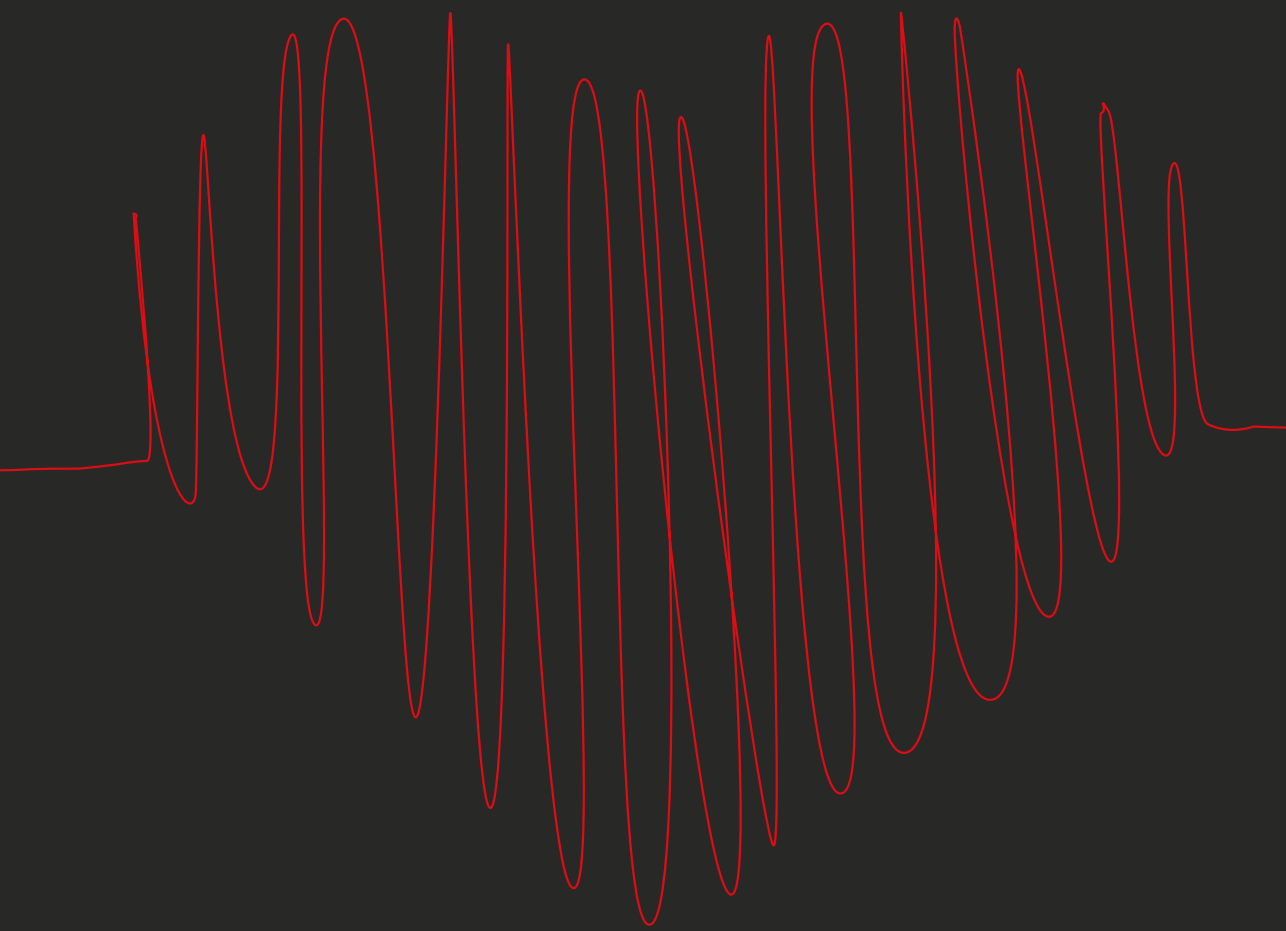
Dietary protein, blood pressure and mortality

The value of repeated measurements

Susanne MAJ Tielemans



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CONTENTS

CHAPTER 1

General introduction

Cardiovascular diseases (CVD) are the main cause of death worldwide. In 2012, about 17.5 million people died from CVD, accounting for 30% of all deaths[1]. High blood pressure (BP) is a major cardiovascular risk factor, which was responsible for 10.4 million deaths in 2013[2]. Around 22% of adults worldwide suffer from hypertension, defined as average systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive medication[1].

Diet and lifestyle are important factors in the etiology of hypertension. Body weight control, physical activity, and a low intake of alcohol and salt are well-established preventive measures[3]. Plant-based diets have been shown to lower BP[4], which may be due to dietary fiber[5,6], polyphenols[7] and minerals such as potassium[8,9] and magnesium[10]. Whether plant protein, or dietary protein in general, is important for maintaining a healthy BP is unclear.

BP is a well-known risk factor of CVD. In individuals aged 40-69 years, each 20-mmHg increment in usual systolic BP has been associated with a 2-times greater risk of coronary heart disease mortality and a more than 2-times greater risk of stroke mortality[11]. When repeated BP measures are available over a longer period of time, e.g. in prospective studies, different analytical approaches can be used for studying the course of BP over time in relation to CVD mortality. Little research has been done on whether these different approaches are relevant to CVD risk prediction.

This thesis is centered on BP as a major cardiovascular risk factor. In the first part, the relation of dietary protein intake with BP was examined. In the second part, repeated BP measurements were analyzed in relation to CVD and all-cause mortality.

DIETARY PROTEIN AND BLOOD PRESSURE

In the Netherlands, habitual mean protein intake is 86 grams/day, accounting for 15% of total energy intake[12]. Main sources of dietary protein are meat, dairy and cereals (Table 1). About two-thirds of dietary protein originates from animal sources.

Dietary assessment methods to estimate protein intake include the food frequency questionnaire, dietary history or 24-hour dietary recall. These

TABLE 1. Top-3 contributors (% energy) of food groups to protein intake in the Netherlands, based on dietary intake of Dutch individuals aged 7 to 69 years between 2007 and 2010[12]

Total protein	Plant protein	Animal protein
Meat and meat products (29%)	Cereals and cereal products (57%)	Meat and meat products (47%)
Dairy products (23%)	Potatoes and other tubers (7%)	Dairy products (38%)
Cereals and cereal products (22%)	Vegetables (7%)	Fish and shellfish (6%)

instruments rely on self-report, which could lead to misclassification of participants according to their dietary intake. A more objective method to estimate protein intake is a nutritional biomarker. A marker of dietary protein is 24-hour urinary total nitrogen excretion, comprising ~80% of dietary nitrogen in individuals with a stable nitrogen balance[13]. A related marker of protein intake is 24-hour urinary urea excretion, which makes up ~80% of urinary total nitrogen[13,14].

In a systematic review of cross-sectional studies, a weak inverse association between dietary protein, especially plant protein, and BP was observed[15]. In the INTERSALT Study among ~10 000 adults from 32 countries, higher 24-hour urinary excretion of urea and nitrogen was significantly associated with lower BP[14]. The INTERMAP Study reported an inverse association between plant protein intake and BP, whereas no association was observed for animal protein[16].

The association of dietary protein with incident hypertension and long-term BP changes was investigated in several prospective cohort studies. Most of these studies observed no associations of total and animal protein intake with hypertension[17-19] or BP change[20,21]. For plant protein intake, findings were inconsistent. In 1714 male participants of the Chicago Western Electric Study, plant protein intake was inversely associated with changes in BP during 8 years of follow-up[22]. Moreover, plant protein intake was associated with a 50% lower 2-year hypertension risk in 5880 Spanish adults[17]. However, these inverse associations were not confirmed by two Dutch prospective cohort studies[18,19].

The effect of protein intake on BP response has been investigated in a number of randomized controlled trials (RCTs)[15]. In the OmniHeart Study, a cross-over trial in 164 American adults, the effect of three diets that differed in macronutrient composition on BP was investigated[23]. After six weeks, the protein-rich diet reduced

BP compared with a carbohydrate-rich diet, but not compared with a diet rich in monounsaturated fat. In another cross-over trial in 352 American adults, the BP effect of supplementation with soy protein, milk protein and carbohydrates was investigated[24]. After 8 weeks, supplementation of protein reduced BP compared with carbohydrates[24]. No BP difference was observed between soy protein and milk protein.

In conclusion, studies investigating the association between dietary protein and BP yielded inconsistent results. Longitudinal studies using nutritional biomarkers of dietary protein are lacking. Further research is needed to conclude whether dietary protein, or more specifically plant and animal protein, could play

TABLE 2. Overview of studies on dietary protein and BP, presented in the first part (chapters 2 – 4) of this thesis

Study type	Study	Dietary exposure	BP outcome	Thesis chapter
Cross-sectional	Meta-analysis	Total protein, plant and animal protein	BP level	4
Prospective	PREVEND Study	Total protein, estimated by 24-hour urinary urea excretion	Incident hypertension*	2
Prospective	Zutphen Elderly Study	Total protein, plant and animal protein	5-year change in BP	3
Prospective	Meta-analysis	Total protein, plant and animal protein	Incident hypertension*	4
RCTs	Meta-analysis	Total protein, plant and animal protein	BP response	4

Abbreviations: BP, blood pressure; PREVEND, Prevention of Renal and Vascular Endstage Disease; RCTs, randomized controlled trials.

* Defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive medication.

a role in hypertension prevention. Therefore, the association of dietary protein with incident hypertension and long-term changes in BP was investigated in two prospective cohort studies (Table 2). Furthermore, a meta-analysis summarizing evidence on dietary protein and BP from observational studies and RCTs was performed.

BLOOD PRESSURE AND CARDIOVASCULAR MORTALITY

Blood pressure is an established risk factor for cardiovascular morbidity and mortality. An individual's BP can be expressed in different ways, such as single BP (measured at one moment in time, also known as casual BP), single BP adjusted for regression dilution, average BP, and trajectories of BP. It is at present unclear which of these measures can best be used for CVD risk prediction. In this thesis, different analytical approaches of BP as a predictor of CVD and all-cause mortality were compared, as summarized in Table 3.

As BP exhibits large intra-individual variation, for example resulting from biological fluctuations, a single BP measurement does not adequately represent the true BP[25]. Relatively high or low observations are likely to be followed by observations that are closer to the mean, called "regression to the mean". This phenomenon may lead to an underestimation of the BP-CVD association, also known as regression dilution. By using replicate measurements of BP in a representative sample of individuals in a prospective cohort study, the regression dilution effect can be estimated[26], as was done in the reported "usual BP" values by the Prospective Studies Collaboration[11]. Another approach to take into account the intra-individual variation in BP is by taking repeated BP measurements within a specified time interval of e.g. monthly or yearly examinations. Using the average BP level reduces the intra-individual variation and is another example of calculating usual BP values.

Lastly, trajectories of BP (i.e. patterns that describe the course of BP over time) can be modeled. In the CARDIA Study, heterogeneity in BP trajectories among groups of individuals was observed[27]. Higher BP trajectories from young adulthood through middle age were associated with a greater risk of coronary artery calcification, a marker of subclinical CVD[27]. Trajectories of BP over time may

TABLE 3. Analytical approaches of blood pressure (BP) as a predictor of mortality in prospective cohort studies, presented in the second part (chapters 5 – 6) of the thesis

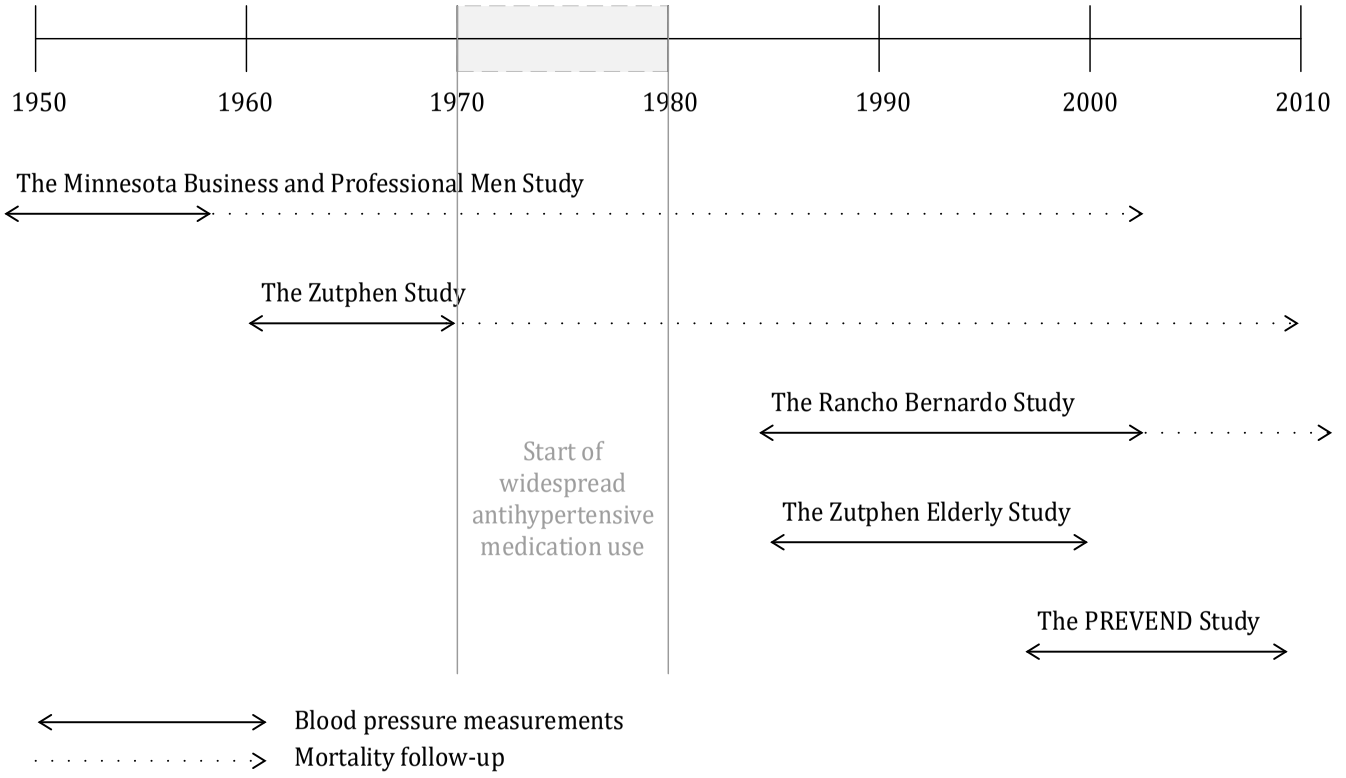
BP measure	Definition
Single BP	BP level at a single moment in time (also known as casual BP)
Usual BP	BP level at a single moment in time, adjusted for regression dilution
Average BP	Average of BP levels in a specified time interval
BP trajectories	Group-based trends of BP in a specified time interval

have greater power in predicting CVD than the above-mentioned approaches, as both BP level and change in BP over time are captured in these trajectories.

The timeline of the prospective studies that form a part of this thesis is shown in Box 1. Two cohorts, the Minnesota Business and Professional Men Study and the Zutphen Study, were conducted before the use of antihypertensive medication was widely distributed[28]. During 10 years of follow-up yearly BP measurements were taken in Minnesota from 1947-1957 and in Zutphen from 1960-1970. Although some drugs for lowering BP were available in the 1960s, for example thiazide diuretics, their effect on morbidity and mortality in hypertensive individuals had not yet been proved. Therapeutic nihilism persisted until the 1970s, when the first RCTs on the efficacy of antihypertensive treatment were published[28]. Therefore, the natural course of BP with ageing could be studied in the cohorts of Zutphen and Minnesota men, as only exceptionally high levels of BP were treated. Moreover, both are "extinction" cohorts, as (nearly) all participants had died at the end of follow-up. These cohorts provide a unique opportunity to study not only the predictive value of BP trajectories in relation to risk of CVD and all-cause mortality, but also in relation to life years lost.

In the other cohort, the Rancho Bernardo Study, data collection started in 1984. This prospective cohort study consisted of male and female residents aged ≥50 years from the upper-middle class of Rancho Bernardo, California, USA. About half of the cohort used antihypertensive medication between 1984 and 2002, which consisted mainly of diuretics, beta blockers and calcium channel blockers. In this study, average BP and BP trajectories were examined in relation to CVD and all-cause mortality, taking into account antihypertensive medication use.

BOX 1. Timeline of the prospective studies described in this thesis



THESIS OUTLINE

The first research objective was to investigate the association between dietary protein intake and BP. In the PREVEND Study, the association of 24-h urinary urea excretion as a biomarker of total protein intake with 9-year incidence of hypertension was investigated (**Chapter 2)**. In the Zutphen Elderly Study, the intake of repeatedly measured plant and animal protein was investigated in relation to 5-year changes in BP (**Chapter 3**). Furthermore, a meta-analysis to summarize the evidence from observational studies and RCTs of dietary protein and BP was conducted (**Chapter 4**).

The second research objective was to investigate the association of repeatedly measured BP with CVD and all-cause mortality. In the Minnesota Business and Professional Men Study and the Zutphen Study, BP trajectories were characterized and examined in relation to CVD mortality, all-cause mortality, and life years lost (**Chapter 5**). Moreover, mortality risk predictions based on single BP, average BP, usual BP, and BP trajectories were compared. In the Rancho Bernardo Study, average BP and BP trajectories were studied in relation to CVD and all-cause mortality, accounting for antihypertensive medication (**Chapter 6**). The last chapter discusses the main findings and their implications (**Chapter 7**).

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CHAPTER 2

Twenty-four hour urinary urea excretion and 9-year risk of hypertension: the PREVEND Study

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ABSTRACT

Objectives: It is not yet clear whether dietary protein could help maintaining a healthy blood pressure (BP). We investigated the association between total protein intake, estimated from 24-h urinary urea excretion, and incident hypertension in Dutch men and women.

Methods: We analyzed data of 3997 men and women (aged 28–75 years) who participated in the Prevention of Renal and Vascular End-stage Disease (PREVEND) Study, a prospective cohort study. Urea excretion was assessed in two consecutive 24-h urine collections at baseline and approximately 4 years later, from which total protein intake was estimated using the Maroni method. Participants were followed for 9 years for hypertension incidence, defined as BP at least 140/90 mmHg or initiation of antihypertensive medication. Hazard ratios (HR) were obtained in sex-specific quintiles of protein intake using time-dependent Cox regression, adjusted for age, sex, BMI, smoking, alcohol use, and 24-h urinary excretions of sodium and potassium.

Results: Baseline BP was on average 119/70 mmHg and 976 participants developed hypertension during follow-up. Mean protein intake (in g/kg ideal body weight) was 1.18±0.26 for men and 1.12±0.25 for women. Estimated protein intake was nonlinearly inversely associated with incident hypertension in the fully adjusted model, with non-significant HR of 0.77, 0.75, 0.82, and 0.83 in consecutive quintiles compared with the lowest quintile (P-trend: 0.52).

Conclusion: Protein intake, as assessed by urinary urea excretion, was not significantly associated with 9-year hypertension incidence in Dutch men and women.

INTRODUCTION

Diet and lifestyle play an important role in the aetiology of hypertension[1,2]. Established measures to lower blood pressure (BP) are weight loss, enhanced physical activity, reduced intake of alcohol and salt, and the DASH-diet that is rich in fruits and vegetables[1,2]. Whether dietary protein is important in BP regulation is not yet clear[3]. In the OmniHeart study, a randomized cross-over trial among 164 US adults with untreated (pre)hypertension, a 6-week protein-rich diet reduced BP by -1.6/-1.4 mmHg when compared with a carbohydrate-rich diet[4]. When compared with a diet rich in monounsaturated fat, however, no effect of dietary protein on BP was observed[4].

Prospective epidemiological studies in this field have yielded conflicting results[3]. Total protein intake estimated by a food frequency questionnaire was weakly positively associated with change in SBP (+0.05 mmHg/year per energy%; P=0.04) during 8 years of follow-up in 1714 US men[5]. In 4146 young normotensive US adults, habitual protein intake was not significantly associated with BP change during 7 years of follow-up[6]. In 5880 university graduates of the SUN cohort, a 20% lower 2-year risk of hypertension was observed for high protein intakes, but this was not statistically significant[7].

Assessment of protein intake by means of dietary questionnaires is based on self-report, which could lead to misclassification of participants and, consequently, attenuated associations with risk of hypertension. Twenty-four hour urinary excretion of urea, which makes up ~82% of urinary total nitrogen, provides a valid and objective biomarker of total protein intake that can be used in epidemiological studies[8,9]. In a cross-sectional analysis of the INTERSALT study among 10 020 adults from 32 countries, significant inverse associations between this biomarker and BP levels were found after adjustment for age, sex, BMI and 24-h urinary mineral excretions[8]. In the INTERMAP study in 4680 Japanese, Chinese, UK and US adults, 24-h urinary urea nitrogen (estimated from urinary urea) was also related to lower BP levels, but findings were not statistically significant[10]. Cirillo *et al.*[11] examined urea excretion in an overnight urine sample in 3705 Italians, and found an inverse association with BP only in participants with a high sodium excretion. They hypothesized that a high protein intake could counteract the sodium-dependent BP rise via stimulation of renal sodium excretion.

Studies investigating associations between protein intake and blood pressure have yielded inconsistent results. Moreover, longitudinal data on protein intake and incident hypertension using objective urinary markers of protein intake are scarce[3]. We therefore assessed the association of total protein intake estimated from 24-h urinary urea excretion with 9-year risk of hypertension in ~4000 participants of the Prevention of Renal and Vascular Endstage Disease (PREVEND) Study in the Netherlands. Additionally, we examined whether the association could be modified by urinary sodium excretion or other participant characteristics, e.g. sex and BMI.

METHODS

Study design

Analyses were based on data of participants of the PREVEND Study, a prospective Dutch cohort study. The primary aim of this study is to investigate urinary albumin excretion in relation to renal and cardiovascular outcomes in the general population. The design of the study has been described elsewhere[12,13]. Briefly, from 1997 to 1998, all inhabitants of Groningen (The Netherlands) aged 28–75 years, were sent a questionnaire and a vial to collect a first morning void urine sample. Of the 85 421 individuals invited, 40 856 responded. From this group, 30 890 individuals had a urinary albumin concentration of less than 10 mg/l and 9966 participants had a urinary albumin concentration of at least 10 mg/l in their morning urine sample. After exclusion of pregnant women and individuals with diabetes mellitus type I, all consenting subjects with a urinary albumin concentration of at least 10 mg/l (n=7768) were invited to participate. In addition, a randomly selected group with a urinary albumin concentration of less than 10 mg/l (n=3394) was invited to participate in the cohort. Of these 11 162 invitees, 8592 participants completed the first screening round (1997/1998) and were enrolled in the PREVEND cohort.

Participants were invited to the outpatient clinic of the University Medical Centre Groningen for measurements approximately every 3 years. Measurement rounds took place in 1997–1998 (baseline), 2001–2003, 2003–2006, and 2006–2009 and consisted of two visits to the outpatient clinic, with a 3-week interval. The PREVEND study was approved by the local medical ethics committee and

conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent.

Population for analysis

From the cohort of 8592 participants, we excluded 65 participants with missing baseline data on 24-h urinary urea excretion. In addition, we excluded 2896 participants with hypertension at baseline, defined as SBP at least 140 mmHg or DBP at least 90 mmHg or use of antihypertensive medication. A total of 1098 participants with missing data on BP and/or use of antihypertensive medication at baseline (n=117) or during follow-up (n=981) were also excluded. Of the remaining 4533 participants, 212 were excluded because of prevalent cardiovascular disease, use of lipid-lowering medication, renal disease (all based on self-report), and diabetes mellitus (based on self-report, fasting glucose levels and/or medication use). In addition, 324 participants were excluded because of missing data on these disorders or missing data on potential confounders. A total of 3997 participants was left for the present analysis.

Data collection

At each measurement round, participants completed a selfadministered questionnaire on demographic data, medical history, lifestyle factors and use of antihypertensive, lipid-modifying and antidiabetic drugs. Information on medication use was completed with data from community pharmacies. Smoking was defined as current smoking or cessation less than 1 year. Alcohol use was classified in three categories, namely nondrinking or occasional drinking, less than one drink per day, or at least one drink per day. Body weight and height were measured at the outpatient clinic and BMI was calculated as weight (kg) divided by height squared (m^2). Overweight was defined as BMI ≥25 kg/m^2 and obesity as BMI ≥30 kg/m^2. BP was assessed during both visits at each measurement round by research assistants according to a strict protocol.

BP was measured in the supine position, every minute for 10 and 8 min respectively, with an automatic Dinamap XL Model 9300 series device (JohnsonJohnson Medical, Tampa, Florida, USA). The mean of the last two recordings from each visit was used. Incident hypertension was defined as SBP at least 140 mmHg or DBP at least 90 mmHg or the initiation of antihypertensive

medication (as assessed by questionnaire or pharmacy data).

Participants collected two 24-h urine collections during two consecutive days at baseline and during the first followup round (2001–2003) in the week prior to measurements. Participants were instructed orally and in writing, and were asked to refrain from heavy exercise and to postpone urine collection in case of fever, urinary tract infection, or menstruation. Urines were stored at 4°C for a maximum of 4 days before the visit. Fasting blood samples were obtained and stored at -80°C.

Laboratory determinations

Urea, sodium, and potassium were determined in urine with a MEGA clinical chemistry analyzer (Merck, Darmstadt, Germany). Urea was determined by a photometric test with the urease-GIDH method, sodium and potassium by indirect potentiometry. Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany). Excretions of urea, sodium, potassium, and albumin are given as the mean of the two 24-h urinary excretions. Serum total cholesterol, serum triglycerides, and plasma glucose were determined fromfasting blood samples by using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York).

