risk.[8] If one of the equations was zero, the formula of Haldane was used.[8] The HLA-locus antigene frequencies in the hypertensive and normotensive parents presented in part A and B of table 1 do not show a positive or a negative association respectively with hypertension. In part C of table 1 a positive association with hypertension was observed for HLA-B13, and a negative association for HLA-B14 and HLA-Dr4. However, if adjusted for multiple comparisons[7] (8 for the HLA-A locus, 16 for the HLA-B, 6 for the HLA-C and 10 for the HLA-DR locus), not one HLA-locus antigen showed a significant higher or lower frequency among hypertensive subjects compared to normotensive subjects. However, the number of hypertensive (84) and normotensive (60) individuals tested for HLA-locus antigen were small compared to other studies with more hypertensive individuals and much more normotensive controls.[4,5] Therefore, although our crude results suggest that blood pressure may be associated with HLA-B13, B14, and Dr4, these findings remain to be confirmed by future studies.

To test whether HLA-haplotype was associated with blood pressure within families, segregation of HLA-haplotype with blood pressure was studied in 45 families. This could be done in families with at least two participating children; 16 families with two hypertensive parents with a total of 40 children, 15 families with one hypertensive and one normotensive parent with a total of 37 children, 14 families with two normotensive parents with a total of 36 participating children. In each family the HLA-haplotypes were recorded to "ab" alleles of the father and "cd" alleles of the mother. For each child it was examined

Table 1 Comparison of the number and percentage of hypertensive and normotensive parents with a specific HLA-locus antigen; Part A for HLA-locus antigens that showed a positive association with hypertension in other studies, part B with a known negative association, and part C of HLA-locus antigens showing an association in our data, uncorrected for multiple comparisons.

	hypertensive parents		normotensive parents		Relative	Chi-sq	p
	n	(%)	n	(%)	risk		
A							
A28	5	(6)	7	(12)	0.48	1.55	0.18
B12	20	(24)	16	(27)	0.86	0.17	0.69
B15	19	(23)	12	(20)	1.17	0.13	0.72
B18	5	(6)	5	(8)	0.70	0.36	0.56
BW22	3	(4)	3	(5)	0.70	0.23	0.64
B27	6	(7)	4	(7)	1.08	0.004	0.91
Dr7	13	(15)	9	(15)	1.04	0.003	0.91
B							
A2	44	(52)	29	(48)	1.18	0.23	0.64
B5	6	(7)	9	(15)	0.44	2.34	0.12
Dr2	22	(26)	15	(25)	1.06	0.02	0.86
C							
B13	5	(6)	0	(0)	8.37	3.78	0.05
B14	1	(1)	5	(8)	0.13	4.13	0.04
Dr4	22	(26)	27	(45)	0.43	5.46	0.02

whether it had HLA-haplotypes from alleles ac, ad, bc or bd. For the segregation analysis of the HLA-haplotypes with blood pressure in a family the blood pressure level of the child was characterized as "hypertensive" or "normotensive"; criteria of a relatively high or relatively low systolic and/or diastolic blood pressure were defined, as shown in table 4.3 using age and gender specific blood pressure percentiles. For the segregation analysis, children were defined "hypertensive" if they had a systolic and/or diastolic blood pressure in the upper quartile, and "normotensive" if their systolic and diastolic blood pressure were below the 75th centile. For each pair of two children in one family, it was assessed if they had 0,1 or 2 alleles in common and whether the children had a similar or dissimilar hypertension status. In this way, for each family it was calculated how many times a pair of children from that family had 0,1 or 2 alleles in common and were concordant for hypertension or normotension status or disconcordant. In a family with two children one

pair could be tested, in a family of 3 children 3 pairs, with four children 6 pairs, with 5 children 10 pairs. Amongst the 45 families with a total of 113 children, 26 families had two children participating, 16 families three children, two families of four and one of five participating children. Therefore the analysis was done with 96 sib-pairs.

The segregation pattern of HLA-haplotypes with blood pressure in the 45 families is given in table 2. Departure from expected mendelian segregation was evaluated by a goodness-of-fit chi-square test.[6] Sib-pairs with a disconcordant blood pressure status, a total of 43 from 96 sibpairs, did show a tendency for either 0 (14/43=32%) or 2 (16/43=37%) concordant HLA-haplotypes in favour of 1 (13/43=30%) concordant HLA-haplotype (Chi-square = 6.9, $p<0.05$). However, as both 0 and 2 concordant HLA-haplotypes occurred more often than expected in sibships with a disconcordant blood pressure status, one can not draw any conclusion from that finding. In 45 normotensive sib-pairs, the number of observed concordant HLA-haplotypes was not different from expected. In the 45 families only 8 affected, i.e. "hypertensive" sibpairs could be observed among the 96 sibpairs (4 sibpairs in the sibships of two hypertensive parents, 4 with one hypertensive and one normotensive parent, none in the families of two normotensive parents). Therefore, the power is too small to conclude that the HLA-haplotype does not segregate with hypertension in these families.

Another problem in the interpretation of our findings is the fact that hypertension is not yet expressed in the offspring, and that therefore the subdivision of "hypertensive" and "normotensive" children is artificial and may not be appropriate in the future. This may have disturbed the segregation analysis. Affected sibpair analysis may partly circumvent this problem. However this analysis was only possible for 8 affected sibpairs (see above). The number of affected sibpairs was too low to draw any conclusions on the possible co-segregation of HLA-haplotypes with hypertension in families.

Table 2 Segregation pattern of HLA-haplotypes and blood pressure in 46 families; 16 families of two hypertensive parents, 16 families of one hypertensive and one normotensive parent and 14 families of two normotensive parents.

Blood pressure status of the offspring			Number of concordant haplotypes for the											
	"hyper-" tensive	"normo-" tensive	Hypertensive sib pairs				Hypertensive Normotensive sib pairs				Normotensive sib pairs			
			N*	0	1	2	N	0	1	2	N	0	1	2
two hypertensive parents														
- 3 sibs	bc, bd	ad	1	0	1	0	2	1	1	0	0			
- 2 sibs	ac	bc	0				1	0	1	0	0			
- 2 sibs		bc, bd	0				0				1	0	1	0
- 2 sibs		bc, bd	0				0				1	0	1	0
- 2 sibs	ac	bc	0				1	0	1	0	0			
- 2 sibs	ac	ac	0				1	0	0	1	0			
- 3 sibs	bd	ac, bd	0				2	1	0	1	1	1	0	0
- 2 sibs		bc, bc	0				0				1	0	0	1
- 2 sibs	ad	bc	0				1	1	0	0	0			
- 3 sibs	ad	bc, bc	0				2	2	0	0	1	0	0	1
- 2 sibs	bc	ad	0				1	1	0	0	0			
- 3 sibs	bd, ad	bc	1	0	1	0	2	1	1	0	0			
- 3 sibs	bd	bd, bd	0				2	0	0	2	1	0	0	1
- 3 sibs	ac, bc	bd	1	0	1	0	2	1	1	0	0			
- 3 sibs	ac, ad	ac	1	0	1	0	2	0	1	1	0			
- 3 sibs	ad	ac, ad	0				2	0	1	1	1	0	1	0
Observed			*4*	*0*	*4*	*0*	*21*	*8*	*7*	*6*	*7*	*1*	*3*	*3*
one hypertensive, one normotensive parent														
- 3 sibs		bc, bc, bd	0				0				3	0	2	1
- 2 sibs	ad, bd		1	0	1	0	0				0			
- 3 sibs		bd, bc, ad	0				0				3	1	2	0
- 2 sibs		bd, bd	0				0				1	0	0	1
- 2 sibs	ad	bc	0				1	1	0	0	0			
- 2 sibs	ad	ad	0				1	0	0	1	0			
- 2 sibs	ac	ac	0				1	0	0	1	0			
- 3 sibs	ac, ad	ad	1	0	1	0	2	0	1	1	0			
- 2 sibs	ad, ad		1	0	0	1	0				0			
- 2 sibs		bd, ac	0				0				1	1	0	0
- 5 sibs	ad	bd, bc, ad, ac	0				4	1	2	1	6	2	4	0
- 2 sibs	ad	ad	0				1	0	0	1	0			
- 3 sibs	bc, bc	ad	1	0	0	1	2	2	0	0	0			
- 2 sibs	ac	bd	0				1	1	0	0	0			
- 2 sibs		ad, ac	0				0				1	0	1	0
Observed			*4*	*0*	*2*	*2*	*13*	*5*	*3*	*5*	*15*	*4*	*9*	*2*

* N = number of sib-pairs for that condition

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Blood pressure status of the offspring			Number of concordant haplotypes for the											
	"hyper-" tensive	"normo-" tensive	Hypertensive sib pairs				Hypertensive Normotensive sib pairs				Normotensive sib pairs			
			N*	0	1	2	N	0	1	2	N	0	1	2
two normotensive parents														
- 2 sibs		ad, ad	0				0				1	0	0	1
- 2 sibs	ad	bd	0				1	0	1	0	0			
- 4 sibs		ac, ac, ac, bd	0				0				6	3	0	3
- 3 sibs	bc	ad, bc	0				2	1	0	1	1	1	0	0
- 3 sibs		ad, bd, bd	0				0				3	0	2	1
- 3 sibs	bd	bd, ad	0				2	0	1	1	1	0	1	0
- 2 sibs		bd, bd	0				0				1	0	0	1
- 2 sibs		ac, ad	0				0				1	0	1	0
- 2 sibs	bd	bd	0				1	0	0	1	0			
- 3 sibs		bc, ac, ac	0				0				3	0	2	1
- 2 sibs	bc	bd	0				1	0	1	0	0			
- 2 sibs	ad	ad	0				1	0	0	1	0			
- 2 sibs	ad	ad	0				1	0	0	1	0			
- 4 sibs		bc, bc, bc, bd	0				0				6	0	3	3
Observed			*0*				*9*	*1*	*3*	*5*	*23*	*4*	*9*	*10*

For all 45 families with 96 sib-pairs: Number of concordant haplotypes for the

	Hypertensive sib pairs				Hypertensive Normotensive sib pairs				Normotensive sib pairs			
	N*	0	1	2	N	0	1	2	N	0	1	2
Observed	8	0	6	2	43	14	13	16	45	9	21	15
Expected	8	2	4	2	43	10¾	21½	10¾	45	11¼	22½	11¼
Chi-square			*3.0*				*6.9***				*1.8*	

* N = number of sib-pairs for that condition
** df=2, $p < 0.05$.