Protein intake

Total protein intake was estimated from 24-h urinary urea excretion by the method of Maroni *et al*[14]. This method takes nonurinary nitrogen excretion (estimated 31 mg nitrogen/kg per day) and proteinuria (g per day) into account, after which the conversion factors 0.4667 (to convert from urinary urea in mmol/24-h to urea nitrogen in mmol/24-h), 0.06 (to convert from mmol/24-h to g/24-h), and 6.25 (to convert from nitrogen in g/24-h to protein in g/24-h) were used. This method was previously used in the PREVEND study[15]. Dietary total protein intake was expressed in g/kg ideal body weight (IBW) per day. For every participant, the IBW was derived by using a BMI value of 22 kg/m^2 as a reference.

Statistical analysis

Baseline characteristics were analyzed (mean±SD) in sex-specific quintiles of total protein intake at baseline. Quintile cut-off points for baseline protein intake were 0.96, 1.10, 1.22, and 1.38 g/kg IBW per day for men and 0.90, 1.04, 1.16, and 1.31 g/

kg IBW per day for women.

Because participants with microalbuminuria were overrepresented in the PREVEND study, we used a complex samples design with weighing factors to correct for oversampling. Time-dependent Cox proportional hazards analysis was used to examine the association between sex-specific quintiles of estimated total protein intake (in g/kg IBW per day), estimated from 24-h urinary urea excretion, and 9-year incidence of hypertension. Incident hypertension during early follow-up (1997–2003) was examined in relation to baseline protein intake, whereas for incident hypertension during later follow-up (i.e. after 2003) the average protein intake of the first two rounds was used. Data are presented as hazard ratios (HR) with 95% confidence intervals (95% CI).

Person-time of follow-up (in days) was computed for each participant in the cohort from baseline until the date of the last measurement round that participants attended or the incidence of hypertension, whichever occurred first. The exact time point at which incident hypertension occurred was unknown and we therefore allocated a survival time of 2 years when hypertension was diagnosed during the second measurement round (at 4 years), 5 years when diagnosed during the third round (at 6 years), and 7.5 years when diagnosed during the fourth round (at 9 years).

Adjustments were made for age and sex (model 1), for age, sex, BMI, smoking, and alcohol use (model 2) and additionally for 24-h urinary excretions of sodium and potassium (model 3). Age, BMI, smoking and alcohol use were updated at measurement round two with the most recent value. Similarly as for protein intake, values of the first and second measurement rounds were averaged for 24-h urinary excretions of sodium and potassium. Linear trends across the quintiles were assessed by modelling median intake within quintiles as a continuous variable. In a posthoc analysis, we combined the second through fifth quintiles and obtained the HR for incident hypertension compared with the lowest quintile, using the fully adjusted model. In addition to the above-mentioned analysis, supplementary analyses were performed with protein intake expressed in g per day, rather than g/kg IBW per day.

Potential interaction of protein intake with participant characteristics was examined by repeating the analysis in strata of sex, baseline BMI (normal weight vs. overweight/obese which was defined as BMI ≥25 kg/m2), baseline urinary

albumin excretion (below/above the median of 8 mg/24-h), and baseline 24-h urinary sodium excretion (below/above the median of 136 mmol/24-h). The median sodium excretion corresponded to a daily intake of salt (sodium chloride) of ~8g.

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS), version 19.0 (SPSS Inc., Chicago, Illinois, USA). Two-sided P-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics in sex-specific quintiles of total protein intake are shown in Table 1. Participants were on average 45±10 years old, and 1821 (46%) were men. BP levels were on average 123/72 mmHg for men and 115/68 mmHg for women. Mean estimated total protein intake (g/kg IBW per day) at baseline was 1.14±0.26 for the total study sample, 1.18±0.26 for men and 1.12±0.25 for women. Mean urinary urea excretions (mmol/24-h) were 397±103 in men and 322±86 in women and mean estimated urinary total nitrogen (g/24-h) was 13.6±3.5 for men and 11.0±2.9 for women. Participants with higher protein intake were slightly older, had more overweight, and were less likely to be a smoker (Table 1). At baseline, BP levels, serum cholesterol, plasma glucose and urinary excretions of sodium, potassium, and albumin were positively associated with total protein intake (all P-trend <0.01).

During follow-up, 976 cases of hypertension occurred, and the incidence of hypertension was 32.6 per 1000 person-years. Table 2 shows HR (95% CI) for incident hypertension in sex-specific quintiles of baseline dietary protein, which pointed in the direction of a nonlinear association (all P-trend >0.10). Compared with the lowest quintile, the risk of hypertension was reduced by more than 20% in all remaining quintiles after adjustment for age, sex, BMI, smoking, and alcohol use (Table 2). Further adjustment for 24-h urinary excretions of sodium and potassium attenuated the associations. When combining quintiles 2 through 5 in a posthoc analysis using the fully adjusted model, the HR for incident hypertension was 0.78 (95% CI: 0.60–1.02, P=0.067) compared with the lowest quintile (data not in table). The HR of the analyses with protein intake expressed in g per day did not

TABLE 1. Baseline characteristics for 3997 participants of the PREVEND Study according to quintiles of baseline protein intake (in g/kg IBW per day) without hypertension or usage of antihypertensive medication at baseline

	Sex-specific quintiles of total protein intake[a]					
	Quintile 1 (n=799)	Quintile 2 (n=799)	Quintile 3 (n=801)	Quintile 4 (n=799)	Quintile 5 (n=799)	P-trend
Total protein intake						
Men (g/kg IBW per day)	0.85 ± 0.10	1.03 ± 0.04	1.16 ± 0.03	1.30 ± 0.05	1.56 ± 0.16	
Women (g/kg IBW per day)	0.79 ± 0.09	0.98 ± 0.04	1.10 ± 0.03	1.23 ± 0.04	1.49 ± 0.15	
Sex (% male)	46	46	46	46	46	0.99
Age (y)	43.6 ± 10.5	44.1 ± 10.6	44.3 ± 10.7	45.1 ± 10.1	45.8 ± 9.7	<0.01
Body mass index (kg/m^2)	23.4 ± 3.1	23.9 ± 3.0	24.6 ± 3.0	25.7 ± 3.5	27.4 ± 4.3	<0.01
Overweight or obese (%)	25	32	42	55	68	<0.01
Current smoking (%)	52	42	36	32	33	<0.01
Alcohol use ≥1 drink/day (%)	26	23	25	25	26	0.71
Family history of hypertension (%)[b]	32	30	35	33	31	0.31
SBP (mmHg)	118 ± 11	117 ± 11	118 ± 11	119 ± 11	120 ± 10	<0.01
DBP (mmHg)	69 ± 7	70 ± 7	70 ± 7	70 ± 7	71 ± 7	<0.01
Plasma glucose (mmol/L)	4.5 ± 0.5	4.5 ± 0.6	4.5 ± 0.6	4.7 ± 0.6	4.7 ± 0.6	<0.01
Serum total cholesterol (mmol/L)[c]	5.4 ± 1.1	5.4 ± 1.1	5.4 ± 1.1	5.5 ± 1.1	5.6 ± 1.1	<0.01
Serum triglycerides (mmol/L)[d]	1.0 (0.8-1.4)	1.0 (0.8-1.4)	1.0 (0.7-1.4)	1.0 (0.8-1.4)	1.1 (0.8-1.6)	0.06
Urinary excretions						
Urea (mmol/24h)	238 ± 50	307 ± 45	353 ± 50	399 ± 56	485 ± 81	<0.01
Sodium (mmol/24h)	108 ± 38	128 ± 39	142 ± 42	154 ± 46	174 ± 50	<0.01
Potassium (mmol/24h)	58 ± 18	68 ± 17	74 ± 18	78 ± 19	87 ± 22	<0.01
Albumin (mg/24h)	6.8 (5.1-10.3)	7.6 (5.7-11.3)	8.2 (6.1-11.9)	8.1 (6.1-12.7)	9.5 (6.7-15.0)	<0.01

Values are expressed as mean±SD, median (25th-75th percentile) or percentage. IBW, ideal body weight. [a] Quintile cut-off points (in g/kg/d): 0.96, 1.10, 1.22, and 1.38 for men and 0.90, 1.04, 1.16, and 1.31 for women. [b] Data are available for n=3303. [c] Data are available for n=3988. [d] Data are available for n=3915.

TABLE 2. Sex-specific quintiles of protein intake in g/kg IBW per day (using time-dependent variables) in relation to 9-year incidence of hypertension in 3997 Dutch adults participating in the PREVEND Study

	Model 1[a]		Model 2[b]		Model 3[c]	
	HR	95% CI	HR	95% CI	HR	95% CI
Quintile 1	1.00	Reference	1.00	Reference	1.00	Reference
Quintile 2	0.80	0.60 – 1.07	0.75	0.56 – 1.01	0.77	0.57 – 1.04
Quintile 3	0.78	0.58 – 1.04	0.71	0.53 – 0.96	0.75	0.54 – 1.03
Quintile 4	0.91	0.69 – 1.20	0.77	0.58 – 1.03	0.82	0.60 – 1.13
Quintile 5	1.06	0.82 – 1.40	0.75	0.56 – 1.01	0.83	0.59 – 1.16
P-trend[d]		0.32		0.15		0.52

CI, confidence interval; HR, hazard ratio; IBW, ideal body weight.

[a] Adjusted for age and sex.

[b] Additionally adjusted for body mass index, smoking, and alcohol use.

[c] Additionally adjusted for 24-h urinary excretions of sodium and potassium.

[d] P-trend was estimated by modeling median protein intake of quintiles.

materially differ from the HR with protein intake expressed in g/kg IBW per day (Supplementary Tables 1 and 2).

In Table 3, associations between total protein intake and incident hypertension are presented in strata of sex, BMI, and 24-h urinary excretions of sodium and albumin. Among men, we observed a significant inverse association between protein intake and incident hypertension (HR 0.59; 95% CI: 0.41–0.86), whereas no association was observed in women (HR 1.00; 95% CI: 0.70–1.44). Associations between protein intake and hypertension did not differ between the other strata. A posthoc analysis in strata of urinary albumin using a higher cut-off point of 10 mg/24-h also showed similar results.

TABLE 3. Hazard ratios (95% confidence interval) for incident hypertension in categories (based on sex-specific quintiles: lowest quintile versus the other 4 quintiles) of protein intake (using time-dependent variables) in the PREVEND cohort, stratified by sex, BMI, urinary sodium and urinary albumin

	Category protein intake (g/kg IBW per day)			
	Quintile 1		Quintile 2 – 5	
	HR	95% CI	HR	95% CI
Sex				
Male (n=1821)	1.00	Reference	0.59	0.41 – 0.86
Female (n=2176)	1.00	Reference	1.00	0.70 – 1.44
Body mass index				
<25 kg/m^2 (n=2218)	1.00	Reference	0.87	0.62 – 1.23
≥25 kg/m^2 (n=1779)	1.00	Reference	0.86	0.56 – 1.33
Urinary sodium excretion[a,b]				
<136 mmol/24h (n=1998)	1.00	Reference	0.86	0.63 – 1.18
≥136 mmol/24h (n=1999)	1.00	Reference	0.84	0.53 – 1.34
Urinary albumin excretion[a]				
<8.0 mg/24h (n=1998)	1.00	Reference	0.83	0.64 – 1.08
≥8.0 mg/24h (n=1999)	1.00	Reference	0.85	0.66 – 1.08

Multivariable HR were obtained by time-dependent Cox proportional hazards analysis, adapted for complex samples (except for stratified analyses on urinary albumin excretion that was an oversampling factor); HR are adjusted for age, sex, body mass index, smoking, alcohol use and 24-h urinary excretions of sodium and potassium (except when used as a stratification factor).

CI, confidence interval; HR, hazard ratio; IBW, ideal body weight.

[a] Divided according to the median of the distribution at baseline.

[b] The cut-off value of 136 mmol/24h corresponds to a daily salt intake of ~8 grams.

DISCUSSION

In a population of Dutch men and women, we found no significant association between total protein intake, estimated from 24-h urinary urea excretion, and incident hypertension during ~9 years of follow-up. Our data did not support the hypothesis of an interaction between urinary urea and sodium in BP regulation, as suggested by Cirillo *et al.*[11]. Previously, a direct association between total protein intake (estimated from urinary urea) and risk of coronary heart disease was observed in the PREVEND study, whereas this association was absent for renal disease[15]. Based on the present analysis, it is unlikely that the previously observed association with coronary heart disease in the PREVEND study was mediated by BP.

The present study has several strengths. We assessed total daily protein intake on basis of 24-h urinary urea excretions, which is considered a valid biomarker of protein intake[8,9]. Results of the Dutch National Food Consumption Survey in 22 to 50 year-old Dutch adults in 1997–1998 showed a daily total protein intake of 1.2±0.4 g/kg body weight[16], which is in line with our data. Because urinary urea was determined in four 24-h urine collections, we were able to well characterize individuals for habitual protein intake. We had no urinary urea data at measurement round three, but total protein intake is relatively stable over time[17,18] and this is unlikely to have influenced the results. Incident hypertension was diagnosed on basis of initiation of antihypertensive medication and/or measured BP values according to a strict protocol, and hypertensive status was updated during three measurement rounds. The PREVEND cohort is well phenotyped, which enabled adjustment for relevant confounders such as BMI, smoking, alcohol consumption, and mineral intakes. A strength of the present study is that salt intake, a major confounder, could be adequately assessed on basis of 24-h urinary samples. After accounting for nonurinary losses (~10%) the salt intake was approximately 9 g in this cohort, which agrees well with other estimates for the general Dutch population[19].

The study also has limitations. First, participants with mildly elevated levels of urinary albumin, an indicator of kidney impairment, were overrepresented in the PREVEND study. However, similar results were obtained after stratification for urinary albumin (cut-off value of 8 mg/24-h) showing that mild kidney impairment

is unlikely to modify the association of protein intake with incident hypertension. Second, no data were available on physical activity, a major BP determinant, and we cannot exclude residual confounding by this factor. If protein intake was inversely associated with physical activity, this could explain part of the 20% difference in risk of hypertension between participants with lowest protein intake and those with higher intakes.

In line with our findings, previous prospective cohort studies did not observe a significant association between protein intake and incident hypertension[6,7,20,21]. The cross-sectional INTERSALT Study, however, showed a significant inverse association between urinary urea nitrogen (estimated from 24-h urinary urea) and BP[8]. The mean level of urinary urea nitrogen was lower in INTERSALT than in our study (10.0 vs. 12.2 g/24-h). In a posthoc analysis, we did observe a borderline reduced risk of hypertension of ~20% when compared with the lowest level of protein intake. We therefore cannot exclude the possibility that dietary protein is related to BP at (very) low intake. The mean protein intake in the lowest quintile in the PREVEND study was around 0.8 g/kg IBW, which still meets average daily requirements[22]. In our study, we observed a significant inverse association between protein intake and incident hypertension in men only. Compared with women, men had a higher overall excretion of urea and sodium in the PREVEND study. An explanation for the differential BP associations in men and women may be related to an interaction of urinary urea with sodium, as suggested by Cirillo *et al.*[11]. A sex-specific effect of dietary protein on BP, however, has not been found in previous observational studies[3]. Also, the confidence intervals of the risk estimates in our stratified analyses were wide. We therefore cannot exclude the possibility that the sex-related differences in our study are due to chance.

Trial data showed that exchanging carbohydrates for proteins could reduce BP[4,23]. This effect, however, may not be specific to protein as BP was also reduced when carbohydrates were replaced by mono-unsaturated fat in the OmniHeart Study[4]. In the present study, we could not examine which macronutrients were exchanged for protein, and how this impacted the risk of hypertension. Also, from our findings for urinary urea we cannot draw conclusions on specific types of protein, that is, animal or plant protein, which could have differential effects on BP[3].

In conclusion, findings from the present study do not support an important

role for dietary protein in long-term BP control within the range of protein intake generally consumed in the Netherlands. We cannot exclude the possibility that lower levels of protein intake are related to hypertension. Further research on different types of protein and protein replacement by specific macronutrients is warranted to conclude whether dietary protein could play a role in hypertension prevention.

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Conflicts of interest

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 1. Sex-specific quintiles of protein intake in g/day (using time-dependent variables) in relation to 9-year incidence of hypertension in 3997 Dutch adults participating in the PREVEND study

	Model 1[a]		Model 2[b]		Model 3[c]	
	HR	95% CI	HR	95% CI	HR	95% CI
Quintile 1	1.00	Reference	1.00	Reference	1.00	Reference
Quintile 2	0.87	0.66 ; 1.15	0.84	0.63 ; 1.11	0.87	0.65 ; 1.16
Quintile 3	0.76	0.57 ; 1.01	0.74	0.55 ; 0.99	0.78	0.56 ; 1.07
Quintile 4	1.05	0.80 ; 1.36	0.92	0.70 ; 1.22	0.99	0.72 ; 1.37
Quintile 5	0.97	0.73 ; 1.29	0.74	0.55 ; 0.99	0.82	0.57 ; 1.19
P-trend[d]		0.63		0.14		0.57

[a] Adjusted for age and sex;

[b] Additionally adjusted for body mass index, smoking, and alcohol use;

[c] Additionally adjusted for 24h-urinary excretions of sodium and potassium;

[d] P-trend was estimated by modeling median protein intake of quintiles.

SUPPLEMENTARY TABLE 2. Sex-specific quintiles of protein intake in g/day (using time-dependent variables) in relation to 9-year incidence of hypertension in 3997 Dutch adults participating in the PREVEND study

	Model 1[a]		Model 2[b]		Model 3[c]	
	HR	95% CI	HR	95% CI	HR	95% CI
Quintile 1	1.00	Reference	1.00	Reference	1.00	Reference
Quintile 2	0.87	0.66 ; 1.15	0.81	0.61 ; 1.07	0.84	0.63 ; 1.13
Quintile 3	0.76	0.57 ; 1.01	0.70	0.52 ; 0.94	0.75	0.54 ; 1.03
Quintile 4	1.05	0.80 ; 1.36	0.88	0.67 ; 1.16	0.96	0.70 ; 1.32
Quintile 5	0.97	0.73 ; 1.29	0.69	0.51 ; 0.94	0.79	0.54 ; 1.15
P-trend[d]		0.63		0.08		0.47

[a] Adjusted for age and sex;

[b] Additionally adjusted for body weight, smoking, and alcohol use;

[c] Additionally adjusted for 24h-urinary excretions of sodium and potassium;

[d] P-trend was estimated by modeling median protein intake of quintiles.

CHAPTER 3

Associations of plant and animal protein intake with 5-year changes in blood pressure: The Zutphen Elderly Study

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ABSTRACT

Background and aim: The aim of the present study was to investigate the association of plant and animal protein intake with 5-year changes in blood pressure (BP) level.

Methods and results: Analyses were based on 702 observations of 272 men participating in the Zutphen Elderly Study. Men did not use antihypertensive medication and were initially free of cardiovascular disease, diabetes mellitus and cancer. Physical and dietary examinations were performed in 1985, 1990, 1995, and 2000. Diet was assessed using the cross-check dietary history method. Men were categorized into tertiles according to their plant and animal protein intake. BP was measured twice at each examination. The associations of plant and animal protein intake with 5-year changes in BP level were investigated by a random intercept model with first-order autoregressive (AR [1]) serial correlation and a nugget effect. Adjustments were made for age, examination year, BMI, socioeconomic status, smoking, physical activity, prescribed diet, alcohol consumption and intake of energy and nutrients. In 1985, men were 70.1 ± 4.6 years old and had a mean BP of 147/84 mmHg. Mean protein intake was 15 en%, of which one-third consisted of plant protein. The higher-intake tertiles of plant protein intake were associated with a mean 5-year change of -2.9 mmHg (95% CI: -5.6, -0.2) systolic and -1.7 mmHg (95% CI: -3.2, -0.2) diastolic, compared with the lowest-intake tertile. No associations were observed for animal protein intake.

Conclusion: Intake of plant protein, but not animal protein, was inversely associated with 5-year changes in BP level in elderly men.

INTRODUCTION

Elevated blood pressure (BP) increases the risk of coronary heart disease, stroke, congestive heart failure and end-stage renal disease[1]. The World Health Organization estimated that about 62% of stroke and 49% of coronary heart disease are attributable to suboptimal BP (systolic >115 mmHg)[2]. Preventive measures such as maintenance of desirable body weight, physical activity, no or moderate alcohol consumption, and a low salt intake will have a substantial impact on BP, and cardiovascular morbidity and mortality[3]. Apart from these well-known preventive measures, findings from the INTERMAP Study indicate an inverse association between plant protein intake and BP, but not for animal protein[4]. This is also suggested by other cross-sectional studies[5].

The association between protein intake and BP was investigated in a number of prospective cohort studies[6-10]. In the SUN cohort, plant protein intake was associated with a lower 2-year hypertension risk in 5880 Hispanic university graduates[6]. Total and animal protein intake, however, were not associated with incident hypertension. In two Dutch cohorts, no association between protein intake (plant, animal, and total) and incident hypertension was observed[7,8]. The association between protein intake and changes in BP level, rather than incident hypertension, was investigated in two US cohorts[9,10]. In the CARDIA study, an inverse association between total protein intake and changes in BP level during 7 years of follow-up was suggested, however, this was not statistically significant[9]. No distinction was made between plant and animal protein in this study. In the Chicago Western Electric Study, an inverse association between intake of plant protein, but not animal protein, and changes in BP during 8 years of follow-up was observed[10]. However, associations were not adjusted for intake of sodium, potassium, magnesium, and dietary fiber.

The aim of the present study was to investigate the association between protein intake, especially plant protein and animal protein, and changes in BP level in a cohort of elderly Dutch men who were repeatedly examined during a total followup period of 15 years.

METHODS

Design and study population

The Zutphen Elderly Study is the continuation of the Zutphen Study, the Dutch contribution to the Seven Countries Study, which is a longitudinal investigation on chronic disease risk factors initiated in 1960 among middle-aged men[11]. Examinations were carried out between March and June 1985, 1990, 1995 and 2000. In 1985, 555 men in the original cohort (the Zutphen Study) were still alive. In addition, a random sample of 711 other men of the same age and also living in Zutphen was selected. In total, 1266 men aged 65-84 years were invited, of whom 939 (74.2%) participated in the Zutphen Elderly Study.

From the cohort of 939 men, we excluded 114 men who did not participate in dietary and physical examinations. Men with a history of cardiovascular disease (CVD), diabetes mellitus or cancer at baseline or taking antihypertensive medication in 1985 were excluded (n=323). To investigate 5-year BP changes in relation to protein intake, we excluded 230 men with less than two BP measurements. In total, 272 men were left for analysis.

In 1985 and 1990, the study was approved by the Medical Ethics Committee of the University of Leiden, The Netherlands; and in 1995 and 2000, by the Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research (TNO). Informed consent was obtained from all participants.

Dietary assessment

Habitual food and (alcoholic) beverage intake of participants in the month before the interview was recorded during a home visit by trained dietitians who used a cross-check dietary history method[12], adapted to the Dutch setting[13]. The cross-check dietary history method is a reproducible and valid method in an epidemiologic setting[13,14]. Each participant was interviewed, together with the person who usually prepared the food, about his habitual food consumption pattern on weekdays and at weekends. Habitual consumption of foods and (alcoholic) beverages during a week was assessed (first check) and verified with the quantities of foods bought per week (second check). Food and (alcoholic) beverage intake data were encoded by the dietitians according to the Netherlands Uniform food Encoding System.

Daily intake of energy, nutrients and alcohol was calculated using Dutch food composition databases close to the year of measurement[15]. Plant protein intake was defined as dietary protein from grains, potatoes, fruits, vegetables, nuts, legumes, soy, and plant protein from mixed dishes. Animal protein was defined as dietary protein from meat, dairy, fish, eggs, and animal protein from mixed dishes.

Blood pressure measurements

Training of the BP measurements was done according to the INTERSALT protocol[16]. In 1985 and 1990, physicians measured BP twice at the right arm with the men in supine position, using a random zero sphygmomanometer. In 1995 and 2000, trained technicians measured BP twice on the left arm with the men in sitting position, using an ordinary mercury sphygmomanometer. The mean of the recordings was used.

Covariates

Men were physically examined at each examination year. Height and body weight were measured according to standardized procedures. BMI was calculated as weight (kg) divided by height squared (m^2). Data on socioeconomic status, physical activity, smoking status, and use of antihypertensive medication were collected by questionnaire. Physical activity was expressed in minutes per week participating in high intensity activities (≥4 metabolic equivalent intensity level). Standardized questionnaires were used for history of cancer, diabetes mellitus and CVD. The physicians' conclusions on the prevalence of these chronic diseases were verified by information obtained by general practitioners and hospital registries.

Statistical analysis

Both protein intake (plant, animal, and total) and BP level were assessed every 5 years between 1985 and 2000 (Supplementary Figure 1). For each individual, changes in BP over time were computed by fitting a regression line through the available measurement points of BP level. Measured protein intake at the start of each 5-year period (i.e., 1985-1990, 1990-1995 and 1995-2000) was categorized into tertiles and linked to the slope of the regression line (Figure 1). For data representation, we expressed changes in BP per 5-year interval. To avoid biased

associations, we did not include BP levels in our analyses after a diagnosis of CVD, diabetes or cancer or initiation of antihypertensive medication. In total, 702 observations from 272 men were available for analysis.

To investigate the association between tertiles of protein intake and 5-year changes in BP level, a random intercept model (using SAS PROC MIXED) with first-order autoregressive (AR [1]) serial correlation, a nugget effect (to account for measurement error), and a dietary protein*time interaction term was used (SAS syntax in supplementary file). The interaction term between protein intake and time evaluated whether protein intake was a predictor of the longitudinal changes in the dependent variable (5-year changes in BP level). Estimates in the highest-intake tertile were subtracted from those in the lowest-intake tertile to examine whether the 5-year changes in BP level differed for high vs. low protein intake. The basic model included age, energy intake, and examination year (all continuous). The extended model additionally included BMI (<25 vs. ≥25 kg/m^2), socioeconomic status (low, medium-low, medium-high, and high), smoking (smoker vs. non-smoker), physical activity (low, medium, high, and missing), alcohol consumption (0 g/d, ≤20 g/d and >20 g/d), prescribed diet (yes vs. no), and intake (all continuous) of carbohydrates, SFA, total PUFA, trans-fatty acids, dietary fiber, sodium (from foods only), potassium, magnesium, calcium and the other type of protein than the one under study (animal protein in plant protein analysis and plant protein in animal protein analysis). All analyses were performed using SAS version 9.2 (SAS Institute, Inc.).