Haplotypes of complement factor 3 (C3) were studied for the children. The frequency distribution of the C3F, C3S, C3FS protein haplotype is given in table 3. No differences between the groups of offspring for distribution of C3 haplotype were present.

Circulating immune complexes, expressed as % of the children with a positive test (above 10 μEq of aggregated IgG), or as a mean level of equivalents of aggregated IgG in all children or only in those with a positive test, did not differ between the groups (table 4).

Table 3 Frequency distribution of complement C3 haplotype in the three groups of offspring.*

	offspring of two normotensive parents	offspring of one hypertensive parent	offspring of two hypertensive parents
% C3F	12	13	14
% C3S	52	39	46
% C3FS	37	48	40

* Chi-square=2.03, df=4, p<0.8.

The concentration of circulating immune complexes was weakly and positively associated with systolic blood pressure in all children (0.011 mmHg/μEq, SE=0.006, p=0.06) and even stronger in those with a positive test (0.021 mmHg/μEq, SE=0.007, p<0.01). If for each group separately the association between circulating immune complexes (if positive) and systolic blood pressure was studied, a significant association was seen only in the offspring of two hypertensive parents (0.017 mmHg/μEq, SE=0.008, p=0.05). To asses if the findings on similar circulating immune complexes were related to the blood pressure difference between the groups, the level of circulating immune complexes was adjusted for systolic blood pressure. Both in the analysis of the total group and in the analysis of the positive tests only, no difference between the groups was observed after adjustments; difference between offspring of hypertensive parents and offspring of normotensive parents for total group analysis was 20.5 μEq (95% confidence interval -27.1 to 68.1), and for positive tests only -0.7 μEq (-145.7, 144.3).

Although an intriguing association between circulating immune complexes and systolic blood pressure was seen, especially in the offspring of two hypertensive parents, no differences in the level of circulating immunecomplexes were found between the three groups of offspring with or without correction for blood pressure differences between the groups.

In summary, no clear association was seen for HLA-haplotype with blood pressure; neither in the parents, nor in families using segregation analysis. Similar negative results were found for haplotypes of complement factor, and for circulating immune complexes.

Table 4 Circulating immune complexes in the three groups of offspring; mean level per group, mean level per group of those positive, % positive.*

	offspring of two normotensive parents (A)	offspring of one hypertensive parent (B)	offspring of two hypertensive parents (C)	difference between B and A	difference between C and A
Mean level in all					
µEq of aggregated IgG	24.2 (17.3)	63.4 (16.7)	59.9 (15.6)	39.2 [-8.9, 87.3]	35.7 [-20.8, 82.2]
Mean level if positive					
µEq of aggregated IgG	127.4 (62.8)	193.5 (46.2)	207.8 (42.3)	66.1 [-89.9, 222.1]	80.4 [-71.1, 231.9]
% positive					
% with level >= 10 µEq	17.3	30.9	30.8	**	**

* Given are means with SEM in parentheses, and differences between means with 95% confidence intervals between brackets.

** Chi-square = 3.4, df=2, p=0.18

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5 DUTCH HYPERTENSION AND OFFSPRING STUDY: CONCLUSIONS AND HYPOTHESES

5.1 INTRODUCTION

In this thesis the results of the Dutch Hypertension and Offspring Study are presented in view of the hypotheses on hypertensive mechanism reviewed in chapter 1. Possible associations between blood pressure regulating mechanisms in the groups of offspring will be discussed in each section if appropriate. In view of the family history approach (chapter 2) and the results of the selection of the youngsters of the Dutch Hypertension and Offspring Study (chapter 3), it is assumed that a substantially greater part of the youngsters of two hypertensive parents compared to youngsters of two normotensive parents will become hypertensive. Therefore, the offspring of two hypertensive parents as a group is considered pre-hypertensive, while the youngsters of two normotensive parents as a group is considered to remain normotensive.

5.1.1 HAEMODYNAMIC CHARACTERISTICS

In our comparison of the three groups of youngsters at different risk for primary hypertension, no support for the existence of a hyperkinetic circulation in the pre-hypertensive phase of primary hypertension could be found. The slightly increased (calculated) peripheral resistance in those at highest risk for hypertension, points to a possible primary increase in peripheral resistance during the developmental phase of hypertension.

The absence of an increased cardiac output in the early phase of primary hypertension might point to a more pronounced effect of pressor stimuli on the vascular muscle cells, compared to cardiac muscle cells. This was assumed by Korner[1] to explain the transitional phase from a supposed increased cardiac output to an increased peripheral resistance. However, this might also explain why there is no hyperkinetic circulatory phase. If pressor stimuli have a more pronounced effect on the vasculature, the increase in blood pressure and afterload will outweigh the increase in cardiac contractile power and heart frequency. In this way, if pressor stimuli or sensitivity to pressor stimuli are elevated in the offspring of hypertensive parents, an increase in peripheral resistance without a change in cardiac output or heart frequency can be expected.

Whether an increased peripheral resistance in the early phase of primary hypertension, originates from structural or functional differences remains unclear. However, from our findings it can be speculated that functional rather then structural vascular changes are primarily involved.

First, although studied in only a small male group, no differences in arterial diameter of the common carotid artery could be found between the groups. This does not exclude structural changes in the arterial wall as the diameter might remain similar through remodelling of the vessel. Yet, the functional properties of the arterial wall of the common carotid artery during the heart cycle at rest did not show an increased wall stiffness or a decreased cross-sectional compliance, which makes a structural difference less likely.

Second, the differences in blood pressure between the groups were more pronounced during daytime and daily activities, with small nonsignificant differences during the night. This similarly points to functional vascular changes, or to an increased stimulation during daily activities in those at highest risk for primary hypertension. Interestingly the increase in heart rate during day time and daily activities was the same for the three offspring groups, suggestive for a similar increase in sympathetic stimulation during activity. The more pronounced blood pressure increase during daily activities in those at highest risk for hypertension, might therefore arise from hyperresponsive vessels, either by structural or functional changes. In the absence of clear blood pressure differences during the night, it seems more likely that the changes in the vessel wall are of a functional nature.

This is partly supported by others, who found no differences in vascular structure in vitro between offspring groups at different risk for hypertension.[2] Still, in vitro no difference in functional responses to stimulation could be detected either.[2] Others however, demonstrated an enhanced blood pressure responsiveness to noradrenaline infusion and not to angiotensin infusion in vivo in offspring of hypertensive parents compared to offspring of normotensive parents.[3] This favours specific functional differences in response to adrenergic stimuli, and not differences in vascular structure. If differences in vascular structure had existed, an increased response to angiotensin would also be expected. In a study where only one vasoactive substance was tested, an increased response of blood pressure to angiotensin infusion has been described in offspring of hypertensive parents.[4]

In contrast with our results for the peripheral vasculature are observations on the heart muscle, in particular the findings of an increased left ventricular mass in the offspring at highest risk, independent of casual blood pressure at rest, or mean 24-hour ambulatory blood pressure. The difference in left ventricular mass index between the offspring groups was observed also in the lower blood pressure strata or the youngest age groups. No correlation between left ventricular mass and ambulatory blood pressure measured either during daytime

or during daily physical activities was found. Therefore, the relatively elevated left ventricular mass index in the offspring of two hypertensive parents appears not to be explained solely by the elevated mean 24-hour blood pressure or the exaggerated blood pressure increase during daily activities.

Both the slightly increased peripheral resistance and increased left ventricular mass index in the offspring of hypertensive parents might originate from an increased functional response to pressor stimuli at the cellular level. Such increased responsiveness might be dependent on calcium influx as shown by Distlers group,[5] and discussed in chapter 1. Moreover, cellular contractile factors are known to have trophic influences[6] and therefore could lead to structural cardiac changes. In the myocardial wall, the muscular tissue forms a relatively greater part of its total wall compared to the vessel wall. Therefore, structural changes may become apparent sooner in the myocardial wall compared to the vessel wall.

Early structural cardiac changes with a decrease in left ventricular compliance could also explain the early decline of cardiac output found in primary hypertension, as suggested by Lund Johanssen.[7]

Our findings are compatible with the view that in offspring of hypertensive parents relative to offspring of normotensive parents, functional changes in cardio-vascular muscle cells could lead to an increased vasoconstrictive response to normal pressor stimuli that circulate during the day at rest and during daily activities, without measurable differences in response of heart rate or cardiac output. These functional changes, together with an increased responsiveness may eventually give rise to structural changes, especially of the heart.

5.1.2 THE KIDNEY

The findings on renal hemodynamics and the renin-angiotensin-aldosterone system are in favour of an increased efferent renal vasoconstriction in the offspring of two hypertensive parents. An increase in efferent renal vascular resistance could explain the observed decrease in renal blood flow, with an increased filtration fraction and equal glomerular filtration. This is also compatible with the reduced plasma renin activity in the offspring of hypertensive parents. In support of such a relative increased renal vascular tone in offspring of hypertensive parents are findings by others, who reported a rise in renal blood flow after calcium antagonists[8,9] or an angiotensin-converting-enzyme inhibitor.[10]

Suggestive for an exaggerated responsiveness of the renal vasculature to

vasoconstrictive stimuli are findings of an enhanced decline in renal blood flow after a mild psychological stimulus[11] or angiotensin-II infusion in offspring of hypertensive parents.[4] As the difference in renal blood flow between the offspring groups can be produced by various stimuli it is hypothesised that the renal vascular cell in the offspring of hypertensive parents is hyperresponsive, either due to early structural changes or due to intrinsic cellular mechanisms.

The 24-hour urinary sodium excretion was similar in the three groups of offspring. Given the elevated blood pressure in the offspring of hypertensive parents, the decreased renin and aldosterone concentration probably compensates for the lower renal blood flow in that group. We have performed our studies on a liberal sodium intake, on average similar in the three groups, and the findings indicate that the sodium excretion in the offspring of hypertensive parents is maintained at a higher level of blood pressure.

Whether the decrease in renal blood flow is secondary to an increase in blood pressure is unclear.[12] As the relative decrease in renal blood flow in the offspring of hypertensive parents was already apparent in the youngest age tertile, at a mean age of 11 years, the decrease in renal blood flow may well precede the increase in blood pressure. Alternatively, renal blood flow and blood pressure may change together through the same vascular cellular mechanism that give rise to vasoconstriction in the peripheral and renal vasculature.

In accordance with the supposed increased renal efferent vasoconstriction in the offspring of hypertensive parents is the lower plasma venous renin concentration. However, a similar decrease in angiotensin-II was not observed. The similar venous plasma levels of angiotensin-II in the groups of offspring despite differences in plasma renin activity, might be explained by recent findings that part of the venous angiotensin-II is formed from locally tissue produced angiotensin-I. Although speculative, the angiotensin-II production from conversion of locally produced angiotensin-I could be relatively increased in the offspring of hypertensive parents, as they showed lower plasma renin activity, but similar venous plasma angiotensin-II concentration. An increased renal tissue angiotensin-I production could then explain the increased renal efferent vasoconstriction.

In accordance with the lower renin concentration in the offspring of hypertensive parents, is the lower concentration of aldosterone. This is found despite the similar levels of venous plasma angiotensin-II. It might be postulated that the adrenal aldosterone production in the offspring of hypertensive parents is less sensitive to circulating angiotensin-II, in

accordance with the findings in so called "non-modulating" hypertension.[13]

The mean 24-hour sodium excretion was similar for the three groups of offspring, despite the lower concentration of venous plasma aldosterone and renine in the offspring of hypertensive parents. This suggest a sodium retaining mechanism in the offspring of hypertensive parents, that is compensated by a decrease in renin and aldosterone, and an increase in blood pressure.

Sodium retention could result from the decrease in renal blood flow as this may give rise to an increased proximal renal tubular sodium reabsorption. This was indeed reflected by a small decrease in fractional uric acid excretion in the offspring of hypertensive parents. Alternatively, a primary membraneous alteration could be implied. Finally, if the increased cellular $\alpha_2/ß_2$-adrenergic receptor ratio is also present in various tissues of the kidney, this could also explain the lower renal blood flow, the increased proximal tubular sodium reabsorption and the decreased renin concentration.

5.1.3 THE SYMPATHETIC NERVOUS SYSTEM

No clear differences in measures for sympathetic nervous system activity between the groups of offspring were observed. Venous catecholamines and 24-hour urinary excretion of catecholamines were similar in the groups. While these negative results might reflect limitations of the methodology applied, it is interesting to note that a slightly elevated noradrenaline was observed in a two hour urine portion sampled during a stay in the research centre. The activity level in the groups during this time-period of two hours was the same and the negative results of the 24-hour period might have been resulted from differences in activity during the 24-hour sampling period. Moreover, others have found an elevated arterial noradrenaline level in offspring of hypertensive parents,[14] suggestive for an increased noradrenaline production or a decreased clearance.

Nonetheless, even similar noradrenaline levels might give different responses in youngsters at different risk for hypertension. An increased adrenergic responsiveness became apparent in research by others, during infusion of catecholamines in offspring of hypertensive parents.[3] Such a response could result from differences in functional cellular characteristics as α_2- and $ß_2$- adrenoreceptor densities, calcium influx and second messengers.

If the density of platelet α_2-adrenoreceptors is an indicator of receptor densities correlated to other cellular structures, as was discussed in section 4.3.1, the increased platelet

α_2-adrenoreceptor density in the offspring of hypertensive parents may represent an increased vascular and renal α_2-adrenoreceptor density. As was discussed in section 4.3.1., various reports have shown an α_2-adrenoreceptor mediated effect on peripheral[15] and renal vascular[16] constriction and renin production.[17] In the Dutch Hypertension and Offspring Study, an increased platelet α_2-adrenoreceptor density might explain the elevated blood pressure, the diminished renal blood flow, the increased proximal tubular sodium reabsorption and the decreased plasma renin activity in the offspring of two hypertensive parents.

5.1.4 ELECTROLYTE HOMOEOSTASIS

Sodium

The fact that several indicators of sodium homoeostasis such as plasma and cellular levels of sodium and potassium and sodium-potassium-ATP-ase activity, were similar in the different groups of offspring, might imply that no change in sodium-regulating mechanisms exists or that the liberal sodium intake has obscured possible differences between the groups. A positive association was present between 24-hour urinary sodium excretion and blood pressure in the offspring of hypertensive parents, and an inverse relation between 24-hour urinary excretion and atrial natriuretic peptide in that group, that was not apparent in the offspring of two normotensive parents. This points to a possible diminished response of a rise of a natriuretic and blood pressure lowering hormone, secondary to increased sodium intake in the offspring of hypertensive parents.

The finding of a lower plasma renin and aldosterone in the offspring of hypertensive parents, is suggestive for a tendency to sodium retention in that group. However, we have no measures for total body sodium in our study. And as discussed above, the lower renin might be secondary to an increased renal vasoconstriction and/or changes in adrenoreceptor density. If, however, the sodium intake should increase further, the amount of sodium that can be excreted through lowering of renin and aldosterone is limited in the offspring of hypertensive parents. At a high sodium intake, sodium retention may occur in the offspring of two hypertensive parents, either by the lower renal blood flow, plasma renin and aldosterone, or diminished rise of atrial natriuretic peptide to a higher sodium intake, or by a postulated membrane alteration. Such a sodium retention was not expressed in an increased intracellular sodium concentration, as it might be compensated by a slightly increased sodium-potassium-ATP-ase activity in the offspring of hypertensive parents. Accordingly, sodium retention could

first increase cellular sodium concentration and secondary sodium-potassium-ATP-ase activity. In our study no clear differences were observed for the total nonesterified fatty acids; a measure of a circulating sodium transport inhibitor. The theory of a circulating sodium transport inhibitor secondary to sodium and volume retention, that decreases sodium-potassium-ATP-ase activity, and thereby increases renal sodium excretion and cellular sodium concentration, is not supported by our data.

Calcium

Unchallenged, a small decrease in plasma calcium in the offspring of hypertensive parents was accompanied by a small increase in parathyroid hormone level. During a calcium challenge the normal physiologic responses of an increase in plasma calcium level and a decrease in plasma parathyroid hormone level were found, but no difference in changes between the groups of offspring. While the magnitude of the decrease in plasma parathyroid hormone level after the calcium challenge was similar, plasma parathyroid hormone became significantly lower in the offspring of hypertensive parents, in accordance with a somewhat more pronounced increase in plasma calcium. It is hypothesised that the offspring of hypertensive parents have a more pronounced negative feedback of plasma calcium on parathyroid hormone after a rise of plasma calcium secondary to increased calcium intake. The depressed plasma parathyroid hormone level might result in a relatively increased urinary calcium excretion. Actually, this was observed; the relative increase in urinary calcium excretion after the calcium challenge was higher in the offspring of hypertensive parents (4 times increase) compared to the offspring of two normotensive parents (2 times the increase). This is in agreement with our finding of a slightly higher urinary calcium excretion with similar dietary calcium intake. During periods of low calcium intake this may result in a somewhat lower serum calcium with an increased positive feedback on plasma parathyroid hormone as was seen during the first two phases of the study, during a fasting state.

It is postulated that the small differences in calcium homoeostasis between the groups of offspring could occur from either an increased (positive and negative) feedback of plasma calcium on plasma parathyroid hormone; from a decreased renal sensitivity to plasma parathyroid hormone; or from a membrane defect that leads to calciuresis. In our study no differences in cellular membrane calcium handling between the groups of offspring were observed.

The differences in calcium homoeostasis might lead to blood pressure elevation through an increase of intra-cellular calcium. However, no differences in intra-lymphocytic calcium concentration between the groups were observed, and no association with parathyroid hormone could be found.

Sodium and calcium

Others have observed an increased urinary calciuresis in offspring of hypertensive parents compared to offspring of normotensive parents on a high sodium diet, but not on a low sodium diet, without differences in natriuresis during the high sodium diet.[18] A high sodium diet may provide a stimulus for an increased calciuresis in the offspring of hypertensive parents. In our study, the increased calciuresis after a calcium challenge in the offspring of hypertensive parents was observed during the fasting state, after a liberal sodium intake with similar 24-hour sodium excretion the day before in the groups of offspring. Although unlikely, it cannot be excluded, that sodium intake and excretion have influenced our results. On a high sodium diet the findings of small differences in calcium homoeostasis between the groups could have been more pronounced. A more pronounced increase of calciuresis on a high sodium diet may mediate the above mentioned association between 24-hour urinary sodium excretion and blood pressure.

5.1.5 IMMUNO-GENETIC FACTORS

From our results on frequency of HLA-haplotypes among the hypertensive and normotensive parents, no clear association of HLA-haplotype and blood pressure category was apparent.
In the segregation analysis of blood pressure category and haplotypes of HLA in the sibships of 45 families, the number (0, 1 or 2) of similar haplotypes between a sibship was not significantly different from that expected, either if the sibs were in a similar or in a dissimilar blood pressure category.