RESULTS

In 1985, mean (±SD) age was 70.1 ± 4.6 years and men had an average BP of 147/84 mmHg (Table 1). Of these 272 men, 27.9% were blue collar workers, 41.2% were white collar workers, 19.1% were small business owners and 11.8% were professionals, managers and teachers. At baseline, mean (±SD) protein intake was 15.2 ± 2.6 en%, of which 67% originated from animal sources.

From all 272 elderly men at baseline, 50 men (18.4%) completed all four examination years. When comparing all examination years (1985-2000), the proportion of obese men decreased (8.8% in 1985 to 4.0% in 2000) (Table 1). Mean

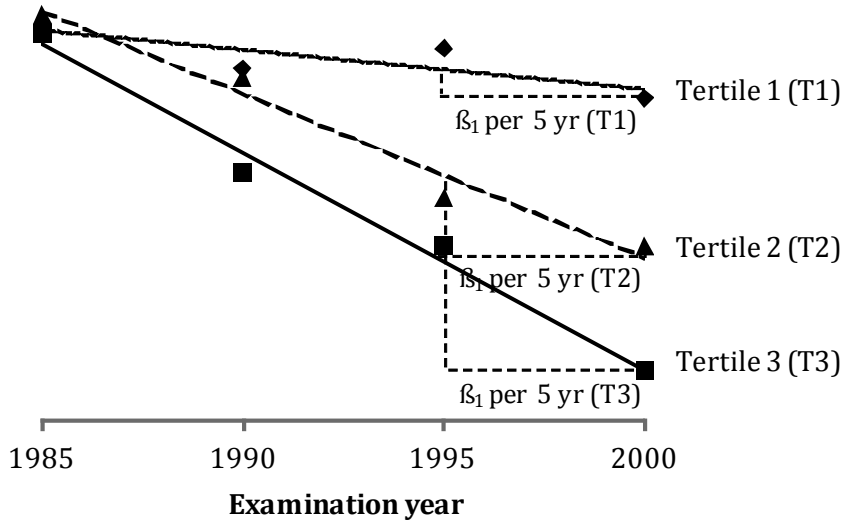

FIGURE 1. Graphical overview of the fitted model (fictive data).

BMI remained stable across the years. Fewer men were smokers (1985: 40.1% to 2000: 10.0%) and the proportion of men with a prescribed diet decreased from 10.3% to 6.0% in 15 years. The contribution of the macronutrients to energy intake remained stable. The intake of trans-fatty acids and dietary fiber decreased and the intake of the different minerals showed a slight decrease (Table 2).

Table 3 shows the 5-year changes in BP level per tertile of plant, animal and total protein intake. Also, the differences in 5-year changes in BP level between the highest and lowest-intake tertiles are presented. Baseline BP levels were similar among tertiles of plant, animal and total protein intake (Supplementary Table 1). We observed no significant differences in changes in BP level between highest- and lowest-intake tertiles of animal protein and total protein intake. For plant protein intake, the mean 5-year change in BP level was -2.64 mmHg (95% CI: -5.76, 0.47) systolic and -1.75 mmHg (95% CI: -3.49, -0.02) diastolic in tertile 3, compared to tertile 1. Moreover, the two higher-intake tertiles, tertile 2 and 3, were significantly inversely associated with 5-year changes in BP level (mean 5-year BP change of -2.94 mmHg [95% CI: -5.63, -0.25] systolic and -1.70 mmHg [95% CI: -3.19, -0.20] diastolic), compared with tertile 1.

TABLE 1. Characteristics of participants of the Zutphen Elderly Study by examination year

	Examination year			
	1985 (n = 272)	1990 (n = 257)	1995 (n = 123)	2000 (n = 50)
Age (years)[a]	70.1 ± 4.6	75.1 ± 4.6	79.3 ± 4.5	82.8 ± 3.2
Systolic BP (mmHg)	147.1 ± 19.3	147.4 ± 20.6	145.3 ± 18.0	139.7 ± 21.0
Diastolic BP (mmHg)	84.1 ± 9.9	80.8 ± 11.0	78.9 ± 9.6	72.6 ± 10.1
BMI (kg/m^2)	25.6 ± 2.9	25.5 ± 3.1	25.3 ± 3.3	25.6 ± 2.7
Overweight	47.8	45.9	49.6	52.0
Obese	8.8	8.2	6.5	4.0
Smoking	40.1	33.1	25.2	10.0
Physical activity[b]				
Low (0 min/wk)	38.2	38.4	29.8	24.5
Medium (1-149 min/wk)	25.1	24.9	28.9	30.6
High (≥150 min/wk)	36.7	36.7	41.3	44.9
Alcohol consumption				
Non-drinking	21.3	24.1	17.1	8.0
≤20 g/d	55.5	57.6	63.4	66.0
>20 g/d	23.2	18.3	19.5	26.0
Prescribed diet	10.3	9.0	7.3	6.0

Values are mean ± SD or percentage.

[a] Defined as the subject's age on December 31st of the year preceding the examination year.

[b] Data are available for 259 men in 1985.

TABLE 2. Energy and nutrient intake (mean ± SD) of participants free of disease of the Zutphen Elderly Study by examination year

		Examination year			
		1985 (n = 272)	1990 (n = 257)	1995 (n = 123)	2000 (n = 50)
Energy intake	MJ/d	9.3 ± 2.1	8.7 ± 2.0	8.8 ± 2.03	8.6 ± 2.0
	kcal/d	2224 ± 496	2083 ± 477	2089 ± 484	2060 ± 474
Total protein	en%	15.2 ± 2.6	15.4 ± 2.6	15.9 ± 3.0	15.6 ± 2.7
	g/d	83.4 ± 18.1	79.3 ± 18.3	81.8 ± 19.4	79.1 ± 18.6
Plant protein	en%	5.0 ± 1.0	5.1 ± 1.0	5.1 ± 0.9	5.2 ± 0.9
	g/d	27.8 ± 8.5	26.1 ± 7.0	26.1 ± 6.6	26.2 ± 6.1
Animal protein	en%	10.2 ± 2.7	10.4 ± 2.5	10.8 ± 3.1	10.3 ± 2.8
	g/d	55.6 ± 15.0	53.2 ± 15.6	55.8 ± 16.9	52.4 ± 15.9
Carbohydrate	en%	44.0 ± 6.4	46.2 ± 6.0	45.7 ± 5.7	46.8 ± 6.7
Total fat	en%	38.5 ± 5.9	38.4 ± 6.1	40.6 ± 5.8	37.6 ± 5.8
Saturated fat	en%	17.3 ± 3.5	16.4 ± 3.6	18.1 ± 3.7	16.1 ± 3.5
Total PUFA	en%	6.6 ± 2.5	7.2 ± 3.0	7.7 ± 3.2	7.2 ± 3.0
cis-MUFA	en%	10.7 ± 2.4	11.8 ± 2.2	12.7 ± 2.5	13.0 ± 2.6
trans-fatty acids	en%	4.1 ± 1.9	2.9 ± 1.4	1.8 ± 0.6	1.2 ± 0.5
Dietary fiber	g/d	25.4 ± 7.0	23.9 ± 7.0	23.0 ± 6.2	21.2 ± 5.9
Sodium[a]	mg/d	2501 ± 617	2342 ± 594	2402 ± 629	2297 ± 630
Potassium	mg/d	3551 ± 802	3426 ± 807	3437 ± 814	3289 ± 852
Calcium	mg/d	1052 ± 371	1022 ± 412	1052 ± 395	958 ± 331
Magnesium	mg/d	304 ± 75	298 ± 75	291 ± 75	291 ± 75

[a] Sodium intake from foods only.

TABLE 3. Associations of plant and animal protein intake with 5-year changes in BP level in 702 observations of 272 men without chronic diseases

	Baseline median intake (en%)[a]	Change in systolic BP (mmHg per 5 years)		Change in diastolic BP (mmHg per 5 years)	
		Basic model[b]	Extended model[c]	Basic model[b]	Extended model[c]
		β_1 (95% CI)	β_1 (95% CI)	β_1 (95% CI)	β_1 (95% CI)
Plant protein intake					
Tertile 1	4.1	-0.36 (-3.49, 2.76)	-1.32 (-4.70, 2.06)	-0.67 (-2.34, 1.00)	-1.63 (-3.44, -0.17)
Tertile 2	4.9	-3.64 (-6.66, -0.61)	-4.58 (-7.83, -1.33)	-2.37 (-3.99, -0.76)	-3.25 (-4.98, -1.53)
Tertile 3	5.9	-3.04 (-6.10, 0.02)	-3.96 (-7.23, -0.69)	-2.37 (-4.01, -0.74)	-3.39 (-5.13, -1.65)
Tertile 3 vs. tertile 1		-2.68 (-5.77, 0.42)	-2.64 (-5.76, 0.47)	-1.70 (-3.46, 0.05)	-1.75 (-3.49, -0.02)
Animal protein intake					
Tertile 1	7.9	-3.52 (-6.59, -0.44)	-4.40 (-7.71, -1.09)	-2.18 (-3.82, -0.53)	-3.03 (-4.80, -1.27)
Tertile 2	9.7	-2.22 (-5.31, 0.87)	-3.14 (-6.49, 0.21)	-1.58 (-3.23, 0.08)	-2.40 (-4.20, -0.60)
Tertile 3	12.7	-1.79 (-4.83, 1.25)	-2.70 (-5.91, 0.52)	-1.79 (-3.41, -0.17)	-2.61 (-4.31, -0.90)
Tertile 3 vs. tertile 1		1.73 (-1.40, 4.86)	1.70 (-1.46, 4.86)	0.39 (-1.38, 2.16)	0.43 (-1.32, 2.18)
Total protein intake					
Tertile 1	12.8	-2.85 (-5.95, 0.26)	-3.60 (-7.00, -0.21)	-1.82 (-3.49, -0.16)	-2.65 (-4.67, -0.84)
Tertile 2	14.8	-1.90 (-4.96, 1.16)	-2.84 (-6.15, 0.46)	-1.37 (-3.01, 0.27)	-2.23 (-4.00, -0.47)
Tertile 3	17.7	-3.00 (-6.06, 0.04)	-3.56 (-6.76, -0.36)	-2.38 (-4.01, -0.76)	-3.11 (-4.80, -1.41)
Tertile 3 vs. tertile 1		-0.16 (-3.27, 2.95)	0.04 (-3.10, 3.18)	0.56 (-2.32, 1.20)	-0.46 (-2.20, 1.29)

[a] Median intake values at the other examination years are similar to those at baseline.

[b] Adjusted for age, energy intake and examination year.

[c] Additionally adjusted for BMI, socioeconomic status, smoking, physical activity, prescribed diet, alcohol consumption, and intake of carbohydrates, SFA, total PUFA, trans-fatty acids, dietary fiber, sodium (only from foods), potassium, magnesium, calcium and the other type of protein under study.

DISCUSSION

In a cohort of elderly Dutch men, animal protein intake was not related to 5-year changes in BP level. Plant protein intake was inversely associated with 5-year BP changes. The mean 5-year change in BP was -2.94 mmHg for systolic BP and -1.70 mmHg for diastolic BP in the higher-intake tertiles, compared with the lowest-intake tertile.

In a sample of 145 men of the Zutphen Elderly Study, a reproducibility study was carried out that also provided information on the inter- and intra-individual variation of protein intake[13]. Ratios of the inter- to intra-individual variance were 2.5 for plant protein and 1.4 for animal protein, indicating that there was less variation in animal protein intake between subjects but more variation within subjects, compared with plant protein intake. Consequently, findings for animal protein may be more biased towards the null than those of plant protein.

In the Zutphen Elderly Study, extensive data on repeated measurements of dietary intake, lifestyle factors and BP were collected during a total follow-up period of 15 years. Adjustments were made for many potential confounders, including physical activity, intake of dietary fiber and mineral during cooking or at the table. An estimated 79% of salt intake is derived from foods[17], indicating that most sodium intake is taken into account in our study. However, we cannot rule out the possibility of residual confounding by discretionary salt use. The data on repeated dietary and BP measurements allowed us to update protein intake over time and use BP change as a quantitative outcome measure, rather than a dichotomous outcome. A major advantage of this quantitative approach is that changes in the pre-hypertensive range are also taken into account and not only those around a specific threshold. We minimized the possibility of reverse causality by excluding men with baseline CVD, diabetes mellitus, cancer and antihypertensive medication. However, this exclusion resulted in a relatively small study sample, which may have reduced the power to detect associations. Moreover, this limits the generalizability of our findings to the general population, as our study sample included only healthy elderly males. It should be noted that protein intake in this study (15.6 ± 2.7 en%) was similar to the protein intake in 1987-1988 of Dutch males aged 65 years and older (14.8 ± 2.9 en%) [18]. Therefore, we cannot

draw conclusions on the associations of very low or high intakes of protein with BP.

In the present study, an inverse association between plant protein intake and 5-year changes in BP level (-2.94/-1.70 mmHg per 5 years) was observed during 15 years of follow-up. This is in agreement with findings from the Chicago Western Electric Study. In this study, plant protein was observed to be inversely associated with an annual change in BP level of -0.24/-0.14 mmHg during 8 years of follow-up[10]. In both studies, data of men who were repeatedly examined during long-term follow-up were used. Extensive dietary intake was estimated by using a dietary history method. These two studies are currently the only cohort studies that investigated the long-term association of plant and animal protein with BP changes, rather than incident hypertension. Findings of the SUN cohort suggest an inverse association between plant protein and incident hypertension[6], but this is not confirmed by two Dutch prospective cohort studies[7,8].

The mechanisms by which plant protein may beneficially influence BP are largely unknown. Differences in amino acid composition between plant and animal protein may contribute to potential differential associations with BP. In the INTERMAP Study, differences in amino acid content between plant and animal protein were observed. Compared to animal protein, the dietary amino acids that are relatively higher in plant protein are glutamic acid, proline, phenylalanine, serine and cysteine[19]. Another explanation may be that individuals with a high plant protein intake tend to have a healthier diet than those with a high animal protein intake. Although the associations in our study were adjusted for dietary fiber and potassium intake, we cannot completely rule out residual confounding of the observed associations in the present study.

In conclusion, we did not observe associations of animal protein intake with BP changes. We observed an inverse association between plant protein and changes in BP level in elderly Dutch men during long-term follow-up, which warrants confirmation in other prospective studies. Moreover, studies investigating the mechanism(s) which may explain the potential beneficial effect of plant protein on BP are needed.

Conflict of interest

The writing of this report was funded by Top Institute (TI) Food and Nutrition (project number A-1003), Wageningen, The Netherlands. TI Food and Nutrition is a public private partnership of science, industry and government conducting strategic research in food and nutrition (www.tifn.nl). None of the authors has a conflict of interest.

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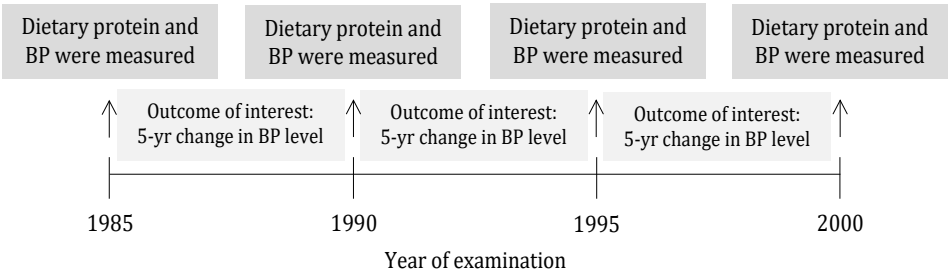
SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE 1. Overview of the study design

SUPPLEMENTARY FILE

Below are the SAS syntax used for the fully-adjusted random intercept model. The association between protein intake (in tertiles) and 5-year changes in systolic BP level is used as an example.

PROC MIXED DATA=zutphen;
CLASS ID year tertprotein SES alcohol physact;
MODEL SBP= tertprotein tertprotein*round age kJ BMI SES smoking physact alcohol diet carbp tfap safap pufap fib sodium potassium magnesium calcium/CL SOLUTION;
RANDOM INT/SUBJECT=ID TYPE=UN;
REPEATED year /SUBJECT=ID TYPE=AR(1) local;
RUN;

Variable	Label
Age	Age (years)
Alcohol	Alcohol intake in 3 categories (0 g/d, ≥20 g/day and >20 g/day)
BMI	Body mass index in 2 categories (<25 and ≥25 kg/m^2)
Calcium	Calcium intake (mg/day)
Carbp	Carbohydrate intake (en%)
Diet	Prescribed diet (yes/no)
Fib	Dietary fiber intake (g/day)
ID	Identification number
KJ	Energy intake (in kJ)
Magnesium	Magnesium intake (mg/day)
Physact	Physical activity (low, medium, high and missing)
Potassium	Potassium intake (mg/day)
Pufap	Poly-unsaturated fat intake (en%)
Round	Measurement round (1, 2, 3 and 4)
Safap	Saturated fat intake (en%)
SBP	Systolic blood pressure (mmHg)
SES	Socioeconomic status (low, medium-low, medium-high and high)
Smoking	Smoking status (yes/no)
Sodium	Sodium intake (mg/day)
Tertprotein	Protein intake (in en%) in tertiles
Tfap	Trans-fatty acid intake (en%)
Year	Year of measurement (1985, 1990, 1995 and 2000)

SUPPLEMENTARY TABLE 1. Mean (± SD) baseline blood pressure (BP) levels per tertile of baseline plant, animal and total protein intake in 272 men

		Tertiles of protein intake			P
		Tertile 1 (n = 90)	Tertile 2 (n = 91)	Tertile 3 (n = 91)	
Plant protein	Systolic BP (mmHg)	145.1 ± 18.7	148.5 ± 20.4	147.6 ± 19.0	0.47
	Diastolic BP (mmHg)	84.9 ± 9.8	83.8 ± 9.7	83.5 ± 10.2	0.62
Animal protein	Systolic BP (mmHg)	146.4 ± 20.0	147.5 ± 18.9	147.3 ± 19.3	0.93
	Diastolic BP (mmHg)	83.4 ± 9.8	84.6 ± 9.1	84.3 ± 10.7	0.68
Total protein	Systolic BP (mmHg)	145.8 ± 19.4	146.0 ± 18.2	149.4 ± 20.4	0.37
	Diastolic BP (mmHg)	84.0 ± 9.0	83.1 ± 9.7	85.2 ± 10.8	0.35

CHAPTER 4

Intake of total protein, plant protein and animal protein in relation to blood pressure: a meta-analysis of observational and intervention studies

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ABSTRACT

There is growing evidence from epidemiological studies that dietary protein may beneficially influence blood pressure (BP), but findings are inconclusive. We performed a meta-analysis of 29 observational studies and randomized controlled trials (RCTs) of dietary protein and types of protein in relation to BP or incident hypertension, published until January 2012. The analysis included eight cross-sectional studies (n=48 985), four prospective studies (n=11 761) and 17 RCTs (n=1449). A modest inverse association between total protein intake and BP (-0.20 mmHg systolic (95% CI: -0.39, -0.01) per 25 g (~1 SD)) was found in cross-sectional studies, but not in prospective studies (relative risk of 0.99 (95% CI: 0.96, 1.02)). For RCTs that used carbohydrate as a control treatment, the pooled BP effect was -2.11 mmHg systolic (95% CI: -2.86, -1.37) for a weighed mean contrast in protein intake of 41 g per day. A non-significant inverse association of -0.52 mmHg systolic (95% CI: -1.10, +0.05) per 11 g (~1 SD) was found for plant protein in cross-sectional studies, whereas animal protein was not associated with BP. In prospective studies and RCTs, however, the associations of plant protein and animal protein with BP were broadly similar. These findings suggest that increasing the intake of protein at the expense of carbohydrates may have a beneficial effect on BP. The BP effect of specific types of protein remains to be established.

INTRODUCTION

Elevated blood pressure (BP) is a major risk factor for cardiovascular diseases. In 2002, the World Health Organization estimated that about 62% of cerebrovascular disease and 49% of ischemic heart disease worldwide were attributable to suboptimal BP (that is, systolic BP levels >115 mmHg)[1,2]. Prevention of high BP by healthy lifestyle and diet, therefore, can have a substantial public health impact; it has been estimated that a population-wide reduction in systolic BP of only 2 mmHg is expected to result in a 6% reduction in fatal stroke and a 4% reduction in fatal coronary heart disease[3].

Some of the well-established measures to lower BP are weight reduction in overweight and obese individuals, reduced salt intake, moderation of alcohol intake (among those who drink) and an increased potassium intake[3,4]. In addition, data from the large DASH trial among 459 (pre)hypertensive adults showed that BP can be substantially reduced by a diet rich in fruits, vegetables and low-fat dairy products compared with a typical US diet, with reductions in systolic BP being -5.5 mmHg in the total DASH population and -11.4 mmHg in hypertensive participants[5]. More recently, interest has grown in the influence of diet composition and macronutrient intake on BP. Whether total protein content of the diet and/or types of protein are important for human BP, however, is not clear.

In a previously published systematic review, we concluded that dietary protein could have a small beneficial effect on BP[6]. Observational (mainly cross-sectional) data suggested a more beneficial role for plant protein than for animal protein. More data on dietary (types of) protein and BP have recently been published[7–13]. We, therefore, performed a series of meta-analyses on total protein, plant protein and animal protein in relation to BP or incident hypertension, based on observational and trial data presented in the literature until January 2012.

MATERIALS AND METHODS

Study selection

For the systematic literature review that has recently been published[6], a systematic

MEDLINE search (1 January 1966 to 1 June 2010) was performed. Search terms on dietary protein and BP or hypertension were used to search for words in title or abstract, and Medical Subject Headings (Supplementary Table 1 in the online issue for detailed query syntax). An additional manual search was performed using reference lists of original research and review articles. For the present meta-analysis, an updated secondary search was conducted until January 2012, using the same query syntax.

We selected any observational or intervention study that examined dietary protein in relation to BP in generally healthy adults. All titles, abstracts and full papers of potentially relevant studies were assessed for eligibility based on predefined inclusion and exclusion criteria. Papers were excluded (1) if data on exposure (total protein, plant protein and animal protein) or outcome (BP, hypertension) were not sufficiently reported; (2) if no estimate for the relationship between exposure and outcome was reported; and (3) if the exclusive effect of protein could not be calculated (for example, studies that focused on dietary patterns, soy combined with isoflavones, or no isocaloric macronutrient replacement in trials). Finally, we excluded studies based on biomarkers, because the biomarkers used were too heterogeneous. Moreover, there were insufficient numbers of studies (<3) to perform a meta-analysis on trials investigating plant protein versus animal protein, and to perform a meta-analysis on studies examining the BP effect of protein sources (for example, dairy protein, meat protein or grain protein). A flowchart of the screening and selection process is provided in the online issue (Supplementary Figure 1).

Statistical methods

From each included paper, we extracted data on protein intake, source of protein and BP values or estimated risk of hypertension according to a predefined standard form. In addition, we extracted data on design, country of study, number of participants, population characteristics (including initial BP, gender and age), dietary assessment method (food frequency questionnaire, 24-h recall, food diary), adjustment for confounders and measures of variation. Data were verified by two authors (ST, WAK). To allow better comparison of results from observational studies, we expressed associations in these studies by standard units of protein intake that correspond to ~1 SD of protein intake in the Dutch population, that is,

25 g per day (3.5 en%) for total protein, 11 g per day (1.4 en%) for plant protein and 23 g per day (2.9 en%) for animal protein[14]. For parallel trials, we calculated the net systolic BP change by subtracting change from baseline in the intervention group from that in the control group. For crossover trials, the net systolic BP change was calculated as the final systolic BP in the intervention period minus the final systolic BP in the control period.

We used STATA version 11.0 (StataCorp, College Station, TX, USA) for meta-analysis using the METAN command. All statistical tests were two-sided with α=0.05. Forest plots were created per study type for total protein, plant protein and animal protein in relation to systolic BP difference or hypertension incidence. For observational studies, we used the results from the main multivariable model that included most confounders. Between-study heterogeneity was assessed using the I^2 statistic[15], which expresses the percentage of variation attributable to between-study heterogeneity. An I^2-value ≥50% was considered to indicate substantial heterogeneity between studies.

For the meta-analysis on randomized trials with a carbohydrate control, we also conducted a meta-regression analysis on protein dose and study duration using the GLST command, with the generalized least-squares method for trend estimation of summarized dose–response data, based on the Greenland and Longnecker method[16]. Furthermore, to check whether systolic BP response to dietary protein was modified by subject characteristics, we conducted a meta-regression analysis on age, gender (% males), body mass index and initial systolic BP level.

RESULTS

Study characteristics

An overview of the eight cross-sectional studies[9,17–23] included in our meta-analysis is given in Table 1. In total, data from 48 985 individuals (from six cross-sectional studies) were available for the analysis on total dietary protein and systolic BP. The mean (±SD) age of the study populations was 47±6 years (range: 38–53 years), mean baseline systolic BP was 124±11 mmHg (range: 107–135 mmHg) and mean protein intake was 82±7 g per day (range: 74–91 g per day).

TABLE 1. Design and study population characteristics of cross-sectional studies on protein intake and BP that were included in the meta-analysis

Source	Country	N	Type of population	Age, y	Men, %	Mean intake, g/d	Mean BP, mmHg	Covariaties
Total protein								
He et al[25]	China	827	Men	38	100	88	107/66	Age, BMI, alcohol, urinary Na, energy intake, region
Stamler et al[26]	USA	11342	Men	46	100	87	125/84	Age, race, BMI, education, smoking, serum cholesterol, antihypertensive drugs, intake of Na, K, alcohol, caffeine
Masala et al[27]	Italy	7601	Women	51	0	91	123/79	Age, BMI, waist circumference, smoking, education, exercise, energy intake
Wang et al[19]	USA	810	HTN	50	38	74	135/85	Age, race, gender, treatment, education, income, weight, waist circumference, exercise, intake of Ca, K
Umesawa et al[14]	Japan	7585	General	53	46	74	135/82	Age, gender, BMI, smoking, alcohol, community, use of antihypertensive medication, intake of Na, K, Ca
Altorf et al[9]	Netherlands	20820	General	42	45	84	120/76	Age, gender, BMI, education, smoking, alcohol, intake of energy, SFA, carbohydrates, fiber, Ca, Mg, K
Plant protein								
Joffres et al[28]	Hawaii	615	Men	55	100	28	135/79	Age, BMI
He et al[25]	China	827	General	38	100	72	107/66	Age, BMI, alcohol, urinary NA, energy intake, region
Elliott et al[18]	China, UK, Japan, USA	4680	General	49	50	36	119/74	Age, sex, weight, height, exercise, alcohol, sample, history CVD or DM, family history of hypertension, special diet, supplement use, urinary Na, K, intake of Ca, SFA, PUFA, cholesterol, fiber
Masala et al[27]	Italy	7601	Women	51	0	29	123/79	Age, BMI, waist circumference, smoking, education, exercise, energy intake

Wang et al[19]	USA	810	HBP	50	38	23	135/85	Age, race, gender, treatment, education, income, weight, waist circumference, exercise, intake of Ca, K
Umesawa et al[14]	Japan	7585	General	53	46	38	135/82	Age, gender, BMI, smoking, alcohol, community, use of antihypertensive medication, intake of Na, K, Ca
Altorf et al[9]	Netherlands	20820	General	42	45	32	120/76	Age, gender, BMI, education, smoking, alcohol, intake of energy, SFA, carbohydrates, fiber, Ca, Mg, K, animal protein
Animal protein								
Elliott et al[18]	China, UK, Japan, USA	4680	General	49	50	45	119/74	Age, sex, weight, height, exercise, alcohol, sample, history CVD or DM, family history of hypertension, special diet, supplement use, 24h urinary Na, K, intake of Ca, SFA, PUFA, cholesterol, fiber
Masala et al[27]	Italy	7601	Women	51	0	59	123/79	Age, BMI, waist circumference, smoking, education, physical activity, energy intake
Wang et al[19]	USA	810	HBP	50	38	50	135/85	Age, race, gender, treatment, education, income, weight, waist, PA, intake of Ca, K
Umesawa et al[14]	Japan	7585	General	53	46	35	135/82	Age, gender, BMI, smoking, alcohol, community, antihypertensive medication, intake of Na, K and Ca
Altorf et al[9]	Netherlands	20820	General	42	45	52	120/76	Age, gender, BMI, education, smoking, alcohol, intake of energy, SFA, carbohydrates, fiber, Ca, Mg, K, plant protein

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DM, diabetes mellitus; General, general population; HBP, untreated pre- or mild hypertensives; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

TABLE 2. Design and study population characteristics of prospective studies on protein intake and BP that were included in the meta-analysis

Source	Country	N	Type of population	Age, years	Men,%	Habitual intake, en%	BP, mmHg[a]	Follow-up time, years	Covariates
Total protein									
Alonso et al[16]	Spain	5880	Grads	36	39	18	NR	2	Age, gender, BMI, exercise, alcohol, smoking, hypercholesterolemia, intake of total energy, Na, fruit, vegetables, fiber, caffeine, Mg, K, low-fat dairy, MUFA, SFA
Altorf et al[8]	Netherlands	2241	Older	65	43	16	122/68	6	Age, gender, BMI, baseline SBP, smoking, alcohol, education, intake of total energy, K, Na, Ca, Mg, fiber, CH, SFA, PUFA
Altorf et al[7]	Netherlands	3640	General	44	44	15	118/76	10	Age, gender, BMI, education, smoking, alcohol, baseline SBP, intake of total energy, SFA, PUFA, CH, fiber, Ca, Mg, K
Plant protein									
Alonso et al[16]	Spain	5880	Grads	36	39	NR	NR	2	Age, gender, BMI, exercise, alcohol, smoking, hypercholesterolemia, intake of total energy, Na, fruit, vegetables, fiber, caffeine, Mg, K, lowfat dairy, MUFA, SFA
Wang et al[19]	Australia, EUR, USA	810	Healthy	50	38	5	135/85	0.5	Age, gender, race, weight, waist, exercise, education, income, antihypertensive drugs, study site, baseline BP, alcohol, intake of Ca, K, urinary creatinine and Na

Altorf et al[8]	Netherlands	2241	Older	65	43	6	122/68	6	Age, gender, BMI, baseline SBP, smoking, alcohol, education, intake of total energy, K, Na, Ca, Mg, fiber, CH, SFA, PUFA, animal protein
Altorf et al[7]	Netherlands	3640	General	44	44	6	118/76	10	Age, gender, BMI, education, smoking, alcohol, baseline SBP, intake of total energy, SFA, PUFA, CH, fiber, Ca, Mg, K, animal protein
Animal protein									
Alonso et al[16]	Spain	5880	Grads	36	39	NR	NR	2	Age, gender, BMI, exercise, alcohol, smoking, hypercholesterolemia, intake of total energy, Na, fruit, vegetables, fiber, caffeine, Mg, K, lowfat dairy, MUFA, SFA
Wang et al[19]	Australia, EUR, USA	810	Healthy	50	38	11	135/85	0.5	Age, gender, race, weight, waist, exercise, education, income, antihypertensive drugs, study site, baseline BP, alcohol, intake of Ca, K, urinary creatinine and Na
Altorf et al[8]	Netherlands	2241	Older	65	43	10	122/68	6	Age, gender, BMI, baseline SBP, smoking, alcohol, education, intake of total energy, K, Na, Ca, Mg, fiber, CH, SFA, PUFA, animal protein
Altorf et al[7]	Netherlands	3640	General	44	44	10	118/76	10	Age, gender, BMI, education, smoking, alcohol, baseline SBP, intake of total energy, SFA, PUFA, CH, fiber, Ca, Mg, K, animal protein

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DM, diabetes mellitus; Grads, graduate university students; General, general population; HBP, untreated pre- or mild hypertensives; MUFA, monounsaturated fatty acids; NR, not reported; PUFA, polyunsaturated fatty acids; SBP, systolic BP; SFA, saturated fatty acids. [a] Mean blood pressure at baseline.

TABLE 3. Design and study population characteristics of trials on protein intake and BP that were included in the meta-analysis

Source	Country	Design	N[a]	Men, %	Type of population	Age, years	Duration, week	Δ Protein, g per day	Protein type	Control[b]	Baseline BP intervention/control, mmHg
Sacks et al[29]	USA	X-SB	18	39	Vegan	32	6	56	S-soy/wheat	CH-NR	112/74
Appel et al[30]	USA	X-DB	161	55	HBP	54	6	53	D-mixed	CH-NR	131/77
He et al[10]	USA	X-DB	273	58	HBP	48	8	31	S-soy[c]	CH-mixed[d]	126/81
He et al[10]	USA	X-DB	273	58	HBP	48	8	33	S-milk	CH-mixed[d]	126/82
Pal et al[12]	Australia	P-SB	20/25	14	OV	48	12	63	S-casein	CH-glucose	118/67 vs. 115/66
Pal et al[12]	Australia	P-SB	25/25	14	OV	48	12	74	S-whey	CH-glucose	119/64 vs. 115/66
Hodgson et al[31]	Australia	P-0	29/31	63	HBP[e]	59	8	38	D-red meat	CH-NR	134/79 vs. 138/77
Hendler et al[32]	USA	P-NR[f]	8/9	NR	OV	31	3	60	D-mixed	CH-fructose	120/79 vs. 121/79
Meckling et al[33]	Canada	P-NR[f]	10/8	0	OV	46	12	28	D-mixed	CH-NR	128/79 vs. 127/81
Meckling et al[33]	Canada	P-NR[f]	14/11	0	OV	39	12	56	D-mixed[g]	CH-NR	134/82 vs. 129/82
Burke et al[34]	Australia	P-O	18/18	50	HBP[e]	57	8	60	S-soy	CH-maltodextrin	134/75 vs. 132/76
Leidy et al[35]	USA	P-O[f]	21/25	0	OV	50	12	48	D-mixed	CH-NR	109/68 vs. 114/72
Brinkworth et al[36]	Australia	P-NR[h]	19/19	18	OV;DM2[e]	62	12	69	D-mixed	CH-NR	148/83 vs. 140/76
Larsen et al[11]	Australia	P-DB[h]	53/46	48	OV;DM2	59	52	30	D-mixed	CH-NR	132/82 vs. 127/82
Delbridge et al[37]	Australia	P-NR[h]	42/40	50	OV	44	48	26	D-mixed	CH-NR	135/85 vs. 131/83
Teunissen et al[13]	NL	P-DB	43/51	70	OV+HBP	55	4	61	S-mixed	CH-maltodextrin	143/93 vs. 143/92

Hodgson et al[38]	Australia	P-DB	101/95	0	Older[e]	74	104	28	S-Whey	CH-malto-dextrin	143/70 vs. 143/70
Papakonstantinou et al[39]	Greece	X-SB[f]	17	29	OV+DM2	46	4	56	D-mixed	Fat-MUFA	134/86 vs. 134/80
Appel et al[30]	USA	X-DB	160	55	HBP	54	6	53	D-mixed	Fat-MUFA	131/77 vs. 131/77
Hochstenbach et al[40]	NL	P-SB[h]	19/26	22	OV	43	8	32	D-milk	Fat-NR	118/75 vs. 116/72

Abbreviations: BP, blood pressure; CH, carbohydrates; D, diet; DM2, diabetes mellitus type 2; HBP, untreated pre- or mild hypertensives; MUFA, monounsaturated fatty acids; NR, not reported; OV, overweight or obese; S, supplement.

[a] In parallel studies: number of participants in the intervention group versus number of participants in the control group.
[b] Carbohydrates or fat and type of carbohydrate or fat.
[c] Isoflavones were not removed from the soy protein.
[d] Sucrose, fructose, maltodextrine.
[e] Antihypertensive drug users included.
[f] Weight-loss trial with hypocaloric diets in both groups.
[g] In both intervention and control group also, an exercise intervention was conducted.
[h] Weight maintenance after a period of weight loss.

Table 2 shows the characteristics of the four prospective studies[7,8,20,24] on protein intake and incident hypertension. In total, data from 11 761 individuals (from three prospective cohort studies) were available for the analysis on total dietary protein and hypertension incidence. The mean age of the study populations was 48±15 years (range: 36–65 years), mean baseline systolic BP was 120±3 mmHg, mean habitual total protein intake was 16±2 en% and follow-up lasted 2–10 years.

An overview of 17 randomized controlled trials (RCTs)[10–13,25–36] on protein intake and BP is given in Table 3. The number of participants in these trials ranged from 17 to 273. A total of 1449 individuals were included. The mean age of the trial populations was 50±10 years (range: 31–74 years), the mean baseline systolic BP was 128±10 mmHg (range: 112–144 mmHg), the mean difference in protein intake between intervention groups was 48±15 g per day (range: 26–74 g per day), and the trials had a mean duration of 17±24 weeks (range: 3–104 weeks).

Total dietary protein

In the six cross-sectional studies that were available for investigating the association between total protein and BP (48 985 individuals), the combined results showed a significant, but small, inverse association with a pooled estimate of -0.20 mmHg systolic (95% CI: -0.39, -0.01) per 25 g (~1 SD) of total protein intake (Figure 1). We observed no evidence for statistical heterogeneity (I^2=3.1%, P=0.40).

In our meta-analysis including data from three prospective studies (11 761 participants), total protein intake was not associated with incidence of hypertension with a pooled HR of 0.99 (95% CI: 0.96, 1.02, Figure 2) and no evidence for statistical heterogeneity (I^2=0.0%, P=0.53).

In 14 intervention studies (1208 individuals) that used carbohydrate as a control treatment, the pooled BP effect was -2.11 mmHg systolic (95% CI: -2.86, -1.37, Figure 3) for a weighed mean contrast in protein intake of 41 g per day and no signs of statistical heterogeneity (I^2=0.0%, P=0.98). Meta-regression analyses showed no association of protein dose or study duration with BP response (Table 4). Moreover, the BP response to dietary protein was not modified by subject characteristics, such as age, gender, baseline body mass index and initial systolic BP. We identified three trials with a fat control (mainly monounsaturated fatty acids, Table 3) yielding a pooled estimate of -0.04 mmHg (95% CI: -2.20, +2.12)

and no statistical heterogeneity (I^2=36.2%, P=0.21, data not shown). The type of intervention, that is, increase in protein intake by means of diet versus supplements, did not influence the BP effect (Supplementary Figure 2).

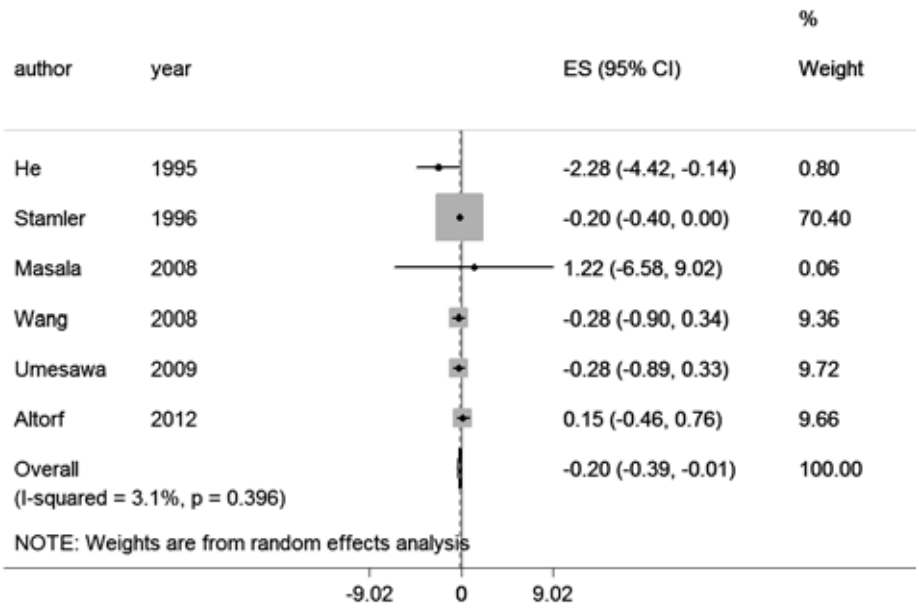

FIGURE 1. Fully adjusted difference (ES, 95% CI) in systolic BP with consumption of 25 grams (~1 SD) higher total protein intake in 6 cross-sectional studies (for details of the studies see Table 1). The I^2 value represents the proportion of variability in point estimates attributable to between-study heterogeneity[23].

Plant protein versus animal protein

Our meta-analysis including seven cross-sectional studies (42 938 participants) showed a small, but non-significant, inverse association of -0.52 mmHg systolic per 11 g (~1 SD) for plant protein (95% CI: -1.10, +0.05, Supplementary Figure 3), whereas animal protein (five cross-sectional studies, 41 496 participants) was not associated with BP (Supplementary Figure 4). We observed substantial heterogeneity for plant protein (I^2=75%, P=0.01) and animal protein (I^2=55%, P=0.07). This was mainly because of the study of Umesawa *et al.*[21] in 7585 Japanese adults, which showed an inverse association with BP for animal protein and a

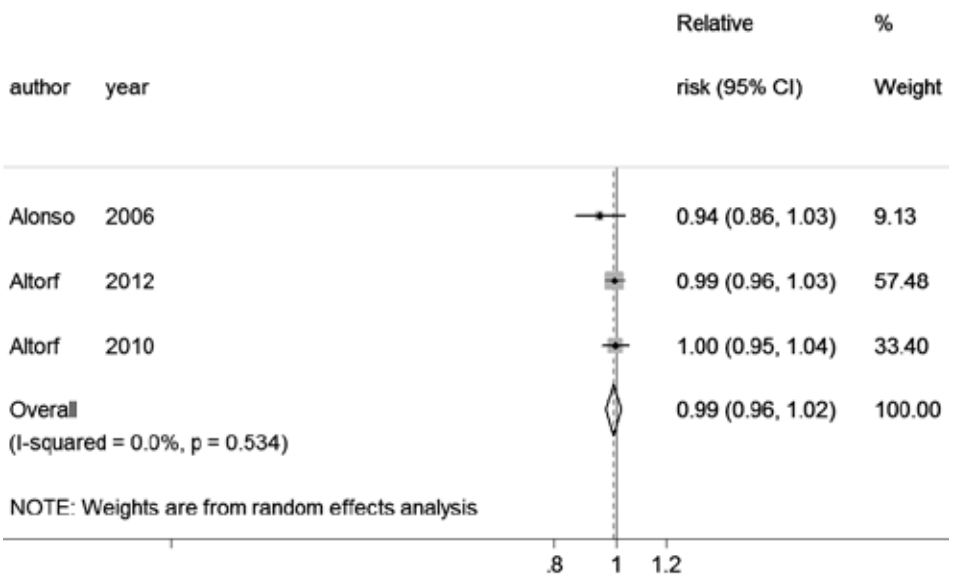

FIGURE 2. Fully adjusted relative risk of hypertension with 25 grams (~1 SD) higher total protein intake in 3 longitudinal studies (for details of the studies see Table 2). The I^2 value represents the proportion of variability in point estimates attributable to between-study heterogeneity[23].

TABLE 4. Characteristics associated with net change in BP in trials with carbohydrates as control: univariate meta-regression analysis

Characteristics	Coefficient	95% CI	P-value
Mean age, y	+0.2	-0.3 to +0.8	0.30
Men, %	-5.7	-20 to +9	0.37
Baseline BMI, kg/m^2	+0.1	-0.9 to +1.0	0.86
Mean baseline systolic BP, mmHg	+0.04	-0.3 to +0.4	0.81
Duration, weeks	-0.1	-0.3 to +0.1	0.30
Δ Protein[a], g/d	-0.1	-0.3 to +0.1	0.30

Abbreviations: BP, blood pressure; BMI, body mass index.

[a] Difference in protein intake between intervention and control group.

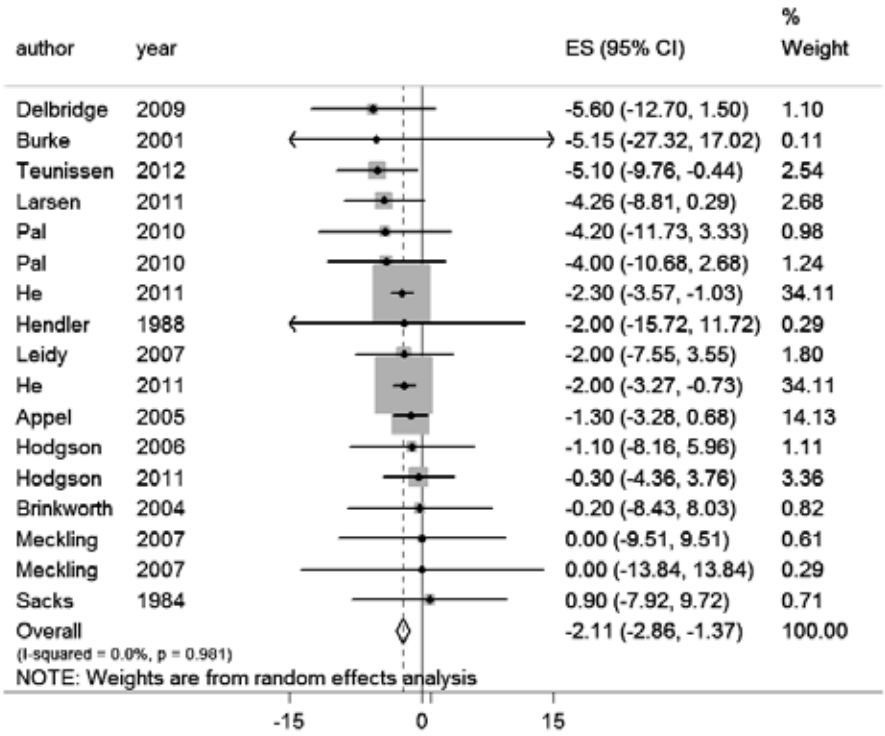

FIGURE 3. Net change (ES, 95% CI) in systolic BP with consumption of protein compared to carbohydrates in 14 randomized controlled trials (for details of the studies see Table 3). The I^2 value represents the proportion of variability in point estimates attributable to between-study heterogeneity.[23] In the studies of Pal *et al.*[12] and He *et al.*[10] two intervention arms were included that were compared to the same control group. In the study of Meckling *et al.*[33] two intervention arms were included that were each compared to their own control group (intervention with and without exercise in both intervention and control group).

direct association for plant protein. After exclusion of that study, heterogeneity was strongly reduced to 17% for plant protein (P=0.31) and 0% for animal protein (P=0.61). In addition, pooled estimates changed towards a larger difference between protein types, that is, -0.73 mmHg systolic per SD (95% CI: -1.08, -0.38) for plant protein and +0.24 mmHg (95% CI: -0.09, +0.57) for animal protein.

In our meta-analysis including data from four prospective studies (12 571 participants), incident hypertension was not related to plant protein (HR: 0.96, 95% CI: 0.89, 1.03; Supplementary Figure 5) or animal protein (HR: 0.98, 95% CI: 0.95, 1.02; Supplementary Figure 6). For these analyses, we observed statistical heterogeneity for plant protein (I^2=63.9%, P=0.04), but not for animal protein (I^2=0.0%, P=0.99). When analyzing trials in strata of plant protein and animal protein, there was no significant difference between the BP effects of protein from plant and animal sources. Our meta-analysis on plant protein included data from three trials (327 individuals) and provided a pooled estimate of -1.95 mmHg systolic (95% CI: -3.21, -0.69) without evidence for statistical heterogeneity (Supplementary Figure 7). The pooled estimate for animal protein including data from four trials (574 individuals) was -2.20 mmHg systolic (95% CI: -3.36, -1.03) without evidence for statistical heterogeneity.

DISCUSSION

The totality of evidence, especially from trials, indicates that dietary protein may have a beneficial effect on BP if consumed instead of carbohydrates, although we did not observe a dose–response relationship. There was no clear differential effect on BP for plant protein or animal protein.

Total dietary protein

In the present meta-analysis, cross-sectional studies showed a small beneficial association of total protein with BP, whereas this was not confirmed in prospective cohort studies. This discrepancy may be explained by lower BP levels in prospective population-based studies owing to the exclusion of hypertensive participants at baseline. Moreover, small associations in these studies may have been missed because of using a dichotomous outcome, that is, incident hypertension defined as

BP ≥140/90 mmHg or initiation of antihypertensive medication. This approach has the advantage that participants who started using medication during follow-up could be retained in the analysis, which reduced the risk of bias. A disadvantage, however, is that BP changes closely around the cut-off point are emphasized, whereas changes further away from the cut-off point are ignored. In addition, small BP differences may have been missed.

Trials with a carbohydrate control provided stronger evidence for a beneficial effect of dietary protein on BP than observational studies did, which may be partly attributable to the inclusion of more vulnerable individuals; for example, those with (pre)-hypertension. In addition, in these trials supplements or fully controlled diets were mostly used, and attenuation of BP effects due to exposure misclassification does not occur. This is in contrast to observational studies in which protein intake is measured using memory-based methods. Finally, the contrast in protein intake was larger in trials with a weighed mean contrast in intake of 41 g per day (range: 26–74 g per day) versus a contrast of 25 g per day (~1 SD) in observational studies. The relatively high doses in trials may also have masked a dose–response relationship with BP.

In isocaloric conditions, a BP effect after intake of protein will be relative to the intake of fat or carbohydrates, or both. The results of our meta-analysis indicate a stronger BP effect of protein when exchanged for carbohydrates rather than for fat (mainly monounsaturated fatty acids). A decreased carbohydrate intake, rather than an increased protein intake, may therefore also be involved in BP reduction. BP effects were more pronounced in trials using glucose or maltodextrin as a control compared with trials that were diet-based and had a mixture of carbohydrates in the control diet. The increase of protein at the expense of carbohydrates (especially 'fast' carbohydrates like sucrose and maltodextrin) reduces the glycemic index of diets, which may result in an attenuated insulin response. As there is some evidence for an unfavorable effect of insulin on BP, this may explain a BP-lowering effect of such diets[37]. However, it cannot be excluded that the more controlled dose in the supplement-based trials, rather than the type of carbohydrates, accounted for the stronger BP effects. In observational studies it was not clear which macronutrients were exchanged, which may explain why no association was found between protein intake and BP.

Plant protein versus animal protein

Early observational studies showed an inverse association for plant protein, but not for animal protein, with BP[20,23,24,38]. In our meta-analysis based on cross-sectional studies, we observed a small and non-significant inverse association of -0.52 mmHg systolic per 11 g plant protein with substantial heterogeneity (I^2=75%, P=0.01). This heterogeneity was mainly because of the study of Umesawa *et al.*[21] in 7585 Japanese adults, which showed an inverse association with BP for animal protein and a direct association for plant protein. After exclusion of that study, the pooled estimate for plant protein became larger and significant (that is, -0.73 mmHg, 95% CI: -1.08, -0.38). No association was observed for animal protein. The deviant estimates in the study of Umesawa *et al.*[21] may be attributable to the eating habits in Japan, where ~24% of animal protein intake comes from fish, whereas in China and in western countries ~6% of animal protein intake is derived from fish[39]. Fish may be more beneficial to BP than meat,[40] which may explain the inverse association between animal protein and BP in the study of Umesawa *et al.*[21]. A more beneficial influence of plant protein compared with animal protein on BP may, therefore, only be present in countries with a more westernized diet. Residual confounding from factors that are strongly correlated to intake of plant protein or animal protein cannot be excluded. Individuals in western countries who consume a diet rich in plant protein, probably have a healthier lifestyle than those who consume much animal protein. Although in most observational studies adjustments were made for nutrients that are indicators of a healthy lifestyle (for example, dietary fiber and potassium), incomplete adjustment for lifestyle factors or dietary factors, such as polyphenols that are abundant in plant food, may have resulted in residual confounding. On the other hand, in the meta-analysis based on prospective studies in western countries, plant protein was not related to incident hypertension (HR: 0.96, 95% CI: 0.89, 1.03).