In these families no distinct blood pressure trait could be observed with the HLA-haplotypes. It is therefore not likely that the immune system is involved in an early phase of primary hypertension. This does not exclude a possible important role for the HLA system in disease progression, by modifying the immune response when for instance endothelial damage due to hypertension has occurred. In that case the immune system and HLA-haplotypes may be related to the complications during the course of hypertension.

5.2 FUTURE LINE OF RESEARCH

Assessment of blood pressure change

The age range of the members in the cohort by the end of 1994 will be 13 to 38 years of age, with a mean age of around 28. With a remeasurement of blood pressure after 1994, an 8 to 19 year follow up period could be reached: 19 years if the subject participated as a child to the screening phase of the EPOZ survey between 1975-1978 (i.e. 133 of the 180 subjects from the DHOS), 8 years if the subject participated for a first time to the Dutch Hypertension and Offspring survey in 1987.

It is very important that the cohort members are remeasured; only then can we proof our assumption that the offspring of two hypertensive parents in the Dutch Hypertension and Offspring Study was at excess risk to develop hypertension, compared to the offspring of two normotensive parents.

Furthermore, then the determinants of the rise in blood pressure can be formulated;

- One could compare the baseline characteristics measured during phase 1,2 and 3 for the offspring stratified according to the blood pressure level reached during follow up,
- Moreover, baseline characteristics could be stratified for the highest blood pressure increase measured during follow up.

Only then we will know if for instance a diminished renal blood flow, or an increased α_2/β_2 adrenoreceptor density ratio is an important determinant for future blood pressure rise in youngsters with hypertensive parents.

Further examination

The youngest group of the Dutch Hypertension and Offspring cohort will reach the age of 13 to 25 at the end of 1994. This group might be suitable to do additional research.

Further examination: α_2-adrenoreceptor density, blood pressure, renal blood flow and renin

To investigate wether the increased trombocytic α_2-adrenoreceptor density in the offspring of hypertensive parents is indicative of an increased response of systemic and renal vascular smooth muscle cells t.. α_2-adrenergic stimulation, and of an increased renal nerve presynaptical and proximal renal tubule response to α_2-adrenoreceptor stimuli, the response to α_2-adrenoreceptor agonists should be studied either in vitro or in vivo.

In vitro, using a muscle biopt to isolate resistance vessels, the response of the resistance

vessels to various agonists an antagonists could be compared with a myograph.[19] However, no in vitro information on renal function and renin production can be gathered. Moreover, in vitro responses might not be indicative of in vivo responses.

For in vivo tests one could choose for a local intra-arterial infusion to study the response in a local vascular bed, without systemic effects, by use of venous occlusion phlethysmography.[20] Combinations of an agonist and an antagonist could be tested as only low doses have to be used to attain a local effect, without the danger of systemic effects. To stimulate the α_2-adrenoreceptor a combination of adrenalin with a ß-blocker is of interest. To exclude a difference between the groups in non-specific vascular reactivity due to possible differences in early structural vascular changes, the responses should be compared to the non-specific stimulus of angiotensin-II.[3]

An in vivo method that would measure both peripheral vascular and renal responses to the stimuli used, is an intravenous infusion of an agonist, that does not cross the blood-brain barrier, and may be infused in different amounts such that the lowest doses give only small or no systemic effects. To study the renal response a prolonged infusion at each dose-interval allows to obtain measurements to calculate the clearance of para-amminohippuric acid. This has been done for 30-minutes clearance-episodes with infusion of angiotensin by Ljungman.[21] Because of the systemic infusion, a specific agonist is preferred, rather than the above mentioned combination. α-Methylnoradrenaline specifically stimulates α_2-adrenoreceptors. This is a long-acting α_2-agonist, a metabolite of α-methyldopa, that does not cross the blood-brain barrier if given systemically.[22,23] If given intra-venously in doses of 0.02 to 2.0 µg/kg α-methylnoradrenaline will give a clear blood pressure rise from 0.3 µg/kg unwards. The lower doses might be used to measure the effect on renal hemodynamics.[22,23] During the several dose steps blood pressure must be measured frequently and blood has to be sampled to measure plasma renin activity, noradrenaline (to monitor a possible effect of α-Methyl-noradrenaline on presynaptic $ß_1$- adrenoreceptors), para-amminohippuric acid, creatinine and uric acid (last three also in timed urine samples to calculate renal plasma flow and fractional excretion of uric acid). To exclude a-specific effects due to differences in vascular reactivity through early vascular changes, the results of the infusion with α-methylnoradrenaline must be compared with results of an infusion with angiotensin-II.

If the hypothesis is correct, one will find a stronger dose-response effect for α-methylnoradrenaline (i.e. on a lower dose, or a stronger effect on a similar dose) on the

decrease of renal plasma flow, of renin, and of fractional uric acid excretion without differences in systemic effects at low doses, and a stronger blood pressure response on high doses in the offspring of hypertensive parents compared to the offspring of normotensive parents.

Further examination: Sodium homoeostasis, calcium metabolism and blood pressure

To study hypotheses on sodium homoeostasis, one cannot escape from the need to study the groups of offspring on a standardized low (50 mmol/day) and standardized high sodium (200 mmol/day) intake. Each diet should be maintained at least 7 days to be sure that a new balance of sodium intake and sodium excretion has been reached. On the eight day several test can be done.

The first 7 days on the diet, body weight, 24-hour sodium excretion and blood pressure can be monitored to study a possible sodium retention and effect on blood pressure. If the diet is also stable in calcium, calciuresis may be measured.

In a four-hour balance study on day 8, a two hour clearance period to measure fractional uric acid excretion as a measure of proximal tubular sodium reabsorption could be included. Moreover, a calcium challenge at day 8 in the middle of the four hour clearance period, gives the possibility to calculate calcium excretion in the two hours before and after the calcium challenge and to test if calciuresis after a calcium challenge is more pronounced in the offspring of hypertensive parents on the high sodium diet.

At day 0 and at day 8 total exchangeable sodium should be measured by a standard-isotope-dilution technique, as a measure for total body sodium.[24] In this way, it can be determined whether the rise of exchangeable sodium during the high sodium diet is increased in the offspring of two hypertensive parents. Moreover atrial natriuretic peptide, intracellular sodium, potassium and calcium could be measured together with plasma electrolytes, parathyroid hormone, and the erythrocytic sodium-potassium-ATPase activity, and plasma renin activity at day 0 and day 8.

After the change from a low to a high sodium diet the offspring of two hypertensive parents are expected to show a more pronounced increase in blood pressure, in exchangeable sodium, in intra-cellular sodium and/or calcium and in erythrocytic sodium-potassium ATP-ase activity. Moreover, a more pronounced decrease in plasma renine activity and fractional uric acid clearance might be found, and a decrease in plasma calcium with an increase in parathyroid hormone. The results should be examined stratified according to α_2/β_2-

adrenoreceptor density ratio, as this has been shown to be a determinant of salt sensitivity in familial hypertension.[25]

Further examination: Structural changes and growth potential of isolated vascular smooth muscle cells

If a muscle biopt can be taken, resistance vessels might be isolated to study their morphology and state if changes in vessel wall thickness and amount of smooth muscle cell layer have occurred. Several studies have now shown that such a biopsy can be done in healthy subjects with minimal inconvenience. Moreover, cells could be brought into culture, and studied after stimulation by various potential growth factors, to study if the intrinsic capacity of vascular smooth muscle cells from offspring of hypertensive parents is increased compared to offspring of normotensive parents.[26]

Finally...

The Dutch Hypertension and Offspring Study is an unique research project, in which many aspects of the early pathogenesis of primary hypertension were studied, in a very well defined cohort of youngsters, selected on bases of multiple parental blood pressure measurements. The idea of the design and the possibility to select the families from a population based survey on cardiovascular risk indicators, originated in the epidemiological department of the Erasmus University Rotterdam. The financial support from the Netherlands Heart Foundation was indispensable. The project has succeeded due to the collaboration of many Dutch and some European investigator: who all gave their expertise and effort. We feel that it is very important to execute the next phase of the Dutch Hypertension and Offspring Study. It gives an unique possibility to study determinants of the blood pressure pattern over time (8 years) in youngsters of hypertensive parents, using the very well defined cohort and the numerous baseline characteristics that have been studied already. Support to make the future lines of research possible, at least to remeasure the blood pressure in the groups of offspring, is of major importance.

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6 SUMMARY

Mortality and morbidity from cardiovascular diseases remain a major public health problem, despite treatment of hypertension and other cardiovascular risk factors. To study the early phase of primary hypertension is of interest to clarify the multifactorial origin of hypertension. The final aim is to prevent elevation of blood pressure and find ways for more individualized and causal treatment of hypertension.

The Dutch Hypertension and Offspring study was designed to study the early phase of primary hypertension by comparing offspring of two hypertensive parents, of one hypertensive and one normotensive parent, and of two normotensive parents in various characteristics deemed to be important in the etiology and pathophysiology of blood pressure elevation.

Chapter One

In chapter one, major hypotheses considered at the time of the start of the Dutch Hypertension and Offspring Study are discussed in five sections discussing haemodynamic characteristics, renal involvement, sympathetic nervous system activity, sodium and calcium homoeostasis and immunogenetic factors respectively.

The hypotheses on haemodynamic changes in the early phase of primary hypertension refer to either a primary increase in peripheral resistance or a primary increase of cardiac output, with more emphasis on the former.

Renal involvement, apparent from kidney transplant experiments, was hypothesised to be dependent on changes in renal perfusion either by an increased or decreased renal vasodilatation and the renin-angiotensin-aldosterone system in relation to renal perfusion and sodium excretion. It was concluded that either a primary increase of renal vasoconstriction by the local renin-angiotensin system or circulating pressor substances was of importance, or a decrease of renal sodium excretory capacity by a membrane abnormality.