To gain more insight in the BP effect of plant protein and animal protein, we conducted a meta-analysis of trials with a carbohydrate control, stratified by plant protein or animal protein in the intervention diet. We did not find a different effect between trials with protein interventions from plant sources or animal sources (data not shown). The protein source in all plant protein trials was soy, and results cannot be generalized to total plant protein. In a western diet, soy protein makes only a small contribution to total intake of plant protein (~2.5% in the Netherlands,

unpublished data). It has been estimated that grain protein contributes ~53% to plant protein intake in the Netherlands, with other important sources being potatoes (10%), vegetables (8%) and fruits (10%). Up to date, no trial has been conducted that examined the BP effect of dietary plant protein originating from these sources compared with a balanced mix of animal protein.

In conclusion, the present meta-analysis indicates a beneficial effect of total dietary protein on BP when consumed instead of carbohydrates. In 14 trials that used carbohydrates as a control treatment, the pooled BP effect was -2.11 mmHg systolic for a weighed mean contrast in protein intake of 41 g per day. This contrast in protein intake is larger than in observational studies (SD ~25 g per day), which suggests that the BP effect in trials is likely to be higher than in the general population. However, a small BP decrease may still have a substantial public health impact. It has been estimated that a population-wide reduction in systolic BP of only 2 mmHg is expected to result in a 6% reduction in fatal stroke, and a 4% reduction in fatal coronary heart disease.[3] In the present meta-analysis, no difference in BP effect for plant protein or animal protein was observed. BP trials of protein from different sources that reflect habitual intakes in western populations are warranted. For now, a prudent diet with adequate amounts of dietary protein at the expense of carbohydrates may be recommended for the prevention of hypertension.

Conflict of interest

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 1. Search strategy for studies published until January 2012, using PUBMED (www.ncbi.nlm.nih.gov/pubmed)

STEP 1 – Exposure (dietary protein)
#1 protein[tiab] OR proteins[tiab] OR peptide*[tiab] OR amino acid*[tiab]
#2 gelatin[tiab] OR casein*[tiab] OR whey[tiab]
#3 dairy[tiab] OR meat[tiab] OR soy*[tiab] OR milk[tiab] OR fish[tiab] OR yoghurt[tiab] OR cheese[tiab] OR vegetable[tiab] OR vegetables[tiab]
#4 casokinin*[tiab] OR IPP[tiab] OR VPP[tiab] OR C12[tiab] OR lactotripept*[tiab] OR tripept*[tiab] OR Ile-Pro-Pro[tiab] OR Val-Pro-Pro[tiab]
#5 peptides[Mesh:NoExp] OR oligopeptides[Mesh:NoExp] OR amino acids, essential[Mesh]
#6 diet[tiab] OR diets[tiab] OR dietary[tiab] OR intake*[tiab] OR suppl*[tiab] OR consumption[tiab] OR food*[tiab] OR drink*[tiab] OR meal[tiab] OR nutrition*[tiab] OR nutrient*[tiab] OR bioactive[tiab] OR biologically active[tiab]
#7 #1 AND #6
#8 #2 AND #6
#9 #3 AND #6
#10 #4 AND #6
#11 #5 AND #6
#12 dietary proteins[mesh]
#13 #7 OR #8 OR #9 OR #10 OR #11 OR #12

STEP 2 – Outcome (blood pressure or hypertension)
#14 blood pressure[Mesh:NoExp] OR hypertension[Mesh:NoExp]
#15 blood pressure [tiab] OR hypertens*[tiab] OR arterial pressure[tiab] OR systolic[tiab] OR diastolic[tiab] OR pulse pressure[tiab]
#16 #14 OR #15

STEP 3 – Combine exposure and outcome
#17 #13 AND #16

SEARCH 4 – Limits (Human BP, English language, adults, no review)

#18 Rats[Mesh] OR Mice[Mesh] OR rat[tiab] OR rats[tiab] OR mouse[tiab] OR mice[tiab]

#19 #17 Limits: Humans, English, All Adult: 19+ years

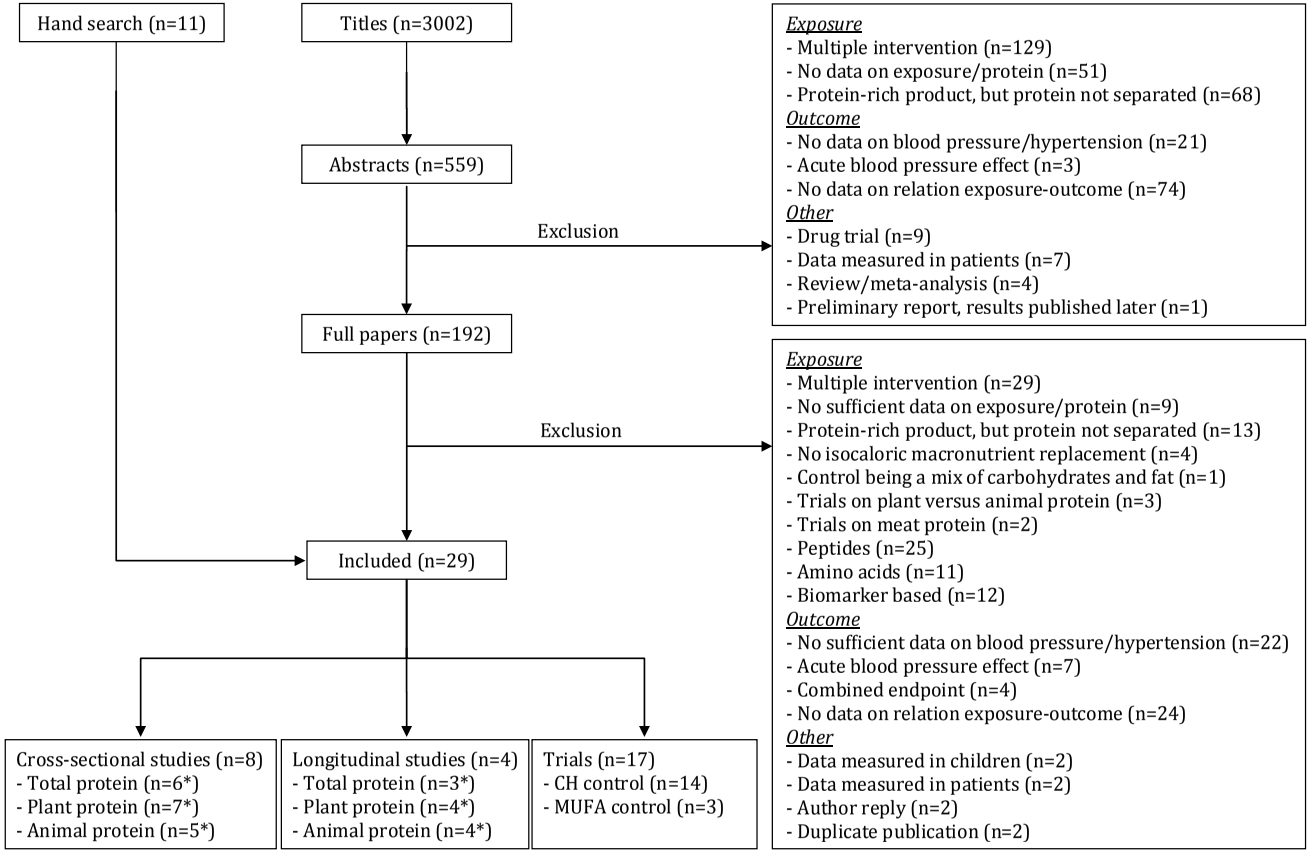
#20 #19 NOT #18

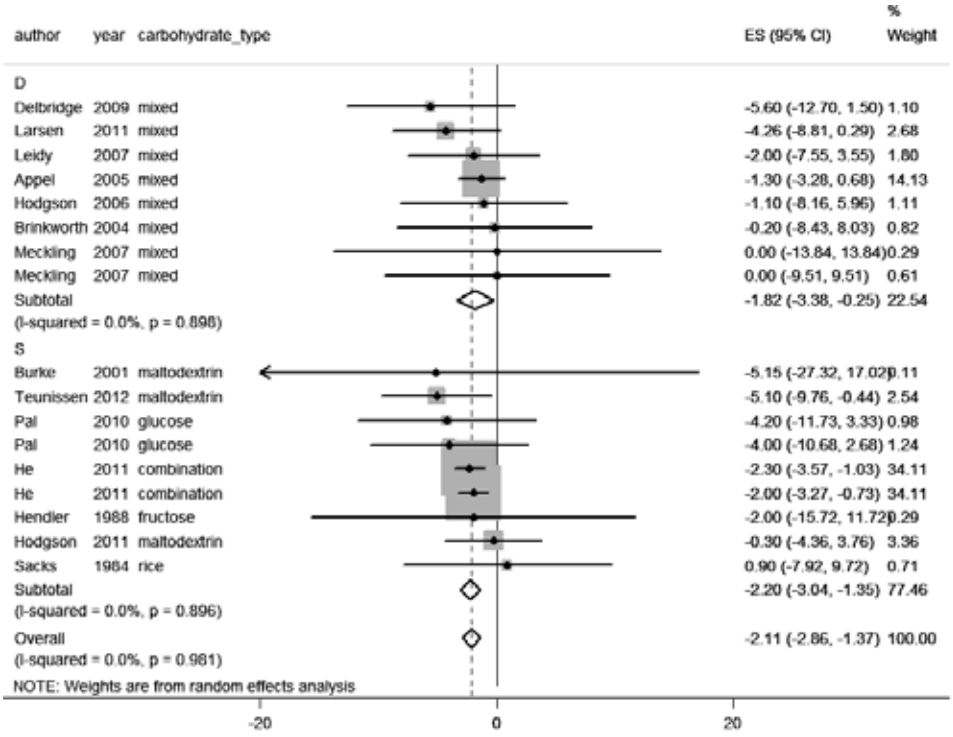
#21 #20 NOT review

#22 pregnancy[Mesh] OR placenta[Mesh] OR pregnan*[tiab] OR placenta[tiab] OR foetus*[tiab] OR fetus*[tiab] OR foetal[tiab] OR fetal[tiab] OR prenatal[tiab] OR perinatal[tiab] OR gestation*[tiab] OR breast feeding[tiab] OR lactation[tiab]

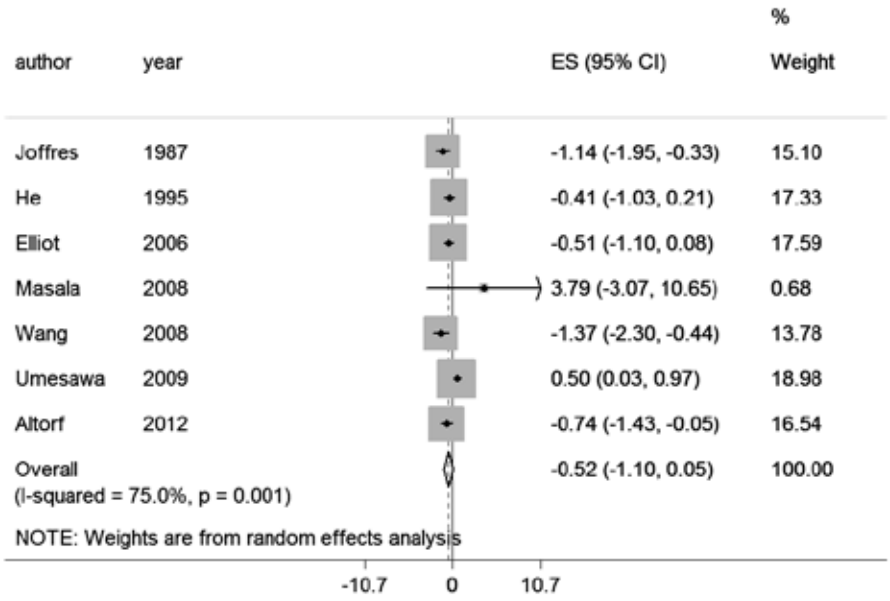
#23 #21 NOT #22

SUPPLEMENTARY FIGURE 1. Flow chart

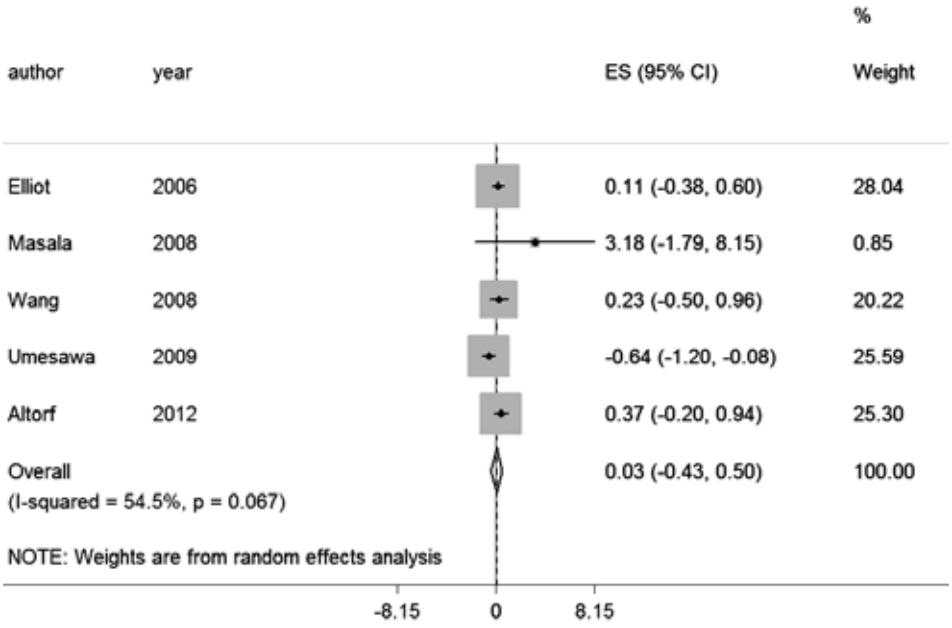




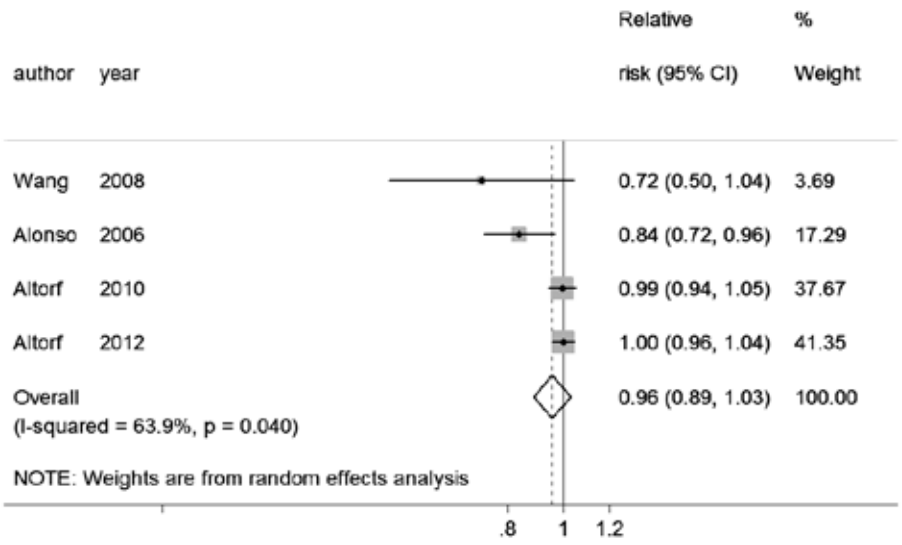
SUPPLEMENTARY FIGURE 2. Net change (ES, 95% CI) in systolic BP with consumption of protein compared to carbohydrates in 14 randomized controlled trials, stratified by intervention type (for details of the studies see Table 3 in the main article). The I^2 value represents the proportion of variability in point estimates attributable to between-study heterogeneity. D=diet based, S=supplement based.



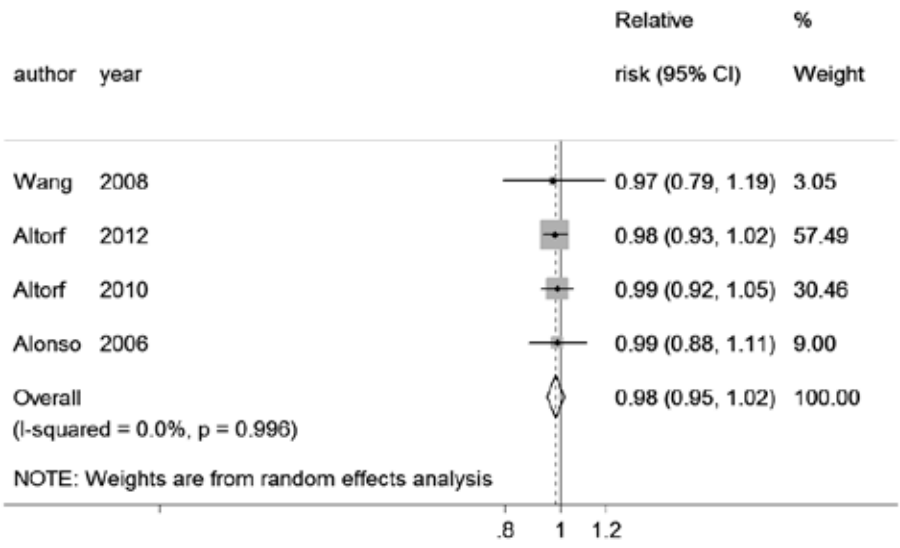
SUPPLEMENTARY FIGURE 3. Fully adjusted difference (ES, 95% CI) in systolic BP with consumption of 11 grams (~1 SD) higher plant protein intake in 7 cross-sectional studies (for details of the studies see Table 1 in the main article). The I^2 value represents the proportion of variability in point estimates attributable to betweenstudy heterogeneity.



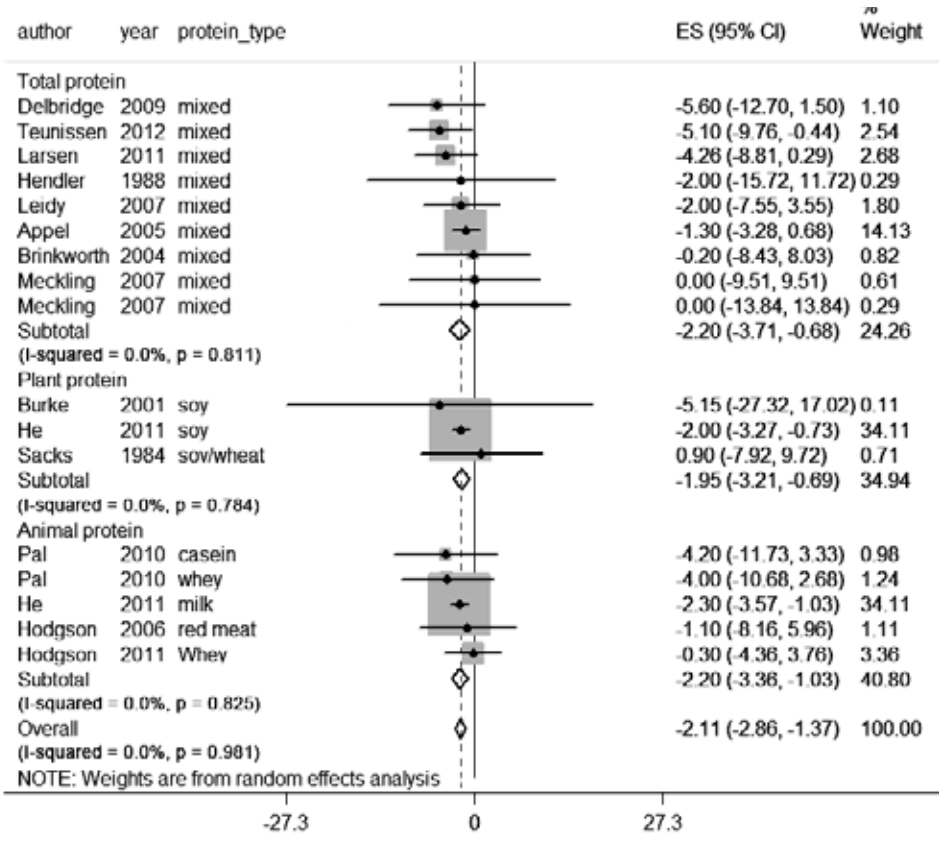
SUPPLEMENTARY FIGURE 4. Fully adjusted difference (ES, 95% CI) in systolic BP with consumption of 23 grams (~1 SD) higher animal protein intake in 5 cross-sectional studies (for details of the studies see Table 1 in the main article). The I^2 value represents the proportion of variability in point estimates attributable to betweenstudy heterogeneity.



SUPPLEMENTARY FIGURE 5. Fully adjusted relative risk of hypertension with 11 grams (~1 SD) higher plant protein intake in 4 longitudinal studies (for details of the studies see Table 2 in the main article). The I^2 value represents the proportion of variability in point estimates attributable to between-study heterogeneity.



SUPPLEMENTARY FIGURE 6. Fully adjusted relative risk of hypertension with 23 grams (~1 SD) higher animal protein intake in 4 longitudinal studies (for details of the studies see Table 2 in the main article). The I^2 value represents the proportion of variability in point estimates attributable to between-study heterogeneity.



SUPPLEMENTARY FIGURE 7. Net change (ES, 95% CI) in systolic BP with consumption of protein compared to carbohydrates in 14 randomized controlled trials, stratified by protein type (for details of the studies see Table 3 in the main article). The I^2 value represents the proportion of variability in point estimates attributable to betweenstudy heterogeneity.

CHAPTER 5

Ten-year blood pressure trajectories, cardiovascular mortality, and life years lost in two extinction cohorts: the Minnesota Business and Professional Men Study and the Zutphen Study

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ABSTRACT

Background: Blood pressure (BP) trajectories derived from measurements repeated over years have low measurement error and may improve cardiovascular disease prediction compared to single, average, and usual BP (single BP adjusted for regression dilution). We characterized 10-year BP trajectories and examined their association with cardiovascular mortality, all-cause mortality, and life years lost.

Methods and Results: Data from 2 prospective and nearly extinct cohorts of middleaged men—the Minnesota Business and Professional Men Study (n=261) and the Zutphen Study (n=632) were used. BP was measured annually during 1947–1957 in Minnesota and 1960–1970 in Zutphen. BP trajectories were identified by latent mixture modeling. Cox proportional hazards and linear regression models examined BP trajectories with cardiovascular mortality, all-cause mortality, and life years lost. Associations were adjusted for age, serum cholesterol, smoking, and diabetes mellitus. Mean initial age was about 50 years in both cohorts. After 10 years of BP measurements, men were followed until death on average 20 years later. All Minnesota men and 98% of Zutphen men died. Four BP trajectories were identified, in which mean systolic BP increased by 5 to 49 mmHg in Minnesota and 5 to 20 mmHg in Zutphen between age 50 and 60. The third systolic BP trajectories were associated with 2-to-4-times higher cardiovascular mortality risk, 2-times higher all-cause mortality risk, and 4 to 8 life years lost, compared to the first trajectory.

Conclusions: Ten-year BP trajectories were the strongest predictors, among different BP measures, of cardiovascular mortality, all-cause mortality, and life years lost in Minnesota. However, average BP was the strongest predictor in Zutphen.

INTRODUCTION

The relation between arterial blood pressure (BP) and cardiovascular disease (CVD) has been investigated in many cohorts. The Prospective Studies Collaboration reported a 1.5-to-2-times higher coronary heart disease mortality risk per 20-mmHg difference in systolic BP level[1]. A previous report from the Seven Countries Study indicated a 1.7-times higher coronary heart disease mortality risk per 20-mmHg difference in systolic BP level[2]. In both studies, BP was based on resting BP obtained on a single occasion in midlife and relative risks were adjusted for regression dilution[3].

Compared to single BP assessment adjusted for regression dilution, patterns of BP over time (i.e., BP trajectories) may have greater power in predicting CVD[4]. In the Minnesota Business and Professional Men Study and the Zutphen Study, BP was recorded annually over a 10-year period[5,6]. The Minnesota Study started in 1947 and the Zutphen Study in 1960, a time in which only exceptionally high levels of BP were treated[7,8]. In 2002, the last Minnesota man died and by 2010, 98% of the Zutphen cohort had died. These "extinction" cohorts provide a unique opportunity to study not only the predictive value of BP trajectories in relation to CVD mortality and all-cause mortality, but also in relation to life years lost.

The aim of the present study was to characterize trajectories of systolic and diastolic BP based on annual resting BP measurements over a 10-year period and to investigate the association of these BP trajectories with CVD mortality, all-cause mortality, and life years lost in two cohorts of middle-aged men followed to "extinction." Moreover, we compared these associations with those of single BP (defined as BP measured at baseline), average BP (defined as the average of all available BP levels during the 10-year period), and usual BP (defined as single BP adjusted for regression dilution) with CVD mortality, all-cause mortality, and life years lost.

METHODS

Design and study populations

An overview of the study design is given in Figure 1. We aimed to replicate the results of the Minnesota cohort in those of the Zutphen cohort. BP was measured annually in the periods 1947–1971 in Minnesota and 1960–1970 in Zutphen. Therefore, a 10-year period was chosen to model BP trajectories in both cohorts.

Minnesota Study

In 1947, business and professional men from the upper socioeconomic class who resided in Minneapolis and St. Paul, MN, were recruited for an epidemiologic program to cover 300 men. Detailed information about the study design is described elsewhere[5]. In total, 285 clinically healthy men, aged 45 to 55 years, participated in the Minnesota Business and Professional Men Study, subsequently referred to as the Minnesota Study. In the first decade, 15 men died and 4 men had had myocardial infarction; we excluded these men from analysis. No one had a medical history of stroke. In addition, 5 men were excluded for having <5 BP recordings during 1947-1957, leaving 261 men for analysis.