Possible involvement of the sympathetic nervous system was considered to be related to an increased activity, either at rest or during stress or both, an increased reactivity or responsiveness at the level of adrenoreceptors, or at the level of the blood vessel wall. The possible associations between the sympathetic nervous system and mechanisms of blood pressure regulation were discussed.

In the fourth section of section two, chapter one, two hypotheses that relate sodium retention to hypertension were considered. The theory of the infinite gain of pressure

natriuresis as a mechanism to control sodium and water excretion by an increase in blood pressure, primarily through an increase in cardiac output, secondarily by an increase in peripheral resistance. Moreover the theory of a postulated circulating sodium transport inhibitor secondary to sodium and water retention, that primarily leads to inhibition of sodium-potassium-ATP-ase activity and secondary to both renal sodium excretion and increase of intracellular sodium concentration. This was hypothesised to lead to an increased intracellular calcium concentration by either voltage-dependent calcium channels or sodium-calcium exchange. Both the infinite-gain-theory of pressure natriuresis and the theory on the circulating sodium transport inhibitor depend on a primary mechanism of sodium retention, that might either be related to renal changes or membraneous abnormalities. Therefore possible changes in membrane characteristics were discussed.

The hypotheses on calcium in blood pressure regulation were related to epidemiological observations on the relation between low dietary calcium intake and hypertension. The hypothesis of a low serum calcium and an increased parathyroid hormone activity, that could lead to a small increase in intracellular calcium, was presented. The low serum calcium was discussed to be related either to an increased urinary calcium excretion, or a deficient calcium intake or uptake. Possible changes in cellular calcium characteristics were discussed for intracellular calcium concentration, calcium fluxes, and calcium membrane binding. Finally, a discussion on a possible interaction between sodium and calcium in hypertension was presented, with regard to renin, renal excretion and dietary intake of sodium and calcium.

In the fifth and last section on major hypotheses in the early phase of primary hypertension, the possible association of immunogenetic factors, as Histocompatibility-Leucocyte-Antigen complex (HLA-type), immunoglobulines and immune complexes, with hypertension was discussed.

Chapter Two

A discussion on the nature of primary hypertension and its mode of familial transmission, either multifactorial or mendelian, is presented recalling a recent classical debate in medical history between Platt and Pickering on the modality of the blood pressure distribution. Several approaches for studies on the early phase of primary hypertension are presented, with emphasis on the family-history design. The prime assumption on which this approach is

based, is an increased risk for high blood pressure in a subject with hypertensive family members. The method to select the families at different risk for hypertension is discussed with a view on a maximal contrast in offspring groups.

Chapter 3

All the details of the methodology of the Dutch Hypertension and Offspring Study are provided here. First a short description of the study design is given, with emphasis on absence or presence of parental hypertension as the determinant for future risk of hypertension in the selected youngsters. In section two the selection method is described, based on blood pressure measurements in a cohort drawn from the general population. The diagnosis of hypertension and normotension in that cohort was based on one-year age and gender specific blood pressure distributions, and defined by a blood pressure in the upper or lower extreme respectively. Three types of families were selected, with;

- two hypertensive parents (offspring at highest risk for hypertension),
- one hypertensive parent and one normotensive parent (offspring at intermediate risk),
- two normotensive parents (offspring at lowest risk for hypertension).

Finally, from 2161 parental couples, 121 families with 291 healthy children aged 5 - 30 years were selected; 51 couples of two hypertensive parents with 111 youngsters, 35 couples of one hypertensive and one normotensive parent with 98 children and 35 normotensive parental couples with 82 offspring.

In section four of chapter three the protocol for each phase of the Dutch Hypertension and Offspring Study is described, followed by a detailed methodology used to assess the various parameters of interest.

General characteristics of the parents and their offspring is given in section 5, with emphasis to the blood pressure distribution in each group of offspring. To compare the selection method with the 'so-called' four-corner approach, the distribution of the selected offspring with a blood pressure centile position at the extreme high, low or two middle quartiles of systolic and/or diastolic blood pressure is given for the three types of families.

Chapter Four

The results of all the unchallenged measurements done in the first three phases of the Dutch Hypertension and Offspring Study are given, grouped according to the five groups of factors

potentially involved in the pathogenesis of early primary hypertension.

Our findings on haemodynamic characteristics do not support the presence of a hyperkinetic circulation in the early phase of primary hypertension, as cardiac output and index were similar in the three groups of offspring. In the offspring of hypertensive parents blood pressure was significantly higher compared to offspring of two normotensive parents, both crude and adjusted for differences in age, height, body weight and proportion of males between the groups. This was confirmed in all analyses, except in the youngest age group in an analysis stratified for age of the 133 youngsters measured at the original survey in 1975-78. The mean change in blood pressure per year between the 1975-78 and the 1987 survey, was most pronounced in the offspring of two hypertensive parents. Ambulatory 24-hour blood pressure measurements did show a higher blood pressure all through the 24-hour period in the offspring of two hypertensive parents compared to offspring of two normotensive parents, but with small differences during the night and most marked differences during daily physical activities. Echocardiographic measurements showed an increased calculated left ventricular mass index in the offspring of hypertensive parents. The calculated total peripheral resistance was slightly, but not significantly, increased in the offspring of hypertensive parents. No difference was found in the dynamic vessel wall properties between male offspring of two hypertensive and two normotensive parents.

An extensive assessment of renal haemodynamics showed a lower renal blood flow in the offspring of two hypertensive parents. This was combined with an increased filtration fraction and calculated renal vascular resistance, and equal glomerular filtration. Similar results were found if adjusted for differences in blood pressure between the groups. Moreover plasma renin activity and aldosterone level were lower in the offspring of hypertensive parents. The difference in plasma renin activity disappeared after adjustments for differences in renal vascular resistance. The level of angiotensin-II did not differ between the groups.

The results of various measures of sympathetic nervous system activity did not show differences between the groups, e.g. in venous or 24-hour urinary excretion of noradrenaline, adrenaline and dopamine. Analysis of a two hour urine sample, however, collected after a two hour leisure period, suggested an increased noradrenaline excretion in the offspring of two hypertensive parents. More clearly, the density of platelet alpha$_2$-adrenoreceptors was increased in the offspring of two hypertensive parents with a similar density between the

groups of lymphocytic $beta_2$-adrenoreceptors. As a result the cellular $alpha_2/beta_2$-adrenoreceptor density ratio in the offspring of two hypertensive parents was raised. Results remained similar when adjusted for differences in blood pressure between the groups. No relation was found between adrenoreceptor densities and blood pressure.

On a liberal sodium intake no differences between the groups were present for 24-hour urinary sodium and potassium excretion, or for plasma levels of these electrolytes. Mean plasma level of atrial natriuretic peptide was similar in the three groups. However, if stratified by 24-hour sodium excretion, at a high 24-hour sodium excretion (above 200 mmol/24 hour) a difference was observed with a relatively lower level of atrial natriuretic peptide in the offspring of hypertensive parents. In view of a role for a for a circulating sodium transport inhibitor, no differences were observed between the groups for total nonesterified fatty acids, with only small differences for palmitoleic acid, slightly elevated in the offspring of one or two hypertensive parents. Examinations of cellular sodium homoeostasis disclosed no clear difference in erythrocytic membraneous sodium-potassium ATPase activity between the groups, although it was slightly increased in the offspring of hypertensive parents. Intra-cellular concentrations of sodium and potassium were the same in the groups. No relation was found between sodium-potassium ATP-ase activity and intra-erythrocytic sodium concentration, but an inverse relation was present between sodium-potassium ATP-ase activity and intra-erythrocytic potassium and intra-erythrocytic sodium/potassium ratio. Renal sodium handling at the proximal tubules estimated from the fractional excretion of both lithium and uric acid, suggested an increased proximal tubular sodium reabsorption by a diminished fractional uric acid excretion in the offspring of two hypertensive parents. Calculation of distal reabsorption of sodium did not show any difference between the groups, despite a lower aldosterone level in the offspring of two hypertensive parents. The findings are compatible with a relative sodium retention on a liberal sodium intake in the offspring of two hypertensive parents.

Small reductions in plasma levels of calcium, magnesium and phosphate were present in the offspring of two hypertensive parents. Plasma intact parathyroid hormone [1-84] was elevated in the group of offspring of two hypertensive parents. Dietary intake of calcium, phosphate or fibre, and plasma levels of 1,25-dihydroxy vitamin D_3 were similar for the three groups. 24-hour Urinary excretion of calcium while on a liberal calcium intake, and the ratio of urinary calcium to daily calcium intake were slightly increased in the offspring of

hypertensive parents. In a three hour clearance period, no differences were found between the groups for the calculated fractional excretion of calcium. Yet fractional excretion of phosphate was decreased in the offspring of hypertensive parents. Moreover, an inverse association was found between plasma parathyroid hormone and plasma calcium. These findings suggest that calcium metabolism in offspring of hypertensive parents is maintained at a higher level of circulating parathyroid hormone. This may be secondary to an increased urinary calcium excretion, either to a membrane alteration or to a diminished sensitivity of the kidney for parathyroid hormone. In further experiments, calcium and phosphate excretion was measured before and after an oral calcium load. Although no difference in plasma levels of calcium and parathyroid hormone existed before the calcium load, the fasting urinary calcium excretion before loading was twice as high in the offspring of two normotensive parents, but became similar after calcium loading. This reflected a four-fold rise in urinary calcium excretion in the offspring of hypertensive parents, compared to a two-fold rise in the offspring of two normotensive parents. Moreover, an enhanced increase in fractional urinary excretion of calcium was observed in the offspring of two hypertensive parents, despite a similar reduction in plasma parathyroid hormone concentration. This would suggest an urinary calcium leak after an oral calcium load. An attenuated effect of parathyroid hormone on renal calcium retention can not be excluded.

Evaluations of the various loci of the histocompatibility-leucocyte-antigens (HLA) did not show a difference in frequency between hypertensive and normotensive parents. Segregation-analysis of HLA-haplotype with blood pressure within 45 families with at least two participating children, did not show trends for segregation of blood pressure with immunogenetic markers as HLA. However, these results remain inconclusive as only very few hypertensive sibpairs could be analyzed. Similar negative results were found for complement C3 haplotype and circulating immune complexes.

Chapter Five

Finally in chapter five, section one, conclusions on the findings are drawn. To quote professor R. Platt in the Lancet (1959;273:159-164) on 'The Nature of Essential Hypertension', adapted freely to the conclusions discussed in the first section of chapter five;

'This section discusses the findings of the Dutch Hypertension and Offspring Study

on the early pathogenesis of primary hypertension. It is quite short. The conclusions cannot be summarized and those who seek to understand the arguments should therefore read the first section of chapter five'.

The second part of chapter five gives recommendations for further research in the Dutch Hypertension and Offspring Study. It is of pivotal importance to remeasure blood pressure in the study population to be able to define those youngsters who have indeed become hypertensive and to test if blood pressure change and blood pressure status after 8 to 19 years may be related to the characteristics studied that were different between the offspring groups during the first three phases of the study. Furthermore, hypotheses that arose from the findings in the first three phases of the Dutch Hypertension and Offspring Study may be explored in more detail. In particular the reactivity of blood pressure, renal haemodynamics and renin after stimulation of alpha$_2$-adrenoreceptors with alpha-methyl-noradrenaline could be examined, or sodium homoeostasis with total exchangeable sodium, blood pressure responses and calcium excretion on a low and high sodium intake. Moreover, in vitro effects of growth factors on isolated vascular smooth muscle cells, may give information on the intrinsic cellular growth potential.

Continuation of research in the Dutch Hypertension and Offspring Study, may provide important information on the determinants of rise and change of blood pressure in youngsters with high and low familial risk for hypertension. The findings in the baseline examinations of the Dutch Hypertension and Offspring Study have provided strong arguments for prolonged follow-up and reexamination of those carefully selected and enthusiastic groups of offspring.

7 SAMENVATTING

Hart- en vaatziekten zijn nog altijd een groot gezondheidsprobleem, ondanks aandacht voor preventie en behandeling van risicofactoren voor hart en vaatziekten, zoals hypertensie. Hypertensie komt bij ongeveer 10% van de mensen voor, bij ouderen kan dit zelfs 20 tot 25% bedragen. Bij het merendeel van de mensen met hypertensie (90%), kan er geen duidelijke oorzaak voor de bloeddruk verhoging worden gevonden; men spreekt dan van een primaire hypertensie. Dit in tegenstelling tot een secundaire hypertensie ten gevolge van een duidelijk aanwijsbare stoornis. Primaire hypertensie komt vaak familiair voor; het risico erop neemt toe als een eerste-graads familielid hypertensie heeft. Door onderzoek te verrichten naar de vroege fase van het ontstaan van de bloeddrukverhoging, probeert men de multifactoriële oorsprong van verhoogde bloeddruk te verduidelijken. Uiteindelijk is het doel om een beter op de oorzaak van de hypertensie afgestemde therapie te kunnen ontwikkelen, dan wel om de bloeddrukstijging te voorkomen.

Het onderzoek beschreven in deze dissertatie, 'The Dutch Hypertension and Offspring Study', werd opgezet om de vroege fase van de bloeddrukverhoging te bestuderen door kinderen van ouders met hoge bloeddruk te vergelijken met kinderen van ouders zonder hoge bloeddruk. Van de factoren die werden onderzocht bij de jongeren werd vooraf verondersteld dat deze mogelijk van invloed waren op de ontwikkeling van de bloeddruk stijging. Deze veronderstelling werd gebaseerd op bevindingen in andere onderzoekingen.

Hoofdstuk 1

Deze veronderstellingen worden in hoofdstuk 1 in vijf onderdelen uiteen gezet. Zoals bijvoorbeeld de rol van de bloedsomloop, de circulatie bij het ontstaan van een verhoogde bloeddruk. het kan zijn dat het hart meer bloed rond pompt dan strikt noodzakelijk, dan wel dat de bloedvaten meer samen knijpen. In medische termen; is er primair sprake van een verhoogd hartminuutvolume of van een verhoogde vaat-weerstand?

Een belangrijke bijdrage van de nier in het ontstaan van hypertensie, wordt verondersteld naar aanleiding van onderzoek bij nier-transplantatie patiënten en dier-experimenten, waaruit blijkt dat als het ware de aanleg voor hypertensie in zekere mate door wordt gegeven met het niertransplantaat. Daarnaast speelt de nier een unieke rol in de water- en zout huishouding en de bloeddruk regulering, zodanig dat water en zout-retentie een bloeddrukverhoging uitlokt die uiteindelijk tot een verhoogde water- en zout excretie lijdt. Daarna moet de bloeddruk weer lager worden. In de nier wordt een aantal systemen

verantwoordelijk verondersteld voor de relatie tussen de water-en zout uitscheiding en de bloeddruk. Deze systemen zouden ook van belang zijn bij het ontstaan van hypertensie. Er wordt gedacht dat de mate van samentrekking van het niervaatbed, van belang voor de doorbloeding van de nier en daarmee de functie van de nier voor water en zout uitscheiding, verhoogd is bij de ontwikkeling van hypertensie. Ook zouden lokaal geproduceerde hormonen, zoals het renine en angiotensine, alsmede drukverhogende stoffen elders uit het lichaam, de niervaatweerstand kunnen beïnvloeden. Het gevolg kan zijn dat de bloeddruk toe moet nemen om de functie van de nier te waarborgen.

In het bloed circuleren een aantal stoffen, zoals noradrenaline en adrenaline, die bloeddruk verhogend werken. Noradrenaline komt vrij bij de zenuw uiteinden van het sympatische zenuwstelsel. Adrenaline komt vrij in de bloedbaan na prikkeling van de bijnier door het sympatische zenuwstelsel. Beide stoffen hebben via hun werking op hartspiercellen en gladde spiercellen in de vaatwand, een bloeddruk verhogend effect. Bij dagelijkse activiteiten wordt het sympatische zenuwstelsel gestimuleerd en zullen deze stoffen vrijkomen. Een toename in de duur en frequentie van stimulering of een toegenomen afgifte van deze stoffen per stimulering veroorzaakt een toegenomen aanwezigheid van deze stoffen in het lichaam. Daardoor kan dit uren later nog tot een bloeddruk verhoging lijden. Aangrijpingspunten voor deze stoffen op de gladde spiercellen in de vaatwand zijn de zgn. adrenoreceptoren. Een verandering in dichtheid van deze receptoren kan de reactie van de vaatwand op bijv. noradrenaline beïnvloeden. Deze receptoren zijn ook van belang voor allerlei aspecten in de nier, zoals o.a. de niervaatweerstand, de renine produktie en de zout uitscheiding.

De zout-huishouding is van belang omdat bij een toegenomen hoeveelheid zout in het lichaam de bloeddruk kan verhogen. Een toename van de totale hoeveelheid zout kan ontstaan door een verhoogde inname, die bij aanvang niet geheel wordt gecompenseerd door een vergrote uitscheiding, dan wel een verandering in de nier waardoor het zout moeilijker uitgescheiden wordt. Er zijn ook veronderstellingen dat bepaalde stoffen gemaakt worden als reactie op een verhoogd water en zout volume. Zo'n stof zou dan naast de gewenste uitscheiding van zout in de nier echter ook de opname van zout in andere cellen bevorderen. Bijvoorbeeld in de gladde spiercel, waardoor indirect via een verhoging van calcium in de cel, weer een bloeddrukverhoging kan ontstaan. Het primaire mechanisme waarmee zout wordt vastgehouden, zou een membraan afwijking kunnen zijn of een verandering in de

nierdoorbloeding.

Ook afwijkingen in de kalk-huishouding van het lichaam, zoals veranderingen in de calcium uitscheiding via de nier en een verhoogde activiteit van de bijschildklier, zouden de bloeddruk kunnen verhogen. Uiteindelijk verloopt dit via een verhoging van het intracellulaire calcium.

Eveneens werd verondersteld dat hypertensie meer zou voorkomen bij mensen, met bepaalde type weefsel markers uit de immuno-genetica (HLA-type), die men op bloedcellen kan meten en die erfelijk bepaald zijn.

Hoofdstuk 2

In dit hoofdstuk wordt weergegeven op grond waarvan aangenomen wordt dat hypertensie familiair voorkomt. Dit naar aanleiding van een beroemde discussie in de medische geschiedenis tussen engelse professoren over de aard van overerving van hypertensie: multifactoriëel met beïnvloeding vanuit de omgeving, of via Mendeliaanse genetische overerving. De discussie had zijn oorsprong bij de vorm van de verdelings curve van de bloeddruk: een één-toppige berg, of een berg met meerdere toppen. De één-toppige bergen op de omslag duiden op de uitslag van deze discussie en geven zgn. Gausse-krommen weer.

Daarna volgt een uiteenzetting van de uitgangspunten voor de selectie van de kinderen in the Dutch Hypertension and Offspring Study. Het verhoogde risico op hypertensie bij een familielid van een persoon met hypertensie geldt als het belangrijkste criterium. Verder wordt besproken hoe een zo groot mogelijk contrast in risico kan worden verkregen, met nadruk op de selectie van de laag risico groep, naast de hoog risico groep. Men selecteert als het ware de families waarvan de ouders een bloeddruk hebben in de extremen van de bloeddrukverdeling, de uitlopers van de berg.