Zutphen Study

In 1960, a longitudinal investigation on coronary heart disease risk factors was begun among middle-aged men in the town of Zutphen, The Netherlands. Out of 1088 randomly selected men, aged 40 to 60 years, 878 men participated. In the first decade, 118 men died and 55 men had had myocardial infarction or stroke; we excluded these men from analysis. In addition, 73 men were excluded for having <5 BP recordings during 1960–1970, leaving 632 men for analysis. Oral informed consent was obtained, as was appropriate at baseline in both cohorts, before the Helsinki Declaration was developed.

Data collection

Minnesota Study

Every year from 1947 through 1957, men were physically examined at the Laboratory of Physiological Hygiene at the University of Minnesota, Minneapolis,

MN[5]. Men came to the Laboratory in the morning without breakfast and had avoided strenuous activity after waking. BP was recorded with men in the supine position with a mercury sphygmomanometer at the beginning of the examination after at least 10 minutes of rest. BP was recorded 3 times about 5 minutes apart. The mean of these 3 recordings in systole and in the fifth phase of diastole was used for analysis. In total, 2738 recordings from 261 men were available.

Height and weight were measured annually. Serum cholesterol levels were determined with the Bloor method from 1947 through 1953 and then converted to Abell–Kendall values[5,9]. Thereafter, Abell–Kendall values were used. Smoking was assessed in 1954 by a questionnaire that included questions that made it possible to reconstruct smoking habits from 1947 onwards. Men were categorized into current former or never smokers. Prevalence of diabetes mellitus was determined by selfreported physician diagnosis (annually) or fasting glucose with a cut-off point of 7 mmol/L (in 1949 and 1952). A 12-lead ECG was taken every year and evaluated according to the Minnesota Code[10].

Zutphen Study

Every year from 1960 through 1970, men were physically examined according to the protocol of the Seven Countries Study[11]. With men in the supine position, BP was recorded with a mercury sphygmomanometer at the end of the examination after at least 5 minutes of rest. Two successive BP recordings were made. The mean of these 2 recordings in systole and in the fifth phase of diastole was used. In total, 6616 recordings from 632 men were available.

Height and weight were measured annually. In 1960 and 1965, serum cholesterol levels were determined with the Abell–Kendall method and in 1970 with the Zlatkis method[12] that were converted to Abell–Kendall values[13]. Smoking was assessed by the Seven Countries Study questionnaire[11]. Men were categorized into current former or nonsmokers. Prevalence of diabetes mellitus was determined by selfreported physician diagnosis in 1960, 1965, and 1970. A 12-lead ECG was taken every year and evaluated according to the Minnesota Code[10].

Outcome ascertainment

In both cohorts, men were followed to "extinction." In Minnesota, mortality follow-up continued until the last man died in 2002. Mortality information was obtained

from death certificates, hospital records, the Minnesota Department of Health, and the National Death Index. In Zutphen, mortality follow-up continued until July 1, 2010, when 12 men aged 90 to 95 years were still alive. Mortality information was obtained from the municipal population registry, hospital disease registry, general practitioners, and Statistics Netherlands.

Causes of death were adjudicated by an expert in cardiovascular epidemiology (A.M.), according to the International Classification of Diseases (ICD). In Minnesota, CVD mortality included ICD-9 codes 401-459 and 798. In Zutphen, CVD mortality included ICD-8 codes 400-458 and 795. In the present analysis, mortality follow-up started 10 years after baseline in both cohorts (Figure 1).

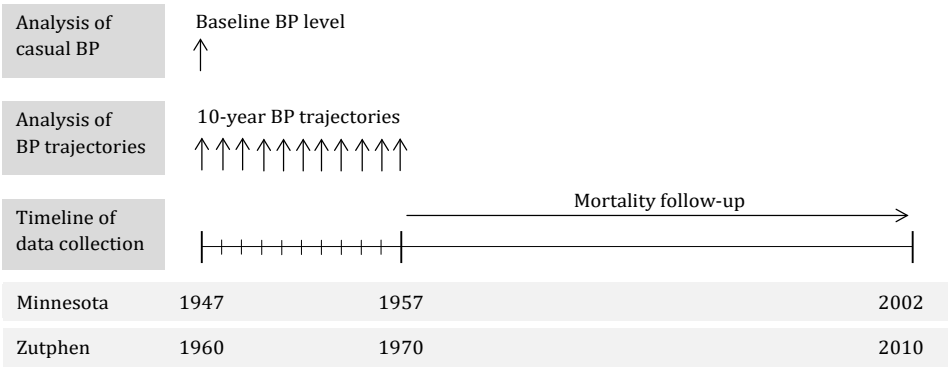

FIGURE 1. Overview of the study design. The age range at baseline is 45-55 years in 1947 in Minnesota and 40-60 years in 1960 in Zutphen.

Statistical analysis

Analyses were done separately for each cohort. Over a 10-year period, systolic and diastolic BP trajectories were identified by using latent mixture modeling within the PROC TRAJ procedure in SAS[14]. This procedure identifies groups of individuals with similar trajectories. We started with a 1-trajectory model and then fitted those up to the optimal number of trajectories by comparing the Bayesian Information Criterion for each number of trajectories[14] and by comparing the number of men per trajectory. Each man was assigned to a trajectory based on the likelihood of correctly classifying men in trajectories (range of mean likelihood across trajectories: 91% to 95%). Linear and quadratic terms were considered and

evaluated based on their significance level, starting with the highest polynomial. Age was used as timescale for the trajectories. Because a small fraction of men (<5% in both cohorts) was assigned to the fourth systolic BP trajectories (SBP4), the present study focused on the comparison of the third trajectories (SBP3) with the first trajectories (SBP1).

Cox proportional hazards analysis was used to investigate the association of BP trajectories with risk of CVD and all-cause mortality. Systolic or diastolic BP trajectories were included as dummy variables in the model using the first BP trajectories (SBP1 and DBP1) as reference.

For all men who had died during follow-up, age at death was calculated. Linear regression analysis was used to investigate the association between BP trajectories and age at death (which was normally distributed), resulting in the estimated number of life years lost per trajectory compared to mean age at death of the reference trajectory. BP trajectories were included as dummy variables in the model using the first BP trajectories as reference. For the 12 men who were still alive in Zutphen on July 1, 2010, their age in 2010 was used as age at death. Using age plus 3 years (the life expectancy for an average 90-to-95-year-old man in 2011) instead did not change the results.

In addition to BP trajectories, Cox proportional hazards analysis was used to investigate the association of single BP (BP measured at baseline), average BP (average of all available BP levels during the 10-year period), and usual BP (single BP adjusted for regression dilution) with risk of CVD and all-cause mortality. Linear regression analysis was used to investigate the association of single, average, and usual BP with the number of life years lost. Estimates were obtained for each unit difference of 10, 25, and 50 mmHg systolic BP and 10, 15, and 30 mmHg diastolic BP. These units were selected because they are similar to the differences in BP level between the reference trajectory and other BP trajectories at age 50 (the mean age at baseline). Average BP level was defined as the mean of all available BP levels during the 10-year period. We calculated the regression dilution ratio[3] from 11 annual BP measurements over the 10-year period to approximate the underestimate of the strength of the association between usual BP and risk of CVD mortality, all-cause mortality, and number of life years lost. The Akaike Information Criterion of proportional hazards models (single BP, average BP, and BP trajectories) were compared to investigate the best model fit.

Prediction of CVD mortality, all-cause mortality, and the number of life years lost by BP trajectories was compared between the 2 cohorts by calculating Z-scores and corresponding P-values. All associations were adjusted for age, serum cholesterol, smoking status, and diabetes mellitus at baseline. Analyses were performed for both cohorts using SAS version 9.2 (SAS Institute, Inc). A 2-sided P-value of <0.05 was considered statistically significant.

RESULTS

Initial mean age was 49.4 years in both cohorts (Table 1). Mean BP level at baseline was 17/16 mmHg lower in Minnesota than in Zutphen. During 10 years, mean BP

TABLE 1. Characteristics of men participating in the Minnesota and Zutphen Study at the first and last year of the BP trajectory. Values are means (SD) unless stated otherwise.

	Minnesota Study		Zutphen Study	
	1947	1957	1960	1970
N	261	231	632	568
Age (years)	49.4 (2.8)	59.4 (2.8)	49.4 (5.4)	59.5 (5.4)
Height (cm)	176.0 (6.2)	175.8 (6.3)	173.7 (6.6)	173.8 (6.6)
Weight (kg)	76.3 (12.1)	78.1 (11.5)[a]	72.6 (9.5)	76.0 (9.5)[b]
BMI (kg/m^2)	24.6 (3.6)	25.3 (3.4)[a]	24.0 (2.7)	25.1 (2.7)[b]
Systolic BP (mmHg)	124.1 (15.4)[c]	134.8 (22.2)	141.4 (18.5)[d]	146.1 (20.2)[e]
Diastolic BP (mmHg)	73.0 (10.9)[c]	81.6 (11.6)	89.0 (11.8)[f]	88.9 (11.5)[e]
Serum cholesterol (mg/dl)	209.4 (33.6)[c]	234.4 (38.5)[a]	232.7 (43.6)[g]	237.6 (42.4)
Current smoking, N (%)	147 (56.3%)[i]	103 (44.6%)[i]	499 (74.3%)	304 (54.3%)[h]
Diabetes mellitus, N (%)	0 (0.0%)	4 (1.7%)	6 (0.9%)	10 (1.8%)

Values are means (SD) unless stated otherwise. BMI indicates body mass index; BP, blood pressure.
Data are available for [a]229 men, [b]565 men, [c]259 men, [d]629 men, [e]567 men, [f]628 men, [g]599 men, and [h]560 men.
[i] Smoking was assessed in 1954 by an extensive questionnaire that included questions about smoking history that made it possible to reconstruct the smoking habits in 1947. Smoking habits in 1954 were used as a proxy for those in 1957.

level increased by 11/9 mmHg in Minnesota and 5/0 mmHg in Zutphen. Mean body mass index was about 25 kg/m^2 in both cohorts at baseline and 10 years later. At baseline, mean serum cholesterol level was 23 mg/dL higher in Zutphen, compared to Minnesota, but levels were similar after 10 years. In Minnesota, fewer men were smokers than in Zutphen. In both cohorts, few men were diabetic (<2%).

Four trajectories of systolic BP (SBP1 to SBP4) and diastolic BP (DBP1 to DBP4) were identified in both cohorts (Figure 2). Trajectories consisted of increasing BP levels fanning out with age, that is, BP increased more rapidly in men with higher

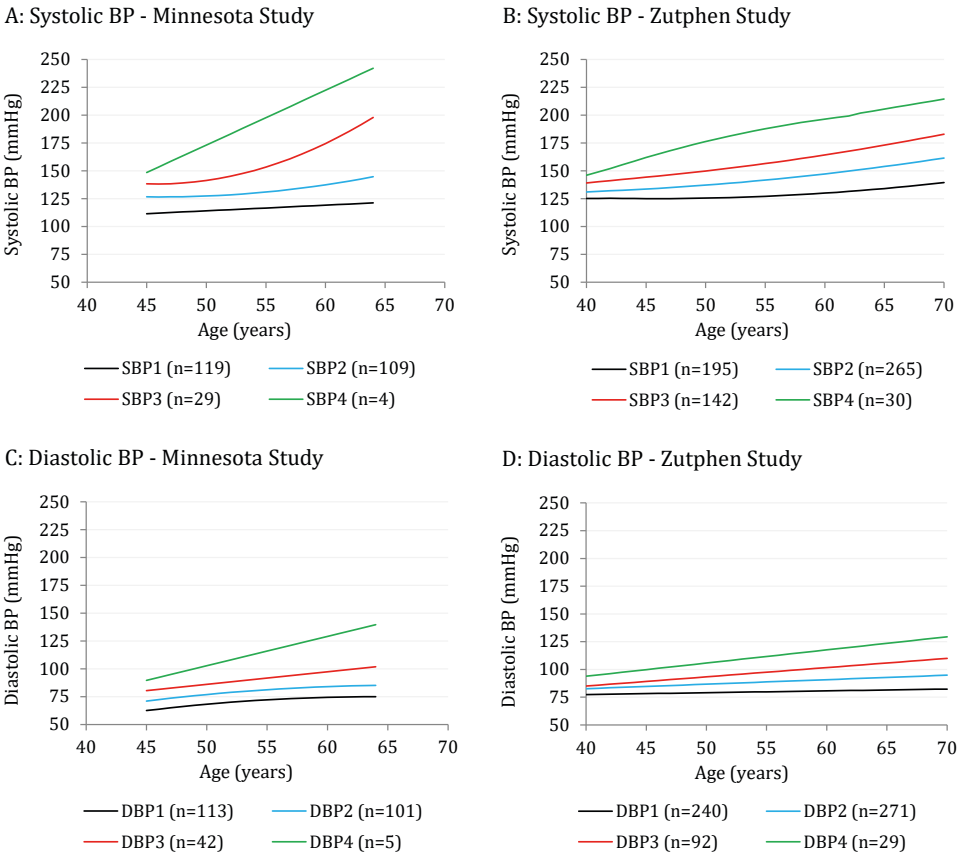

FIGURE 2. Trajectories of systolic (A-B) and diastolic (C-D) BP in 261 American men participating in the Minnesota Study and 632 Dutch men participating in the Zutphen Study.

TABLE 2. Hazard ratios (95% CI) for risk of CVD mortality per trajectory of systolic and diastolic BP in the Minnesota Study and the Zutphen Study

	Minnesota Study (n=261)				Zutphen Study (n=632)			
	N	Unadjusted	Age-adjusted[a]	Fully-adjusted[b,c]	N	Unadjusted	Age-adjusted[a]	Fully-adjusted[b,c]
N cases CVD mortality (%)			137 (52.5%)				282 (44.6%)	
Sum of person-years			5448				11912	
N cases/1000 person-years			25.1				23.7	
Systolic BP trajectories								
SBP1	119	Reference	Reference	Reference	195	Reference	Reference	Reference
SBP2	109	1.68 (1.16 to 2.43)	1.87 (1.28 to 2.72)	1.82 (1.25 to 2.66)	265	1.15 (0.86 to 1.54)	1.38 (1.03 to 1.86)	1.34 (1.00 to 1.81)
SBP3	29	3.11 (1.82 to 5.30)	3.91 (2.26 to 6.74)	3.80 (2.18 to 6.64)	142	1.56 (1.13 to 2.15)	2.13 (1.53 to 2.97)	2.05 (1.47 to 2.87)
SBP4	4	4.35 (1.35 to 14.04)	2.99 (0.92 to 9.75)	3.95 (1.17 to 13.38)	30	1.97 (1.15 to 3.39)	3.06 (1.76 to 5.33)	3.05 (1.74 to 5.33)
Diastolic BP trajectories								
DBP1	113	Reference	Reference	Reference	240	Reference	Reference	Reference
DBP2	101	1.13 (0.78 to 1.65)	1.33 (0.90 to 1.95)	1.34 (0.90 to 1.98)	271	1.19 (0.91 to 1.56)	1.39 (1.06 to 1.82)	1.35 (1.02 to 1.78)
DBP3	42	1.63 (1.01 to 2.64)	2.09 (1.27 to 3.43)	2.10 (1.26 to 3.49)	92	1.16 (0.80 to 1.66)	1.69 (1.16 to 2.46)	1.66 (1.14 to 2.43)
DBP4	5	3.67 (1.32 to 10.17)	3.10 (1.11 to 8.65)	4.07 (1.42 to 11.63)	29	2.17 (1.31 to 3.61)	3.61 (2.13 to 6.10)	3.68 (2.16 to 6.26)

CVD indicates cardiovascular disease; DBP, diastolic blood pressure; SBP, systolic blood pressure.

[a] Adjusted for age.

[b] Adjusted for age, serum cholesterol, smoking status, and diabetes mellitus.

[c] Missing values for serum cholesterol at baseline were replaced by serum cholesterol levels measured at the consecutive year.

TABLE 3. Hazard ratios[a] (95% CI) for CVD mortality risk per single BP unit difference[b] in the Minnesota Study and Zutphen Study

	Minnesota Study (n=261)			Zutphen Study (n=632)		
	Single BP[c]	Average BP[d]	Usual BP[e]	Single BP[c]	Average BP[d]	Usual BP[e]
N cases CVD mortality (%)	137 (52.5%)			282 (44.6%)		
N cases/1000 person-years	25.1			23.7		
Baseline systolic BP						
Per 10 mmHg	1.17 (1.06 to 1.28)	1.17 (1.08 to 1.27)	1.22	1.12 (1.06 to 1.19)	1.24 (1.15 to 1.34)	1.17
Per 25 mmHg	1.47 (1.16 to 1.86)	1.49 (1.22 to 1.82)	1.62	1.34 (1.16 to 1.54)	1.72 (1.43 to 2.08)	1.50
Per 50 mmHg	2.16 (1.35 to 3.45)	2.22 (1.49 to 3.33)	2.64	1.78 (1.34 to 2.38)	2.96 (2.03 to 4.32)	2.23
Baseline diastolic BP						
Per 10 mmHg	1.18 (1.02 to 1.37)	1.29 (1.10 to 1.50)	1.28	1.14 (1.03 to 1.26)	1.40 (1.20 to 1.62)	1.24
Per 15 mmHg	1.28 (1.03 to 1.60)	1.46 (1.16 to 1.83)	1.44	1.21 (1.04 to 1.41)	1.65 (1.32 to 2.07)	1.37
Per 30 mmHg	1.64 (1.06 to 2.55)	2.12 (2.12 to 3.37)	2.08	1.47 (1.08 to 1.99)	2.73 (1.74 to 4.27)	1.89

[a] Adjusted for age, serum cholesterol, smoking status, and diabetes mellitus. Missing values for serum cholesterol at baseline were replaced by serum cholesterol levels measured at the consecutive year; [b] Differences in single BP level are similar to the differences in BP level between the reference trajectory (SBP1) and other BP trajectories at age 50, which is the mean age at baseline; [c] Defined as BP level measured at baseline; [d] Defined as the average of all available BP levels during the 10-year period; [d] Defined as single BP adjusted for regression dilution. Adjustment factors were 1.26 for systolic and 1.48 for diastolic BP in the Minnesota Study and 1.39 for systolic and 1.65 for diastolic BP in the Zutphen Study.

initial BP levels. Mean systolic BP of the 4 trajectories increased by 5 to 49 mmHg in Minnesota and 5 to 20 in Zutphen from age 50 to 60. For diastolic BP, these 10-year increases were 6 to 26 mmHg in Minnesota and 2 to 12 mmHg in Zutphen.

During a mean (±SD) follow-up of 20.9±10.0 years, all Minnesota men had died, of whom 137 (52.5%) died from CVD. During a mean follow-up of 18.8±9.6 years, 623 Zutphen men had died (98.1%), of whom 282 (44.6%) died from CVD. In both cohorts, CVD mortality rate was ~25 per 1000 person-years and all-cause mortality rate was ~50 per 1000 person-years. Mean age at death was 79.7±9.5 years in Minnesota and 78.3±8.8 years in Zutphen.

Systolic BP was directly associated with CVD mortality (Tables 2 and 3). This association was more pronounced in Minnesota than in Zutphen. After adjustment, the third systolic BP trajectory, SBP3, was associated with a CVD mortality risk ratio of 3.8 (95% CI: 2.2 to 6.6) in Minnesota and 2.1 (95% CI: 1.5 to 2.9) in Zutphen, compared to the first trajectory (Table 2). For SBP3, hazard ratios were significantly higher in Minnesota than in Zutphen (P=0.03) (Table 4). Each unit difference of 25 mmHg systolic BP was associated with a CVD mortality risk ranging from 1.5 for single BP to 1.6 for usual BP in Minnesota and from 1.3 for single BP to 1.7 for average BP in Zutphen (Table 3). Similar patterns were found for diastolic BP. In addition to CVD mortality, systolic BP was directly associated with all-cause mortality (Tables 5 and 6). BP trajectories were the strongest predictors of CVD and all-cause mortality in Minnesota, whereas in Zutphen, average BP was the strongest predictor (Table 7).

Systolic BP was directly associated with life years lost (Tables 8 and 9). This association was more pronounced in Minnesota than in Zutphen. After adjustment, the number of life years lost associated with SBP3 was 8.1 (95% CI: 4.4 to 11.8) years in Minnesota and 3.7 (95% CI: 1.8 to 5.6) years in Zutphen (Table 8). For SBP3, the number of life years lost were significantly higher in Minnesota than in Zutphen (P=0.01) (Table 3). The number of life years lost associated with each 25-mmHg difference in systolic BP ranged from 2.9 for single BP to 3.9 for average BP in Minnesota and from 1.7 for single BP to 2.9 for average BP in Zutphen (Table 9). Similar patterns were found for diastolic BP.

TABLE 4. Comparison of associations of systolic BP trajectories with CVD mortality, all-cause mortality and life years lost between Minnesota and Zutphen

	Minnesota Study	Zutphen Study	Z-score	P-value
CVD mortality [hazard ratio (95% CI)]				
SBP2	1.82 (1.25 to 2.66)	1.34 (1.00 to 1.81)	-1.27	0.10
SBP3	3.80 (2.18 to 6.64)	2.05 (1.47 to 2.87)	-1.87	0.03
SBP4	3.95 (1.17 to 13.38)	3.05 (1.74 to 5.33)	-0.40	0.34
All-cause mortality [hazard ratio (95% CI)]				
SBP2	1.44 (1.10 to 1.88)	1.12 (0.93 to 1.36)	-1.47	0.07
SBP3	2.48 (1.62 to 3.79)	1.54 (1.23 to 1.93)	-1.93	0.03
SBP4	2.45 (0.88 to 6.86)	2.44 (1.64 to 3.62)	-0.01	0.50
Number of life years lost [years (95% CI)]				
SBP2	3.06 (0.68 to 5.43)	1.07 (-0.53 to 2.67)	-1.62	0.05
SBP3	8.10 (4.39 to 11.82)	3.68 (1.80 to 5.56)	-2.32	0.01
SBP4	12.75 (3.74 to 21.77)	6.42 (3.11 to 9.74)	-1.38	0.08

CVD indicates cardiovascular disease; SBP, systolic blood pressure.

TABLE 5. Hazard ratios (95% CI) for risk of all-cause mortality per trajectory of systolic and diastolic BP in the Minnesota Study and the Zutphen Study

	Minnesota Study (n=261)				Zutphen Study (n=632)			
	N	Unadjusted	Age-adjusted*	Fully-adjusted†‡	N	Unadjusted	Age-adjusted*	Fully-adjusted†‡
N cases all-cause mortality (%)			261 (100%)				620 (98.1%)	
Sum of person-years			5448				11912	
N cases/1000 person-years			47.9				52.0	
Systolic BP trajectories								
SBP1	119	Reference	Reference	Reference	195	Reference	Reference	Reference
SBP2	109	1.34 (1.03 to 1.74)	1.48 (1.14 to 1.93)	1.44 (1.10 to 1.88)	265	0.93 (0.77 to 1.12)	1.12 (0.93 to 1.36)	1.12 (0.93 to 1.36)
SBP3	29	2.14 (1.42 to 3.23)	3.91 (1.73 to 4.01)	2.48 (1.62 to 3.79)	142	1.15 (0.92 to 1.43)	1.57 (1.25 to 1.96)	1.54 (1.23 to 1.93)
SBP4	4	2.71 (1.00 to 7.36)	1.91 (0.70 to 5.24)	2.45 (0.88 to 6.86)	30	1.47 (1.00 to 2.17)	2.32 (1.56 to 3.43)	2.44 (1.64 to 3.62)
Diastolic BP trajectories								
DBP1	113	Reference	Reference	Reference	240	Reference	Reference	Reference
DBP2	101	1.08 (0.83 to 1.42)	1.26 (0.96 to 1.66)	1.24 (0.94 to 1.64)	271	0.91 (0.77 to 1.09)	1.05 (0.88 to 1.26)	1.04 (0.87 to 1.25)
DBP3	42	1.44 (1.01 to 2.06)	1.83 (1.27 to 2.64)	1.75 (1.21 to 2.55)	92	0.85 (0.67 to 1.09)	1.24 (0.96 to 1.60)	1.22 (0.95 to 1.58)
DBP4	5	2.42 (0.99 to 5.95)	2.05 (0.83 to 5.05)	2.55 (1.02 to 6.38)	29	1.36 (0.93 to 2.01)	2.24 (1.51 to 3.33)	2.38 (1.59 to 3.54)

DBP indicates diastolic blood pressure; SBP, systolic blood pressure.

*Adjusted for age. †Adjusted for age, serum cholesterol, smoking status, and diabetes mellitus. ‡Missing values for serum cholesterol at baseline were replaced by serum cholesterol levels measured at the consecutive year.

TABLE 6. Hazard ratios* (95% CI) for all-cause mortality risk per single BP unit difference† in the Minnesota Study and Zutphen Study

	Minnesota Study (n=261)			Zutphen Study (n=632)		
	Single BP‡	Average BP§	Usual BP|	Single BP‡	Average BP§	Usual BP|
N cases CVD mortality (%)		261 (100%)			620 (98.1%)	
N cases/1000 person-years		47.9			52.0	
Baseline systolic BP						
Per 10 mmHg	1.10 (1.02 to 1.18)	1.13 (1.06 to 1.20)	1.13	1.08 (1.04 to 1.13)	1.16 (1.10 to 1.23)	1.11
Per 25 mmHg	1.26 (1.05 to 1.52)	1.34 (1.14 to 1.58)	1.34	1.21 (1.09 to 1.35)	1.46 (1.27 to 1.68)	1.30
Per 50 mmHg	1.60 (1.11 to 2.30)	1.80 (1.31 to 2.48)	1.81	1.47 (1.19 to 1.82)	1.71 (1.24 to 2.37)	1.71
Baseline diastolic BP						
Per 10 mmHg	1.14 (1.02 to 1.27)	1.23 (1.09 to 1.38)	1.21	1.08 (1.01 to 1.16)	1.20 (1.08 to 1.33)	1.14
Per 15 mmHg	1.21 (1.03 to 1.42)	1.36 (1.14 to 1.62)	1.33	1.12 (1.01 to 1.24)	1.31 (1.11 to 1.54)	1.21
Per 30 mmHg	1.47 (1.06 to 2.03)	1.86 (1.31 to 2.64)	1.77	1.25 (1.01 to 1.55)	1.71 (1.24 to 2.37)	1.45

* Adjusted for age, serum cholesterol, smoking status, and diabetes mellitus. Missing values for serum cholesterol at baseline were replaced by serum cholesterol levels measured at the consecutive year. † Differences in single BP level are similar to the differences in BP level between the reference trajectory (SBP1) and other BP trajectories at age 50, which is the mean age at baseline. ‡ Defined as BP level measured at baseline. § Defined as the average of all available BP levels during the 10-year period. | Defined as single BP adjusted for regression dilution. Adjustment factors were 1.26 for systolic and 1.48 for diastolic BP in the Minnesota Study and 1.39 for systolic and 1.65 for diastolic BP in the Zutphen Study.

TABLE 7. Comparison of the Akaike Information Criterion[a], a measure of the model fit, for each proportional hazards model of systolic BP[b]

	Minnesota Study	Zutphen Study
CVD mortality		
Single systolic BP	1251	3126
Average systolic BP	1248	3111
Systolic BP trajectories	1239	3119
All-cause mortality		
Single systolic BP	2379	6761
Average systolic BP	2374	6747
Systolic BP trajectories	2370	6750

[a] The lower the Akaike Information Criterion (AIC), the better the model fit. [b] The AIC was not obtained for usual BP, because for usual BP single BP was adjusted for regression dilution.

DISCUSSION

In 1 extinct cohort of American middle-aged men and 1 nearly extinct cohort of Dutch middle-aged men, 10-year BP trajectories were identified that were strong predictors of CVD mortality, all-cause mortality, and life years. Compared to other BP measures, 10-year BP trajectories were the strongest predictors of mortality in Minnesota. In Zutphen, however, average BP was the strongest predictor of mortality. The third systolic BP trajectory was associated with 4-times greater CVD mortality risk in Minnesota and 2-times greater risk in Zutphen, compared to the first trajectory. In both cohorts, these trajectories were associated with a 2-times greater risk of all-cause mortality. The number of life years lost between these trajectories was 8 years in Minnesota and 4 years in Zutphen.

In this study, mean initial BP level in Minnesota was lower than in Zutphen. This finding was confirmed by a study in which BP levels in the Minnesota Study were compared to those in other American cohorts[15]. An explanation for these lower BP levels may be that measured BP in Minnesota was closer to basal BP levels because 3 consecutive measurements of BP separated by 5 minutes each were taken after

a long period of rest. Moreover, the Minnesota Study involved highly educated men who were selected for health, whereas the Zutphen Study involved a random population sample of Zutphen inhabitants.

Although BP levels were lower in Minnesota than in Zutphen, we observed relatively similar BP trajectories in both cohorts. In all 4 trajectories, BP levels increased more rapidly in men with higher initial levels. In Minnesota, these increases were more rapid than in Zutphen. For example, from age 50 through 60, mean systolic BP of the 4 trajectories increased by 5 to 49 mmHg in Minnesota and 5 to 20 mmHg in Zutphen. In the Framingham Heart Study, 4 trajectories were identified in 890 men aged 30 to 84 years with a maximum of 15 BP measurements per person. Systolic BP rose linearly by 5 to 23 mmHg per 10 years within all 4 trajectories[16]. These findings were similar to those observed in Zutphen, but the associations between trajectories and cardiovascular outcomes were not investigated.

The CARDIA study recently demonstrated that higher BP trajectories from young adulthood through middle age were associated with an increased risk of coronary artery calcification, a marker of subclinical atherosclerosis[4]. In our study, higher BP trajectories from middle age through old age were associated with a higher risk of CVD mortality. Since the present study focuses on middle through old age, these findings complement those of the younger CARDIA population. Moreover, the association of BP trajectories with a mortality end point, rather than a marker of CVD, was investigated in the present study. Our findings regarding life years lost are in line with those of the Framingham Heart Study. In that study, normotensive men were expected to live 1.7 years longer than prehypertensive men and 5.1 years longer than hypertensive men[17]. The second trajectory in our study, which is comparable to the prehypertensive group in the Framingham Heart Study, was associated with 1.1 (Zutphen) to 3.1 (Minnesota) life years lost compared with the first trajectories. The third trajectory, which is comparable to the hypertensive group, was associated with 3.7 (Zutphen) to 8.1 (Minnesota) life years lost. Although similar results were observed, it should be noted that life expectancy was the outcome of the Framingham Heart Study, whereas life years lost that are based on the actual age at death was the outcome of the present study.

Due to random-within person variation in BP, the strength of the association between BP obtained on a single occasion and mortality will be underestimated.

TABLE 8. Life years lost (years)[a] (95% CI) per trajectory of systolic and diastolic BP in the Minnesota Study and the Zutphen Study

	Minnesota Study (n=261)				Zutphen Study (n=632)			
	N	Unadjusted	Age-adjusted[b]	Fully-adjusted[c,d]	N	Unadjusted	Age-adjusted[b]	Fully-adjusted[c,d]
Age at death (yr)			79.6 ± 9.6				78.3 ± 8.8	
Systolic BP trajectories								
SBP1	119	Reference	Reference	Reference	195	Reference	Reference	Reference
SBP2	109	3.29 (0.89 to 5.69)	3.35 (0.92 to 5.77)	3.06 (0.68 to 5.43)	265	1.32 (-0.27 to 2.91)	0.97 (-0.63 to 2.57)	1.07 (-0.53 to 2.67)
SBP3	29	8.54 (4.79 to 12.29)	8.61 (4.84 to 12.39)	8.10 (4.39 to 11.82)	142	4.15 (2.29 to 6.01)	3.69 (1.82 to 5.56)	3.68 (1.80 to 5.56)
SBP4	4	11.88 (2.67 to 21.08)	11.96 (2.72 to 21.19)	12.75 (3.74 to 21.77)	30	6.69 (3.39 to 10.00)	6.07 (2.75 to 9.38)	6.42 (3.11 to 9.74)
Diastolic BP trajectories								
DBP1	113	Reference	Reference	Reference	240	Reference	Reference	Reference
DBP2	101	2.28 (-0.24 to 4.79)	2.45 (-0.11 to 5.02)	2.28 (-0.25 to 4.82)	271	0.84 (-0.67 to 2.35)	0.48 (-1.04 to 2.00)	0.55 (-0.97 to 2.07)
DBP3	42	6.01 (2.69 to 9.34)	6.25 (2.86 to 9.65)	5.80 (2.46 to 9.14)	92	2.37 (-0.28 to 4.45)	1.67 (-0.45 to 3.79)	1.77 (-0.35 to 3.89)
DBP4	5	11.08 (2.68 to 19.49)	11.40 (2.94 to 19.86)	11.61 (3.36 to 19.86)	29	6.68 (3.33 to 10.02)	5.84 (2.48 to 9.21)	6.37 (3.00 to 9.75)

[a] The number of life years lost is based on the age at death.[b] Adjusted for age.
[c] Adjusted for age, serum cholesterol, smoking status, and diabetes mellitus.
[d] Missing values for serum cholesterol at baseline were replaced by serum cholesterol levels measured at the consecutive year.

TABLE 9. Life years lost (years)[a,b] (95% CI) per single BP unit difference[c] in the Minnesota Study and Zutphen Study

	Cohort					
	Minnesota Study (n=261)			Zutphen Study (n=632)		
	Single BP[d]	Average BP[e]	Usual BP[f]	Single BP[d]	Average BP[e]	Usual BP[f]
Age at death (years)		79.7 ± 9.5			78.3 ± 8.8	
Baseline systolic BP						
Per 10 mmHg	1.16 (0.42 to 1.89)	1.54 (0.84 to 2.25)	1.46	0.66 (0.29 to 1.03)	1.16 (0.71 to 1.62)	0.92
Per 25 mmHg	2.89 (1.05 to 4.73)	3.86 (2.10 to 5.62)	3.64	1.65 (0.73 to 2.57)	2.91 (1.78 to 4.04)	2.29
Per 50 mmHg	5.79 (2.11 to 9.47)	7.71 (4.19 to 11.23)	7.30	3.30 (1.47 to 5.13)	5.82 (3.56 to 8.08)	4.59
Baseline diastolic BP						
Per 10 mmHg	1.58 (0.54 to 2.62)	2.64 (1.39 to 3.88)	2.34	0.68 (0.10 to 1.27)	1.51 (0.67 to 2.35)	1.12
Per 15 mmHg	2.37 (0.80 to 3.93)	3.95 (2.09 to 5.82)	3.51	1.03 (0.15 to 1.90)	2.26 (1.01 to 3.52)	1.70
Per 30 mmHg	4.73 (1.61 to 7.86)	7.91 (4.18 to 11.63)	7.00	2.05 (0.30 to 3.80)	4.53 (2.02 to 7.04)	3.38

[a] Life years lost are based on the age at death; [b] Adjusted for age, serum cholesterol, smoking status, and diabetes mellitus. Missing values for serum cholesterol at baseline were replaced by serum cholesterol levels measured at the consecutive year; [c] Differences in single BP level are similar to the differences in BP level between the reference trajectory (SBP1) and other BP trajectories at age 50, which is the mean age at baseline; [d] Defined as BP level measured at baseline; [e] Defined as the average of all available BP levels during the 10-year period; [f] Defined as single BP adjusted for regression dilution. Adjustment factors were 1.26 for systolic and 1.48 for diastolic BP in the Minnesota Study and 1.39 for systolic and 1.65 for diastolic BP in the Zutphen Study.

By taking the average BP or adjusting for regression dilution (usual BP), associations with CVD mortality, all-cause mortality and life years lost became stronger compared to single BP. Associations regarding BP trajectories were about twice as strong as compared to single BP. The number of life years lost according to BP trajectories was twice as high in Minnesota than in Zutphen. Moreover, we observed that BP trajectories were stronger predictors of mortality than single, average, and usual BP in Minnesota. In Zutphen, however, average BP was the strongest predictor of mortality. BP was based on 3 recordings with longer time intervals in Minnesota and on 2 recordings with shorter time intervals in Zutphen. Therefore, BP may be measured less accurately in Zutphen than in Minnesota, which has led to less valid BP trajectories in Zutphen. Another explanation could be that BP trajectories in Zutphen are quite linear, which is also captured by average BP. Therefore, the model with a long-term continuous BP variable (average BP) was superior to the model with categorical BP variables (BP trajectories) in terms of model fit. These findings indicate that measuring BP accurately is crucial for estimating the association between BP and mortality.

Cumulative BP levels over time (e.g., patterns of BP) are not taken into account in recommendations for management of hypertension. In individuals younger than 60 years, the current recommendation is to start pharmacologic treatment at BP levels of ≥140 mmHg systolic or ≥90 mmHg diastolic[18]. An individual's BP pattern before reaching this BP cut-off level is not included in these recommendations. Our findings emphasize the importance of taking BP increases during time into account. Increases in BP seem especially important in the group that does not yet reach the treatment cut-off level of 140/90 mmHg. We observed that the first trajectories, in which systolic BP increased from 114 to 121 mmHg in Minnesota and 125 to 130 mmHg in Zutphen from age 50 through 60, were the most favorable in terms of life years lost and risk of CVD and all-cause mortality. Trajectories starting at a systolic BP level of 127 mmHg in Minnesota and 137 mmHg in Zutphen at age 50 and increasing with 10 mmHg until age 60 were associated with a 30% to 80% greater risk of CVD mortality, a 10% to 40% greater risk of all-cause mortality and 1.1 to 3.1 life years lost, compared with the first trajectories. These findings suggest that regularly monitoring BP of middle-aged men who have a BP level between 130 to 140 mmHg is important, as these men are usually not treated but already have an increased risk of CVD mortality. Monitoring BP in this group

provides the opportunity to detect hypertension and prevent CVD earlier in life. Moreover, our findings show that average BP is superior to single BP in terms of mortality prediction. For clinical settings, this implies that a BP level based on multiple annual BP recordings is a stronger predictor than a BP level based on a few recordings at a single moment in time.

A major strength of the present study is that detailed data on BP during a long follow-up period were collected in both cohorts. Almost everyone had died at the end of follow-up, which enabled us to investigate the association of BP trajectories with life years lost. A mean of 10 (out of 11) BP measurements per person was included to characterize 10-year BP trajectories, which indicates that the majority of men had complete BP data. Because cohorts were initiated in a time when only very high levels of BP were treated, it is unlikely that the observed associations are confounded by antihypertensive medication. A drawback of the present study is that the characterization of BP trajectories is complex. It is too time-consuming to use BP trajectories as a predictive tool in clinical settings. However, our findings on average BP and mortality emphasize the importance of repeated BP measurements from middle into old age, which is relevant for clinicians. Another drawback is the small number of participants. Few men were assigned to the fourth BP trajectories, which warrants cautious interpretation for these specific findings. It should be noted that the Minnesota men were all from the upper socioeconomic class in the metropolitan area of Minneapolis, St. Paul, which could limit the generalizability of these findings. However, we observed similar findings in the population-based Zutphen Study, which included more men with large differences in socioeconomic class.

PERSPECTIVES

In two cohorts of American and Dutch middle-aged men, four 10-year BP trajectories were characterized. BP trajectories were stronger predictors of CVD mortality, all-cause mortality, and life years lost than single, average, and usual BP in Minnesota, but not in Zutphen. In general, we observed similar associations within these cohorts, emphasizing the replication of the results of one cohort in the other. To our knowledge, this is the first study that investigated the association

of BP trajectories with CVD mortality, all-cause mortality, and life years lost. The novel approach of using data from cohorts in which participants were physically examined and followed to "extinction" provided a unique opportunity to study the association of risk factors in relation to cardiovascular health and life years lost.

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DISCLOSURES

None.

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CHAPTER 6

Repeated blood pressure measurements in relation to cardiovascular and all-cause mortality in individuals with and without antihypertensive medication: the Rancho Bernardo Study

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ABSTRACT

Background: The predictive value of systolic blood pressure (SBP) for cardiovascular disease (CVD) in individuals using antihypertensive medication has not yet been established in epidemiological studies. We investigated the association of average SBP and SBP trajectories with CVD and all-cause mortality, accounting for antihypertensive medication.

Design: Prospective cohort study.

Methods: Data from 762 participants of the Rancho Bernardo Study were used. Five examinations took place from 1984-2002; mortality data were obtained from 2002-2013. Average SBP was defined as the mean of all available SBP levels between 1984-2002 and SBP trajectories over this period were derived using group-based trajectory modeling. Cox proportional hazards analysis was used to investigate associations of average SBP and SBP trajectories with CVD and all-cause mortality, adjusted for age, sex, plasma cholesterol, smoking, diabetes, and antihypertensive medication. We examined whether antihypertensive medication modified these associations by means of interaction terms in the multivariable models.

Results: Mean baseline age was 65.7 years, and 67% were female. For average SBP, each 20-mmHg increment was associated with a 1.4-times greater CVD mortality risk and 1.2-times all-cause mortality risk. These associations were not modified by antihypertensive medication (p for interaction >0.10). For SBP trajectories, the highest trajectories were associated with a 2-to-3-times greater CVD mortality risk and 1.5-times greater all-cause mortality risk, compared with the lowest trajectory. SBP trajectories were not superior to average SBP in predicting CVD and all-cause mortality.

Conclusions: In the general middle-aged and older population of the Rancho Bernardo study, long-term average SBP levels and trajectories were significant predictors of CVD and all-cause mortality, irrespective of prescribed antihypertensive medication in the 1980s and 1990s.

INTRODUCTION

The association between blood pressure (BP) and cardiovascular disease (CVD) is well established in healthy individuals. Findings of the Prospective Studies Collaboration indicate a 2-times greater risk of vascular mortality per 20-mmHg increment in systolic BP (SBP) level in adults aged 60-69 years when adjusted for regression dilution[1]. In the Minnesota Business and Professional Men Study and Zutphen Study, a 1.5-times greater CVD mortality risk per 25 mmHg was observed after adjustment for regression dilution[2].

Recently, two studies reported findings for age-related changes in BP, as reflected in BP trajectories, that were strongly associated with CVD risk[2,3]. In the CARDIA Study, higher SBP trajectories from young adulthood to middle age were associated with a 2-to-5-times greater risk of coronary artery calcification[3]. Also, higher SBP trajectories from middle age to old age were associated with a 2-times greater CVD mortality risk in the Zutphen Study and a 3-to-4 times greater risk in the Minnesota Business and Professional Men Study[2]. Moreover, higher trajectories were associated with a 2-times greater risk of all-cause mortality in both cohorts, 4 – 6 life years lost in Zutphen and 8 life years lost in Minnesota. The Minnesota and Zutphen Study consisted of healthy men without antihypertensive medication[2]. In the CARDIA Study, BP trajectories were modeled for all individuals, regardless of antihypertensive medication use[3].

The aim of the present study was to investigate the association of long-term SBP with CVD and all-cause mortality in older participants of the Rancho Bernardo Study, taking into account validated antihypertensive medication use. For long-term SBP, the average SBP of all available SBP levels over a median period of 15 years were used and SBP trajectories over this period were modeled. Because SBP is superior to DBP in predicting CVD in older individuals[1,4], the present study focuses on SBP in relation to CVD and all-cause mortality.

METHODS

Study design

The Rancho Bernardo Study is an ongoing, prospective, community-based study

of the epidemiology of CVD and other chronic diseases. Between 1972 and 1974, 4717 male and female Rancho Bernardo residents aged 40-79 years (82% of initially invited) of Rancho Bernardo, CA, USA, were enrolled. During 1984-1987, the baseline visit of the present study, 2480 of the 3431 surviving participants of the original cohort returned for a follow-up visit. During a median period of 15 years (from 1984/1987 until 1999/2002), five clinical examinations took place. Mortality data were collected up to 2013. All participants provided written informed consent. The study protocol was approved by the Human Subjects Protections Program at the University of California, San Diego, La Jolla, CA, USA.

Population for analysis

In order to identify SBP trajectories over a 15-year period and to prospectively relate these trajectories to mortality, we excluded participants who died before Jan 1st, 2000, from this analysis (n=1101). Forty-eight were excluded because they were younger than 50 years at baseline, 6 for missing data on plasma cholesterol level, and 3 for unknown smoking status. We excluded 268 participants with prevalent CVD or CVD medication use (other than antihypertensive medication) between 1984/1987 (baseline visit) and 1999/2002 (fifth visit). Moreover, 292 participants with less than 2 BP recordings over time were excluded. In total, 2834 BP recordings from 342 untreated and 420 treated participants were available during 1984-2002, leaving 762 participants for analysis.

Blood pressure and covariates

At all examination rounds, BP was measured in fasting subjects according to the Hypertension Detection and Follow-up Program (HDFP) protocol[5]. This protocol requires that the participant had been seated quietly for at least five minutes, when BP was measured twice at least one minute apart using a mercury sphygmomanometer. The mean of these two measurements was used. Medical histories and information about smoking status were obtained using questionnaires developed by the Rancho Bernardo Research Study. Height and weight were measured with participants wearing light clothing and no shoes, from which body mass index was calculated (weight divided by height squared). Total plasma cholesterol was measured by enzymatic techniques using an ABA-200 biochromatic analyzer (Abbott Laboratories, Irving, TX). Plasma glucose was

determined using a glucose oxidase method. Current medication use was verified by prescriptions, pills, or containers brought to the clinic. Diabetes mellitus was defined by fasting plasma glucose, oral glucose tolerance test, reported physician diagnosis, or use of insulin or diabetes-specific medication. CVD history was defined at each visit as heart attack, angioplasty, bypass, stroke, or transient ischemic attack.

Mortality outcomes

All participants were followed up to January 2013 for vital status using annual mailed questionnaires. Mortality data from 1999 to 2013 were used in the present study. Death certificates were coded by a certified nosologist using the International Classification of Diseases, Ninth Revision (ICD-9). For CVD mortality, ICD-9 codes 390-459 were used.

Statistical analysis

Average SBP was defined as the mean of all available SBP levels over a period of 15 years. Cox proportional hazards analysis was used to investigate the association of average SBP with CVD and all-cause mortality. Average SBP was analyzed per 20-mmHg increment. Analyses with average SBP were performed for all participants, regardless of antihypertensive medication use, and stratified by antihypertensive medication use. For individuals who used antihypertensive medication, BP values were taken into account from the time point when antihypertensive medication use started. Effect modification by antihypertensive medication and sex was tested by including antihypertensive medication×SBP and sex×SBP interaction terms in the models. Associations were adjusted for age, sex, plasma cholesterol, smoking status, diabetes, and antihypertensive medication (if applicable).

SBP trajectories over a period of 15 years were identified by using latent mixture modeling within the PROC TRAJ procedure in SAS[6]. A minimum of 2 and a maximum of 5 BP measurements per person were available. Trajectories were derived by modeling SBP as a function of age and based on significant linear and quadratic terms. Due to limited power, it was not possible to stratify trajectories for antihypertensive medication. The optimal number of trajectories was selected by comparing the Bayesian Information Criterion for each number of trajectories. Cox proportional hazards analysis was used to investigate the association of SBP

trajectories with CVD and all-cause mortality. SBP trajectories were included as dummy variables in the model, and the trajectory with the lowest initial SBP level was used as reference. Effect modification by antihypertensive medication was tested by including antihypertensive medication×SBP interaction terms in the models. Associations were adjusted for age, sex, plasma cholesterol, smoking status, diabetes, and antihypertensive medication. The Akaike Information Criterion of proportional hazards models of average SBP and SBP trajectories were compared to investigate the best model fit.

Analyses were performed using SAS version 9.3 (SAS Institute, Inc.). A two-sided P-value of <0.05 was considered statistically significant.

RESULTS

At baseline, mean (SD) age of participants was 65.7 (8.4) years; 66.9% were female. Mean baseline BP in individuals without BP medication (untreated) was 126/74 mmHg and 136/78 mmHg in treated individuals (Table 1). From 1984 to 2002, mean BP rose by 14/4 mmHg in untreated individuals and by 11/0 mmHg in treated individuals. Mean plasma cholesterol level was higher at baseline than at visit 5 (223 mg/dl vs. 206 mg/dl) and did not differ between untreated and treated individuals. The proportion of smokers was higher in untreated individuals (17% vs. 9% at visit 1), while the prevalence of diabetes mellitus was higher in treated individuals (12% vs. 8% at visit 1). From baseline until visit 5, usage of diuretics and beta blockers decreased, whereas usage of calcium channel blockers increased.

During a median 11.7-year follow-up, 286 participants died; 34.3% of deaths were attributed to CVD. All-cause and CVD mortality rates were higher in treated individuals compared with untreated individuals (Table 2). After adjusting for age, sex, plasma cholesterol level, smoking status, diabetes, and antihypertensive medication, each 20-mmHg increment in average SBP was associated with a hazard ratio (HR) of 1.35 (95% CI: 1.03, 1.77) for CVD mortality and 1.23 (95% CI: 1.07, 1.47) for all-cause mortality. Associations with CVD and all-cause mortality did not differ between treated and untreated individuals (P for interaction >0.40). After adjusting for age, sex, plasma cholesterol level, smoking status, and diabetes, each 20-mmHg increment in average SBP was associated with a HR for CVD mortality

TABLE 1. Characteristics of 762 participants of the Rancho Bernardo Study at the first and fifth visit, stratified by antihypertensive medication use

	1984 – 1987 (first visit)		1999 – 2002 (fifth visit)	
	Untreated	Treated	Untreated	Treated
N	342	420	143	261
Female (%)	230 (67.3%)	280 (66.7%)	91 (63.6%)	160 (61.3%)
Age (years)	65.2 (8.8)	66.0 (8.0)	76.5 (7.5)	78.7 (7.1)
Body mass index (kg/m^2)	24.3 (3.4)[a]	25.4 (3.8)[b]	25.2 (3.8)	26.5 (4.4)
Systolic blood pressure (mmHg)	126.3 (18.0)	136.1 (18.8)	140.1 (19.3)	146.6 (20.4)
Diastolic blood pressure (mmHg)	73.9 (8.4)	77.8 (9.5)	78.3 (8.7)	78.0 (9.4)
Plasma total cholesterol (mg/dl)	222.7 (36.0)	222.9 (38.8)	207.9 (34.9)[c]	205.4 (41.7)
Current smoking (%)	57 (16.7%)	38 (9.1%)	11 (7.7%)	7 (2.7%)
Diabetes mellitus (%)	26 (7.6%)	51 (12.1%)	2 (1.4%)	24 (9.2%)
Antihypertensive medication use (%)	0 (0.0%)[d]	231 (57.2%)[e]	0 (0.0%)	224 (85.5%)
Diuretics (%)	0 (0.0%)[d]	175 (75.8%)[f]	0 (0.0%)	119 (53.1%)[g]
Beta blockers (%)	0 (0.0%)[d]	97 (42.0%)[f]	0 (0.0%)	65 (29.0%)[g]
Calcium channel blockers (%)	0 (0.0%)[d]	8 (3.5%)[f]	0 (0.0%)	79 (35.3%)[g]
Other antihypertensive medication (%)	0 (0.0%)[d]	31 (13.4%)[f]	0 (0.0%)	107 (47.8%)[g]
Lipid-lowering medication (%)	0 (0.0%)[d]	2 (0.5%)§	0 (0.0%)	61 (23.4%)

Data are shown as mean (SD) and number of individuals (percentage).

[a] Data were available for 340 individuals;

[b] Data were available for 419 individuals;

[c] Data were available for 142 individuals;

[d] Data were available for 332 individuals;

[e] Data were available for 404 individuals;

[f] Based on data for 231 individuals with antihypertensive medication at baseline;

[g] Based on data for 224 individuals with antihypertensive medication at visit 5.