Hoofdstuk 3

Hierin worden alle methoden weergegeven gebruikt in het onderzoek. Allereerst de methode van selectie, op basis van de in hoofdstuk twee beschreven uitgangspunten. Voor ons onderzoek hebben we de families geselecteerd uit een groot onderzoek onder de bevolking van Zoetermeer. De families werden geselecteerd op grond van de bloeddruk van de ouders. Deze bloeddruk moest stabiel hoog zijn ten opzichte van leeftijdsgenoten van de zelfde sexe, of stabiel laag. Op grond van de bloeddruk bij beide ouders werden de volgende typen

families geselecteerd; families met
- twee ouders met hoge bloeddruk (kinderen met het hoogste risico)
- een ouder met hoge en een ouder met lage bloeddruk (intermediair risico)
- twee ouders met een lage bloeddruk (kinderen met het laagste risico op hypertensie).

Uit het oorspronkelijke onderzoek van rond de 10.000 personen met 2161 ouderparen, werden op basis van de eerste meting tussen 1975 - 1979, en een hermeting in 1986, 121 families geselecteerd met 291 gezonde kinderen in de leeftijd van 5 tot 30 jaar, anno 1986. Het betrof 51 families met twee hypertensieve ouders met 111 kinderen, 35 ouderparen waarvan een ouder hoge en een ouder lage bloeddruk had, met 98 kinderen, en 35 ouders met een lage bloeddruk met 82 kinderen.

De onderzoekingen die bij de jongeren na toestemming werden verricht, worden uiteengezet in het protocol bij de verschillende fasen van het onderzoek, en bij de beschreven testen en methoden verder gespecificeerd. In samenwerking met vele andere instituten in Nederland konden zo velerlei testen worden verricht.

De bevindingen aangaande bloeddruk, leeftijd, lengte, gewicht etc. van de deelnemende ouders en kinderen worden weergegeven in de vijfde sectie van hoofdstuk 4, met speciale aandacht voor de spreiding van de bloeddruk in de drie groepen.

Hoofdstuk 4 en 5

In hoofdstuk vier worden de resultaten uiteen gezet, gegroepeerd volgens de vijf groepen veronderstellingen, beschreven in hoofdstuk 1. Conclusies die hieruit worden opgemaakt, worden beschreven in hoofdstuk 5, en hierbij kort vermeld.

Bij de jongeren van twee ouders met hoge bloeddruk bestond geen verhoogd hartminuutvolume, maar al wel een (ten opzichte van de jongeren van de twee ouders met lage bloeddruk) licht verhoogde bloeddruk. Hierdoor was de berekende vaatweerstand al enigszins verhoogd bij de jongeren met twee hypertensieve ouders. Aangezien ambulante metingen van de bloeddruk gedurende 24 uur, zeer kleine verschillen gedurende de nacht laten zien tussen de groepen, en grotere verschillen op de dag, en met name tijdens dagelijkse lichamelijke activiteit, worden functionele afwijkingen van het vaatsysteem verondersteld, en geen structurele afwijkingen. Bij structurele afwijkingen van de vaten zouden 's-nachts de verschillen tussen de groepen mogelijk ook wat groter zijn.

Afbeeldingen met ultrageluid van het hart, waarmee onder andere de dikte van de

hartspier van de linker hart kamer kan worden gemeten, lieten een hogere berekende linker hartkamer massa zien bij de kinderen van twee ouders met hoge bloeddruk. Gespeculeerd werd dat bijvoorbeeld een functionele afwijking die vaker tot stimulering van de spiercellen in hart en bloedvaten lijdt bij de jongeren van twee hypertensieve ouders, bij het hart eerder dan in de vaten tot structurele veranderingen lijdt.

De bevindingen bij uitgebreid nier onderzoek, gaven een enigszins verlaagde doorbloeding van de nieren te zien bij de kinderen van twee hypertensieve ouders. Hierbij past de bevinding van een mogelijk secundair verlaagd renine, bij de kinderen van twee hypertensieve ouders. Daarnaast werd een verlaagd aldosteron aangetroffen bij de kinderen van twee hypertensieve ouders, in overeenkomst met het verlaagde renine. Het angiotensine was echter vergelijkbaar tussen de groepen. Verondersteld wordt dat in een vroege fase van de ontwikkeling van een verhoogde bloeddruk, een toegenomen samentrekking van de niervaten tot de gevonden bevindingen zou kunnen lijden. Deze veronderstelde renale vasoconstrictie zou kunnen ontstaan door een verhoogde prikkel door stoffen uit het bloed, dan wel de nier zelf, of uit de sympatische zenuwen. Ook zou een veranderde dichtheid van receptoren op het niervaatbed hierop van invloed kunnen zijn.

De resultaten van de diverse metingen van de activiteit van het sympatische zenuw stelsel, in bloed afgenomen na een half uur rust, en urine verzameld gedurende 24 uur, lieten geen duidelijke verschillen zien tussen de groepen in noradrenaline en adrenaline. Dit zijn vrij grove maten voor de activiteit van het sympatische zenuwstelsel, die idealiter in arterieel verzameld bloed bepaald kan worden. Om praktische redenen hebben wij van zo'n bepaling afgezien. Echter, in urine verzameld gedurende een 2 uur durend rustig verblijf op het onderzoekscentrum, werd wel een licht verhoogde uitscheiding van noradrenaline gevonden bij de kinderen van twee hypertensieve ouders. Eveneens was de dichtheid van adrenoreceptoren op bloedcellen verhoogd, namelijk de zgn $alpha_2$-adrenoreceptoren, zonder verschil in $beta_2$-adrenoreceptoren. Hierdoor ontstaat een verhoogde berekende verhouding van cellulaire $alpha_2$-$beta_2$-adrenoreceptoren. De functie van deze adrenoreceptoren en de verhoogde dichtheid daarvan op de bloedcellen van de kinderen van twee hypertensieve ouders, suggereren een relatie met toekomstige bloeddruk verhoging, alsmede een rol in de verlaagde doorbloeding van de nier en de verlaagde renine produktie.

Bestudering van de zout-huishouding bij de drie groepen jongeren liet geen evidente verschillen zien van zout in bloed of urine, onder een normaal dieet. Het zout gehalte

gemeten in de cellen was eveneens niet verschillend tussen de groepen. Onderzoekingen waarbij de nieruitscheiding en her-opname van zout werd geschat, toonden een verhoogde her-opname van zout in de proximale nier tubulus, en een gelijke uitscheiding distaal in de nier tubuli, ondanks het verlaagde aldosteron. Deze gegevens wijzen op een neiging tot het vasthouden van zout bij kinderen van twee ouders met hoge bloeddruk.

Voor wat betreft de calcium huishouding werd een licht verlaagd gehalte van magnesium, calcium en fosfaat en een verhoogd gehalte aan plasma bijschildklier hormoon gevonden. Er waren geen verschillen in inname van calcium via de voeding. De urine uitscheiding gedurende 24 uur, en gedurende de 3 uren in de ochtend, liet een licht verhoogde uitscheiding zien van calcium bij de kinderen van twee ouders met hoge bloeddruk. Dit zou kunnen betekenen dat de calcium huishouding bij de kinderen van de ouders met hoge bloeddruk, "op peil" wordt gehouden door een licht verhoogde activiteit van de bijschildklier. Dit is mogelijk secundair aan calcium verlies via de nier, dan wel door een verlaagde gevoeligheid van de nier voor het bijschildklier hormoon.

Als laatste worden de resultaten van de immuno-genetica gepresenteerd met gegevens over o.a. het HLA (een genetische weefsel typering). De frequentie van bepaalde HLA-typen was niet duidelijk verschillend tussen de hypertensieve en normotensieve ouders, en er werd geen duidelijk patroon van overdracht van HLA met bloeddruk in de families waargenomen.

Na een bespreking in hoofdstuk 5 van de bevindingen, volgen suggesties voor verder onderzoek. Allereerst wordt voorgesteld om op korte termijn de bloeddruk bij de kinderen opnieuw vast te leggen. Dit is belangrijk om na te gaan in welke mate een hoge bloeddruk inderdaad vaker voorkomt bij jongeren van twee hypertensieve ouders. Ook is het van belang de verandering van de bloeddruk in de tijd vast te leggen, om na te gaan wie er de grootste stijging laat zien. Tevens kan op grond daarvan worden bekeken welke mechanismen beschreven in dit proefschrift, inderdaad determinanten blijken te zijn voor de ontwikkeling van een verhoogde bloeddruk, dan wel een stijging van de bloeddruk. Aanbevelingen voor aanvullend onderzoek op bases van boven beschreven speculaties over onder andere bloeddruk, nierdoorbloeding en alpha$_2$-adrenoreceptoren, werden kort uitgewerkt.

Continuering van onderzoek in The Dutch Hypertension and Offspring Study is van groot belang omdat dit op eenvoudige wijze belangrijke informatie over de determinanten van stijging en verandering van de bloeddruk bij kinderen van ouders met hypertensie kan

verstrekken. De bevindingen in het hier beschreven deel van The Dutch Hypertension and Offspring Study vormen de beste argumenten voor een verlenging van de observatieduur en voor uitbreidingen van het onderzoek bij deze zo zorgvuldig geselecteerde en goed beschreven, enthousiaste jongeren.

8 ABOUT THE AUTHOR

Ingrid Maria Sylvia van Hooft was borne on December 31th, 1959 in 's-Herthogenbosch The Netherlands. She passed her secondary school exam, 'gymnasium-B', at the 'Sint-Jans-Lyceum' in 's-Herthogenbosch in 1978. The same year, she started her medical training in Antwerp, Belgium, at the 'State-University-Centre-Antwerp', and continued this in 1979 in Rotterdam, The Netherlands, at the 'Erasmus University Rotterdam'. She received her doctoral degree in medicine in 1984 and her medical degree in 1986.

She started her professional career at the Department of Epidemiology and Biostatistics of the Erasmus University Rotterdam in 1986 (Head: Prof. Dr. H.A. Valkenburg, succeeded by Prof. Dr. A. Hofman), and between 1986 and 1990 the practical work of the Dutch Hypertension and Offspring Study described in this thesis was done. In 1989 she received the Young Investigator Award for part of the work published in this thesis at the Second International Conference on Preventive Cardiology by the Council of Epidemiology of the American Heart Association and the Council of Epidemiology and Prevention of the International Society and Federation of Cardiology. In 1990 she started her specialist training in internal medicine at the department of Internal Medicine I at the University Hospital Dijkzigt (Head: Prof. Dr. M.A.D.H. Schalekamp), which she decided to adjourn in 1992. She started to work for the pharmaceutical industry; in 1992 as a medical adviser/clinical research associate at the cardiovascular division of the medical department of Lorex Synthélabo, Maarssen, The Netherlands. Since april 1994, she is working as project-leader at the 'Cardio/Bone' division of the medical research department of Sandoz BV., Uden, The Netherlands. Currently she is involved in FLARE, the "Fluvastatine in Angioplasty and Restenosis" trial, and various other projects with Fluvastatine in high risk patients, i.e. patients with diabetus mellitus and/or patients with lipid disturbances. Moreover, she is responsible for projects with new products for treatment of patients with non-insulin dependent diabetes mellitus or gastro-intestinal diseases.

9 DANKWOORD

Meer dan 300 pagina's verder, na 8 jaren werk aan het onderzoek en het proefschrift, inmiddels in mijn vierde baan, lijkt het onmogelijk iedereen te memoreren die voor mij en de verwezenlijking van dit proefschrift onmisbaar waren.

Prof. Dr. H.A. Valkenburg heeft bij mij de interesse gewekt voor het epidemiologisch onderzoek, tijdens het keuzepraktikum in het derde jaar van de studie. Minstens zo belangrijk, zijn zijn verdiensten het Epidemiologisch Onderzoek Zoetermeer op te zetten en zodanig uit te voeren en te automatiseren dat het EPOZ bestand als bases voor de DHOS kon dienen.

Prof. Dr. A. Hofman zag dit als een unieke kans en bereidde het project groots voor met een interuniversitaire stuurgroep en een prachtige subsidie van de Nederlandse Hartstichting, mede te danken aan een puzzel-actie van het damesblad Libelle. Het project werd ingevuld tijdens de veelvuldige werkbesprekingen, aanvankelijk met mijn beide promotores, later voornamelijk met Prof. Dr. D.E. Grobbee.

Bert, het heeft allemaal erg lang geduurd, je hebt zelfs ooit gezegd dat het er nooit van zou komen. Tevens heb je inmiddels je onderzoek op een ander aandachtsveld gericht. Daarom ben ik zeer vereerd dat je alsnog mijn promotor wilde zijn.

Rick, jij vormde de rode draad voor het onderzoek; je was uitstekend op de hoogte van allerlei hypothesen en onderzoek van groepen elders, en legde vaak de contacten met instituten binnen en buiten Nederland voor uitbreiding van het onderzoek. In het afgelopen jaar heb je met veel geduld de maar mondjesmaat binnenkomende manuscripten bestudeerd, en bleef je gematigd optimistisch, waarvoor dank!

De praktische uitvoering van het onderzoek, had nooit zo snel kunnen plaats vinden zonder de uitstekende infra-structuur van het Instituut Epidemiologie. Ik denk aan allerlei zaken die zo vanzelf sprekend waren; een beschikbaar onderzoeks centrum in Zoetermeer met grote naambekendheid onder de lokale bevolking; de inzet van ervaren, enthousiaste en flexibele epidemiologisch medewerksters (Wilma Verbruggen, Ria Rijneveldshoek, Joke Jansen, en Heleen de Bruijn); een goed geoutilleerd laboratorium in het onderzoeks centrum en op de afdeling, bestierd door Ing. A.M. "Ton" de Bruijn in samenwerking met Rene Vermeeren, waar Hilda Kornman en ik altijd terecht konden met uiteenlopende vragen over isolatie en bepalings-methodieken en materialen; een aan het project verbonden chemisch analiste, Hilda Kornman, door wie het mogelijk werd om het EPOZ-laboratorium zodanig uit te rusten dat zij al de materialen adequaat kon bewerken; de automatiseringsafdeling, toen

snel veranderend van "main-frames" naar "personal-computers" onder leiding van Leo Muller, de kenner van het EPOZ-data bestand en de ontwerper van SELFAM, het programma waarmee ouderparen konden worden geselecteerd; de Sahara met Yolanda Bekker en Lilian Verwey, waar het werk in Zoetermeer administratief werd voorbereid en vaak ook data-invoer plaats vond: het secretariaat, destijds met Ien en Celia Kuynders. Het was dus goed werken op het Instituut. Daarnaast was er een prima sfeer, met vele enthousiaste, jonge onderzoekers. Voor mij waren dat Willy-Anne van Stipthout, Jan van Saasse, Bert van Hemert, Jacqueline Witteman, Cock van Duin, Stella de Man en later Cuno Uiterwaal, Jan Schouten, Arno Hoes en Annette Bak. De uitwisseling van ervaringen, werkbesprekingen, proef-presentaties waren altijd prettig en zakelijk. De koffiekamer, kantine en de wandelgangen zorgden voor de rest. Cock, jou dank ik ook met name voor je kritische hulp bij de voorbereiding van hoofdstuk 4.5 van mijn dissertatie.

Hilda, je was veel meer dan een chemisch analist; je hebt samen met mij het hele project gedragen en je in die tijd tot een zeer veelzijdig medewerkster ontplooit. Je was verstandig en rustig, en had altijd wel een oplossing voor een of ander probleem. Ik vind het dan ook vanzelf sprekend, en waardeer het ten zeerste, dat je mijn paranimf bent.

Enny-de Waal Manning, dank voor je enorme werklust waarmee je de ambulante bloeddruk data attaqueerde, om ze geschikt te maken voor analyse. Eveneens bedankt voor jouw interesse in de DHOS en je hulp bij vele manuscripten.

Karen Hulshof, ook jou wil ik danken voor je goede en vlotte werk m.b.t. de voedingsanamnese bij de deelnemers van de DHOS. Het is een belangrijke aanvulling voor het onderzoek geweest.

Hanneke Vermeurs, je toegewijde en flexibele inzet was onontbeerlijk voor de uitvoering en analyse van de echocardiografische metingen bij de jongeren. Dank hiervoor!

Dianne, je was een uitstekende partner in het DHOS onderzoek, waarvan we samen de derde fase hebben voorbereid en uitgevoerd. Voor mij was dat het einde, voor jou het begin van een enorme dataset, waarop je inmiddels al weer gepromoveerd bent. Ik wens je het allerbeste toe in je nieuwe werkkring.

Het onderzoek zou niet mogelijk geweest zijn zonder de enthousiaste deelname van de jongeren en hun ouders uit Zoetermeer. Aan hen heb ik alles te danken.

De leden van de stuurgroep; prof. dr. P. van Brummelen, dr. L.J.P. van Doornen, prof. dr. P.W. de Leeuw, dr. H.A.P Pols, prof. dr. K.H. Rahn, prof. dr. M.A.D.H. Schalekamp, prof. dr. H.A.J. Struijker-Boudier, prof. dr. Th. Thien en prof. dr. R.R.P. de

Vries, wil ik hier allen bedanken voor hun tijd en inzet en de mogelijkheid. om bepalingen of testen via de onderzoekslaboratoria uit te laten voeren. Ook dank ik hen voor het kritisch doornemen van manuscripten.

Prof. Schalekamp, u wil ik ook bijzonder bedanken voor het vertrouwen dat u in mij stelde, voor mijn opleiding tot internist. Het was een genoegen om u niet alleen als wetenschapper mee te maken, maar ook als arts bij de morgensessies en de grote visites. Tevens dank ik u voor de ruimte die u mij gaf om te komen tot het besluit om de opleiding interne geneeskunde te stoppen. Ook op uw afdeling was het goed toeven. Ik denk hierbij aan mijn klinische collega's die mij zo vele adviezen gaven, en altijd zeiden dat ik het proefschrift in ieder geval moest afronden; Minke, je was mijn grote voorbeeld hoe het ook kon; Jeanette, bedankt voor je wijze inzicht in mijn situatie; Jaap, Rene, Willem-Jan en vele anderen; bedankt voor jullie collegialiteit. Lies; het waren voor mij mooie jaren, ik heb veel van je geleerd, wat mij nog dagelijks als referentie kader van pas komt.

Tevens wil ik hier mijn collega's van de afgelopen jaren bedanken voor de belangstelling en aanmoediging die ik mocht ondervinden; Erik, Freek, Ron, Wilma en Trijnie, het was gewoon te gezellig bij jullie. Ineke, er is weer tijd voor sport!

Peter, Frida, Henk en Nathaly bedankt voor jullie begrip als ik eens een dagje afwezig moest zijn de afgelopen maanden. Ook jullie belangstelling voor die moeilijke laatste loodjes was onontbeerlijk.

Anne Mieke, mijn paranimf, maar bovenal vriendin, dank voor al die momenten dat je er voor me was om allerlei gedachten van mij aan te horen, of het nu het onderzoek of mijn carriere-keuzes betrof. Dank ook voor je gastvrijheid bij de logeerpartijen in Rotterdam. Ruim acht jaren geleden gaven we samen ons afstudeerfeest; inmiddels heb jij je droom van toen bereikt. Nu ben je weer van de partij, op deze voor mij zo belangrijke dag! Dank!

Als laatste dank ik vooral mijn familie van wie ik altijd alle vertrouwen kreeg. Mama, dank dat je me hebt laten studeren en je me altijd vrij in mijn keuzes hebt gelaten. Ik denk met veel plezier terug aan de retraites in Rosmalen.

Lieve Tom, ik kan alleen maar zeggen dat ik hoop dat je geduld eindelijk wordt beloond. Tevens spreek ik de hoop uit dat wij weer snel vele vrienden kunnen zien en hen voor een heerlijke avond zullen kunnen uitnodigen. Dit is te lang niet gebeurd!