TABLE 2. Hazard ratios (95% CI) for CVD and all-cause mortality risk per each 20-mmHg increment in average SBP[a] for 762 participants of the Rancho Bernardo Study, stratified by antihypertensive medication use

	Stratified by antihypertensive medication use		
	Untreated (n=342)	Treated (n=420)	All (n=762)
Average (SD) systolic BP	131.1 (15.2)	140.6 (15.5)	136.0 (16.1)
CVD mortality (N)	37	61	98
Sum of person-time (y)	3427	3866	7293
CVD mortality rate (per 1000 person-y)	10.8	15.8	13.4
Crude HR (95% CI)	1.97 (1.33 – 2.92)	1.78 (1.32 – 2.42)	1.87 (1.49 – 2.36)
Adjusted HR (95% CI)[b]	1.49 (0.94 – 2.36)	1.35 (0.96 – 1.89)	1.35 (1.03 – 1.77)[c]
All-cause mortality (N)	114	172	286
Sum of person-time (y)	3427	3866	7293
All-cause mortality rate (per 1000 person-y)	33.3	44.5	39.2
Crude HR (95% CI)	1.62 (1.28 – 2.04)	1.53 (1.27 – 1.84)	1.58 (1.38 – 1.82)
Adjusted HR (95% CI)[b]	1.24 (0.95 – 1.63)	1.29 (1.05 – 1.58)	1.25 (1.07 – 1.47)[c]

[a] Average SBP was defined as the mean of all available SBP levels during 1984 and 2002.

[b] Adjusted for age, sex, plasma cholesterol, smoking status, and diabetes.

[c] Additionally adjusted for antihypertensive medication use.

of 1.49 (95% CI: 0.94, 2.36) in untreated individuals and 1.35 (95% CI: 0.96, 1.89) in treated individuals (Table 2). For all-cause mortality, HR was 1.24 (95% CI: 0.95, 1.63) in untreated individuals and 1.29 (95% CI: 1.05, 1.58) in treated individuals. A significant interaction between sex and SBP was observed for CVD mortality (P=0.03), but not for all-cause mortality (P=0.23). After adjusting for age, plasma cholesterol level, smoking status, diabetes, and antihypertensive medication, each 20-mmHg increment in average SBP was associated with a HR for CVD mortality of 1.53 (95% CI: 1.12, 2.08) in women and 1.00 (95% CI: 0.56, 1.80) in men (Supplementary Table 1).

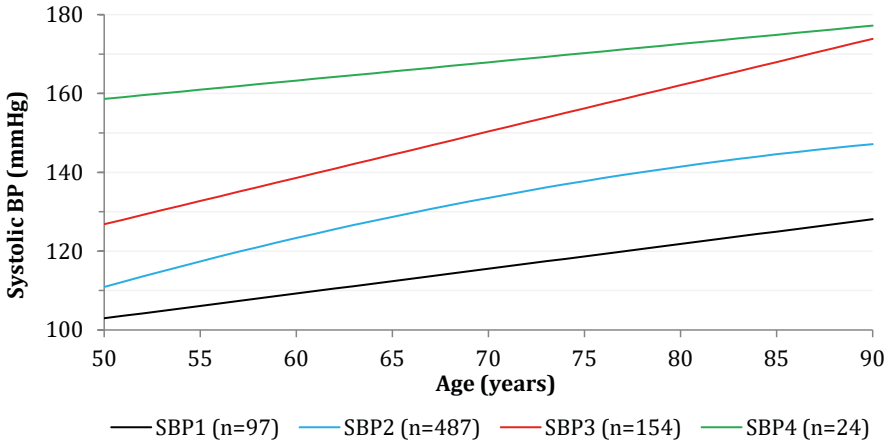

FIGURE 1. SBP trajectories for 762 participants of the Rancho Bernardo Study

TABLE 3. Predicted mean SBP level (95% CI) and slope[a] of each SBP trajectory

		Model parameters		
		SBP at age 65 (mmHg)		Change in SBP
Trajectory	N (%)	Estimate	95% CI	(mmHg/10 years)
SBP1	97 (12.7%)	112.4	109.7, 115.1	6.3
SBP2	487 (63.9%)	128.7	127.0, 130.4	9.1
SBP3	154 (20.2%)	144.5	141.6, 147.3	11.8
SBP4	24 (3.2%)	165.6	160.3, 170.9	4.6

[a] Trajectories are based on significant linear (SBP1, SBP3, and SBP4) and quadratic terms (SBP2).

TABLE 4. Hazard ratios (95% CI) for CVD and all-cause mortality per SBP trajectory in 762 participants of the Rancho Bernardo Study

	SBP trajectories			
	SBP1 (n=97)	SBP2 (n=487)	SBP3 (n=154)	SBP4 (n=24)
CVD mortality (N)	10	57	25	6
Sum of person-time (years)	947	4759	1367	220
CVD mortality rate (per 1000 person-y)	10.6	12.0	18.3	27.3
Model 1	1.00	1.13 (0.57 – 2.21)	1.68 (0.81 – 3.50)	2.60 (0.94 – 7.15)
Model 2[a]	1.00	1.14 (0.58 – 2.24)	2.05 (0.97 – 4.30)	4.17 (1.49 – 11.70)
Model 3[b]	1.00	0.91 (0.46 – 1.80)	1.72 (1.06 – 2.74)	3.34 (1.39 – 7.99)
All-cause mortality (N)	34	168	76	8
Sum of person-time (years)	947	4759	1367	220
All-cause mortality rate (per 1000 person-y)	35.9	35.3	55.6	36.4
Model 1	1.00	0.98 (0.68 – 1.42)	1.51 (1.01 – 2.26)	1.02 (0.47 – 2.20)
Model 2[a]	1.00	0.99 (0.69 – 1.44)	1.73 (1.15 – 2.61)	1.47 (0.67 – 3.19)
Model 3[b]	1.00	0.97 (0.67 – 1.40)	1.62 (1.07 – 2.45)	1.33 (0.61 – 2.91)

[a] Adjusted for age, sex, plasma cholesterol, smoking status, and diabetes.

[b] Additionally adjusted for antihypertensive medication.

Four trajectories of SBP were identified (Figure 1). The two lower trajectories (SBP1 and SBP2) were characterized by an average predicted SBP below 130 mmHg at age 65 and the two higher trajectories (SBP3 and SBP4) by an average predicted SBP above 140 mmHg at age 65 (Table 3). Mean SBP of the four trajectories increased by 5 to 12 mmHg from age 60 to 70. In the first three trajectories, SBP increased more rapidly with higher initial SBP levels. The first and fourth trajectories had similar slopes, despite a 50-mmHg difference in SBP level. Compared with the first trajectory, individuals in the fourth trajectory were about 5 years younger and had a 56-mmHg higher SBP at baseline (P<0.01) (Supplementary Table 2).

SBP trajectories were significantly associated with CVD and all-cause mortality, with the lowest risk in the first two trajectories and the highest risk in the third and fourth trajectories. After adjusting for age, sex, plasma cholesterol level, smoking status, diabetes, and antihypertensive medication, the HR for CVD mortality was 1.72 (95% CI: 1.06, 2.74) for the third trajectory and 3.34 (95% CI: 1.39, 7.99) for the fourth trajectory, compared with the first trajectory (Table 4). For all-cause mortality, a HR of 1.62 (95% CI: 1.07, 2.45) was observed for the third trajectory, compared with the first trajectory. Associations did not differ between treated and untreated individuals (P for interaction>0.10). SBP trajectories were not superior to average SBP in predicting CVD and all-cause mortality (Table 5).

TABLE 5. Comparison of the Akaike Information Criterion*, a measure of the model fit, for each proportional hazards model of systolic BP

	CVD mortality	All-cause mortality
Average systolic BP	1189	3551
Systolic BP trajectories	1188	3550

* The lower the Akaike Information Criterion (AIC), the better the model fit.

DISCUSSION

In 762 participants of the Rancho Bernardo Study, long-term SBP was directly associated with CVD mortality and all-cause mortality. Each 20-mmHg increment in 15-year average SBP was associated with a 35% higher risk of CVD mortality and a 23% higher risk of all-cause mortality. Four SBP trajectories over a 15-year period were identified, which were significantly associated with CVD mortality and all-cause mortality. The strongest associations were observed for CVD mortality, with a 3-times greater risk for the highest trajectory compared with the lowest trajectory. SBP trajectories were not superior to average SBP in predicting CVD and all-cause mortality. Associations of average SBP and SBP trajectories did not differ between individuals with and without antihypertensive medication.

The association between SBP and CVD mortality is well established in healthy individuals. A 2-times greater risk of CVD mortality per 20-mmHg increment in usual SBP in healthy individuals aged 60-69 was reported by the Prospective Studies Collaboration, taking measurement error into account[1]. In the present study, a 20-mmHg increase in average SBP was associated with a 50% higher CVD mortality risk in untreated individuals. Similar findings were observed in those on antihypertensive medication in the 1980s and 1990s, as shown in stratified analyses. Whether current antihypertensive treatment would change the predictive value of SBP for CVD and all-cause mortality warrants further investigation.

Findings of the Minnesota Business and Professional Men Study and the Zutphen Study[2], which consist of men without antihypertensive medication, are in agreement with those of the present study, which consists of men and women with and without antihypertensive medication. Participants were followed from middle age to old age. An increase in average SBP of 20 mmHg was associated with a 27% – 36% higher all cause mortality risk and a 38% – 55% higher CVD mortality risk, similar to what we observed in our study. Moreover, SBP trajectories were strongly associated with CVD and all cause mortality in Minnesota and Zutphen. The higher trajectories were associated with a 2-times higher all-cause mortality risk and a 2-to-4-times higher CVD mortality risk, compared with the lowest trajectory.

In the CARDIA Study of young adults followed to middle age, five distinct SBP trajectories were identified that were strongly associated with coronary

artery calcification[3]. No distinction was made between healthy individuals and those treated for high BP. Findings from the present study suggest that the association between long-term average SBP does not differ between individuals with and without antihypertensive medication and supports the CARDIA analyses combining all individuals and adjusting for antihypertensive medication. Although the CARDIA population consists of young adults, similar findings were observed for older adults in the present study.

In the Prospective Studies Collaboration, similar risks of SBP in relation to ischemic heart disease mortality and stroke mortality were observed[1]. However, in the present study, average SBP was associated with CVD mortality in women only. This discrepancy may be explained by the low number of male CVD mortality cases, resulting in a lack of power to detect an association in men.

In the present study, the largest difference in CVD and all-cause mortality risk was observed when comparing upper with lower SBP trajectories. The two lower trajectories were characterized by an average predicted SBP below 120 mmHg at age 50, followed by an average predicted annual rise in SBP of 0.6 to 0.9 mmHg. In the third trajectory, SBP at age 50 was 127 mmHg and the annual rise was 1.2 mmHg and in the fourth trajectory, SBP at age 50 was 159 mmHg and the annual rise was 0.5 mmHg. These trajectory differences translated into a significant 72% greater risk of CVD and a 62% greater risk of all-cause mortality for the third trajectory and a 3-times greater risk of CVD mortality for the fourth trajectory. Fifty-year-old individuals in the fourth trajectory usually come to medical attention because their SBP exceeds the conventional cut-off value of 140 mmHg. For individuals in the third trajectory with values of ~125 mmHg, however, this is not the case. Monitoring that group and their annual increase in SBP is warranted for CVD prevention.

A major strength of the present study is that we were able to stratify our findings for individuals with and without antihypertensive medication. Also, the availability of extensive and repeated data enabled us to identify SBP trajectories over a 15-year period and to investigate their associations with long-term mortality. However, misclassification of individuals to trajectories cannot be ruled out. The degree of misclassification is probably higher in the present study than in the Minnesota and Zutphen Study. In these two cohorts, a mean of 10.5 (out of 11) BP measurements per person was included to characterize 10-year BP trajectories

compared to a mean of 3.7 (out of 5) BP measurements per person over 15 years in the present study. Mean posterior probabilities of trajectory membership were 0.84 in the present study and 0.95 in Minnesota and Zutphen, supporting the higher chance of misclassification. A limitation of our study is the small number of individuals, which may especially limit the interpretation of the results for the stratified analysis. Also, our findings are not applicable to other ethnic and socioeconomic groups, because nearly all Rancho Bernardo Study participants were white, mainly middle-class, and had health insurance.

In conclusion, average SBP and trajectories over a 15-year period were both significant predictors of CVD and all-cause mortality. This was observed also in individuals with SBP ~125 mmHg at age 50, and in those who received antihypertensive medication in the 1980s and 1990s. The implications of these findings for CVD prevention warrant further consideration.

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Conflicts of interest

None.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 1. Hazard ratios (95% CI) for CVD and all-cause mortality risk per each 20-mmHg increment in average SBP[a] for 762 participants of the Rancho Bernardo Study, stratified by sex

	Women (n=510)	Men (n=252)
Average systolic BP (SD)	137.3 (16.5)	134.3 (15.1)
CVD mortality (N)	74	24
Sum of person-time (years)	4976	2316
CVD mortality rate (per 1000 person-y)	14.9	10.4
Crude HR (95% CI)	2.09 (1.60 – 2.74)	1.21 (0.71 – 2.05)
Adjusted HR (95% CI)[b,c]	1.53 (1.12 – 2.08)	1.00 (0.56 – 1.80)
All-cause mortality (N)	185	101
Sum of person-time (years)	4976	2316
All-cause mortality rate (per 1000 person-y)	37.2	43.6
Crude HR (95% CI)	1.68 (1.41 – 1.99)	1.46 (1.14 – 1.86)
Adjusted HR (95% CI)[b]	1.32 (1.08 – 1.60)	1.14 (0.87 – 1.51)

[a] Average SBP was defined as the mean of all available SBP levels during 1984 and 2002.

[b] Adjusted for age, plasma cholesterol, smoking status, diabetes, and antihypertensive medication.

[c] A significant interaction term for sex × SBP was observed.

SUPPLEMENTARY TABLE 2. Baseline characteristics per SBP trajectory of 762 participants of the Rancho Bernardo Study

	SBP trajectories			
	SBP1 (n=97)	SBP2 (n=487)	SBP3 (n=154)	SBP4 (n=24)
Female (%)	62 (63.9%)	323 (66.3%)	107 (30.5%)	18 (75.0%)
Age (years)	66.6 (7.8)	65.8 (8.7)	65.2 (8.0)	62.0 (6.9)
Body mass index (kg/m^2)[a]	24.5 (2.7)	24.8 (3.8)	25.2 (3.6)	26.2 (4.9)
Systolic BP (mmHg)	111.9 (11.7)	129.4 (15.1)	145.7 (16.3)	168.0 (19.8)
Diastolic BP (mmHg)	68.5 (6.5)	75.4 (8.2)	81.0 (9.0)	87.9 (11.9)
Plasma total cholesterol (mg/dl)	225.1 (41.7	222.2 (36.9	221.6 (37.0	232.7 (36.3
Current smoking (%)	10 (10.3%)	69 (14.2%)	14 (9.1%)	2 (8.3%)
Diabetes mellitus (%)	3 (3.1%)	49 (10.1%)	22 (14.3%)	3 (12.5%)
Antihypertensive medication (%)[b]	17 (19.1%)	135 (29.5%)	66 (3.4%)	13 (56.5%)
Lipid-lowering medication (%)[b]	0 (0.0%)	1 (0.2%)	1 (0.7%)	0 (0.0%)

Data are shown as mean (SD) and number of individuals (percentage);

[a] Data are based on 759 individuals;

[b] Data are based on 722 individuals.

CHAPTER 7

General discussion

This thesis is centered on blood pressure (BP), a major risk factor for cardiovascular diseases (CVD), with 62% of stroke and 49% of coronary heart disease attributable to suboptimal BP[1]. In the first part (Chapter 2, 3 and 4), the relation of dietary protein intake with BP was examined. In the second part (Chapter 5 and 6), repeated BP measurements were analyzed in relation to CVD and all-cause mortality. This chapter discusses the main findings and their implications.

PROTEIN INTAKE AND BLOOD PRESSURE

Table 1 gives an overview of the findings on dietary protein intake in relation to BP. Total protein intake, estimated from 24-h urinary urea excretion, was not associated with incident hypertension during 9 years of follow-up in ~4000 men and women (Chapter 2). In a cohort of elderly men, repeatedly measured plant protein intake was inversely associated with 5-year BP changes, whereas no associations were observed for total and animal protein (Chapter 3). Results from a meta-analysis, particularly from randomized controlled trials (RCTs), showed a beneficial effect of dietary protein on BP if consumed instead of carbohydrates. No differential effect on BP for animal or plant protein was observed (Chapter 4).

Discussion of the main findings

Total protein intake

Total protein intake was not associated with BP in two prospective cohort studies, the PREVEND Study and the Zutphen Elderly Study. In both cohorts, total protein intake was updated over time because repeated measurements were available. In the PREVEND Study, urea excretion was assessed in two consecutive 24-hour urine collections at baseline and approximately four years later, from which total protein intake was estimated. Incident hypertension was chosen as the main outcome of interest rather than BP change, since BP levels could have been influenced by prescribed antihypertensive medication. In the Zutphen Elderly Study, total protein intake was estimated by a cross-check dietary history method four times during 15 years and linked to 5-year changes in untreated BP. In addition to the findings in these two cohorts, a meta-analysis of three other prospective

TABLE 1. Overview of the main findings on dietary protein and BP described in this thesis

EVIDENCE FROM PROSPECTIVE COHORT STUDIES				
Chapter	Population	Exposure	Outcome	Findings
2	3997 Dutch men and women	Total protein intake estimated from 24-h urinary urea excretion	9-year incident hypertension	Non-significant hazard ratios ranging from 0.75 to 0.83 in quintiles of protein intake vs. bottom quintile (P for trend: 0.52).
3	272 elderly Dutch men	Total, animal and plant protein intake	5-year BP change	No associations were observed of total and animal protein with BP. The higher-intake tertiles of plant protein intake were significantly associated with a mean 5-year BP change of -2.9 mmHg systolic and -1.7 mmHg diastolic.
4	Meta-analysis of 11 761 individuals from 4 prospective cohort studies	Total, animal and plant protein intake	Incident hypertension	Non-significant pooled hazard ratios ranging from 0.96 to 0.99 per 25 gram increase in total, animal and plant protein intake.
EVIDENCE FROM RANDOMIZED CONTROLLED TRIALS (RCTs)				
Chapter	Population	Intervention vs. control	Outcome	Findings
4	Meta-analysis of 1208 individuals from 14 RCTs	Protein intake (total, animal and plant) vs. carbohydrate intake	BP response	The pooled systolic BP effect was -2.1 mmHg for a weighed mean contrast in total protein intake of 41 grams per day. No differential BP effect was observed for animal and plant protein.

cohort studies[2-4] showed no association between total protein intake and incident hypertension. For these different approaches on analyzing total protein in relation to BP in this thesis, no associations were observed.

Animal and plant protein intake

Findings in the Zutphen Elderly Study and a meta-analysis of four prospective cohort studies[2-5] showed no association between animal protein intake and BP. No differentiation was made among types of animal protein, e.g. from dairy or meat. For instance, dairy protein was not associated with hypertension in a Dutch prospective cohort study[6]. For high meat protein intake, however, a greater hypertension risk was observed in participants aged ≥70 years, but not in middle-aged individuals[6]. Evidence on animal protein from different sources in relation to BP is scarce and further research is needed to draw conclusions.

Findings for plant protein intake were inconsistent in the studies described in this thesis. In the Zutphen Elderly Study, plant protein intake was inversely associated with 5-year BP changes. However, plant protein intake was not associated with incident hypertension in the meta-analysis of prospective cohort studies[2-5]. No differentiation was made among types of plant protein, e.g. from grains or soy products.

In the meta-analysis of RCTs[7-12], plant protein and animal protein were not differentially associated with BP. An explanation for the inverse association between plant protein intake and BP in Zutphen may be that individuals with a high plant protein intake have a healthier diet than those with a high animal protein intake. Therefore, plant protein intake may be a proxy for an overall healthy dietary pattern and lifestyle. Although the associations in the Zutphen Elderly Study were adjusted for dietary and lifestyle variables such as intake of dietary fiber and potassium, smoking, alcohol use and physical activity, residual confounding of the observed associations cannot be ruled out.

Substitution of macronutrients

While prospective studies showed no association between total protein and BP in this thesis, the meta-analysis of RCTs[7-20] showed that substituting carbohydrates for protein significantly reduced BP. In the OmniHeart Study, the BP effect of three diets with a different macronutrient composition was investigated[20]. After six

weeks, the protein-rich diet reduced BP compared with a carbohydrate-rich diet, but not compared with a diet rich in monounsaturated fat. In another cross-over trial, supplementation of both soy protein and milk protein reduced BP compared with carbohydrates[8]. These findings suggest that a decreased carbohydrate intake, rather than an increased protein intake, may be beneficial for BP. A recent meta-analysis of prospective studies showed an increased risk of hypertension for sugar-sweetened beverages[21] which would support a BP raising effect of simple carbohydrates. The role of these and other types of carbohydrates in BP control could have public health implications, and warrants further investigation.

Discrepancies in the protein-BP relationship were observed when comparing prospective cohort studies and RCTs for which there may be various explanations. In RCTs, the BP effect after altered protein intake was relative to a fixed amount of fat or carbohydrates under isocaloric conditions. In prospective studies, however, it was not clear which macronutrients are exchanged. Also, BP estimates were expressed per 41 grams in RCTs and per 25 grams in prospective cohort studies. The mean study duration of RCTs in the meta-analysis was ~20 weeks, in comparison to multiple years of follow-up in prospective studies. The type of intervention, that is, increase in protein intake either by diet or supplements, did not influence the BP effect in the meta-analysis.

LONG-TERM BLOOD PRESSURE AND MORTALITY

An overview of findings on repeated BP measurements in relation to mortality is given in Table 2. In cohorts of American and Dutch middle-aged men, four 10-year BP trajectories were identified before 1970 (Chapter 5). In the Minnesota Study, systolic BP trajectories were stronger predictors of CVD mortality, all-cause mortality, and life years lost than single, average, and usual systolic BP. In the Zutphen Study, average systolic BP was superior to systolic BP trajectories in predicting the different mortality outcomes. In middle-to-old-aged participants of the Rancho Bernardo Study, average systolic BP and systolic BP trajectories were both strong predictors of CVD and all-cause mortality (Chapter 6). These associations were not modified by antihypertensive treatment use in the 1980s and 1990s. In the three cohorts described in these chapters, systolic BP trajectories had little added value in predicting mortality compared to average systolic BP.

TABLE 2. Overview of the main findings on repeated BP with CVD and all-cause mortality in this thesis

BEFORE ANTIHYPERTENSIVE MEDICATION WAS INTRODUCED					
Chapter	Population	Data collection	BP measures	Outcome	Findings
5	261 American men, aged 45 – 55 years	1947–1957: 11 BP recordings 1957–2003: Mortality data	Single BP Average BP Usual BP BP trajectories	All-cause and CVD mortality Life years lost	Each 25-mmHg increase in average SBP was associated with a 49% greater CVD mortality risk, 34% greater all-cause mortality risk and 4 life years lost. Four trajectories were identified in which systolic BP increased by 5 to 49 mmHg between age 50 and 60. A 4-times greater CVD mortality risk, 2-times greater all-cause mortality risk and 8 life years lost were observed when comparing trajectories. Ten-year BP trajectories were the strongest predictors of mortality and life years lost, among different BP measures.
5	632 Dutch men, aged 40 – 60 years	1960–1970: 11 BP recordings 1970–2010: Mortality data	Single BP Average BP Usual BP BP trajectories	All-cause and CVD mortality Life years lost	Each 25-mmHg increase in average SBP was associated with a 72% greater CVD mortality risk, 46% greater all-cause mortality risk and 3 life years lost. Four trajectories were identified in which systolic BP increased by 5 to 20 mmHg between age 50 and 60. A 2-times greater CVD mortality risk, 2-times greater all-cause mortality risk and 4 life years lost were observed when comparing trajectories. Average BP was superior to ten-year BP trajectories in predicting mortality and life years lost.

AFTER ANTIHYPERTENSIVE MEDICATION WAS INTRODUCED					
Chapter	Population	Data collection	BP measures	Outcome	Findings
6	762 American men and women, aged ≥50 years	1984–2002: 5 BP recordings 2002–2013: Mortality data	Average BP BP trajectories	All-cause and CVD mortality	Each 20-mmHg increase in average SBP was associated with a 35% greater CVD mortality risk and a 25% greater all-cause mortality risk. Four trajectories were identified in which systolic BP increased by 5 to 12 mmHg between age 60 and 70. A 2-3-times greater CVD mortality and a 1.5-times greater all-cause mortality risk was observed when comparing BP trajectories. Long-term average SBP levels and trajectories were both significant predictors of CVD and all-cause mortality, irrespective of prescribed antihypertensive medication in the 1980s and 1990s.

Discussion of the main findings

Measured BP levels are liable to random errors, due to imperfections in BP recordings and biological fluctuations in individuals[22]. This intra-individual variation will lead to an underestimation of the true BP-CVD association, also known as regression dilution bias[23]. Two replicate BP measurements can be used to estimate the amount of error in a single BP measurement, and can be used to adjust for regression dilution. In the Minnesota Study and the Zutphen Study, the error in a single BP measurement was estimated. Adjustment factors were 1.3 for systolic and 1.5 for diastolic BP in Minnesota and 1.4 for systolic and 1.7 for diastolic BP in Zutphen. In the Prospective Studies Collaboration and the Seven Countries Study, adjustment factors were 1.6 for systolic and between 1.7 and 2.1 for diastolic BP[24,25]. The fact that adjustment factors were higher for diastolic BP than for systolic BP suggests that a single measurement of diastolic BP is accompanied by more random error than systolic BP in epidemiological studies, under field conditions. Moreover, systolic BP is superior to diastolic BP in predicting CVD in middle-aged to older individuals in epidemiological studies[24,26]. Therefore, in this thesis the focus is on systolic rather than diastolic BP.

Systolic blood pressure trajectories

In three long-term prospective cohort studies that were carried out in different time periods, the associations of systolic BP trajectories with CVD and all-cause mortality were investigated. In middle-aged men, BP trajectories based on an average of 10.5 readings per person were characterized over a 10-year period in the Minnesota and Zutphen Study. Antihypertensive medication was not common at that time. In the Rancho Bernardo Study, which started 25-40 years later, BP trajectories were based on an average of 3.5 readings over a 15-year period in middle-to-old-age men and women, 55% of whom were treated with antihypertensive medication. In the Zutphen Study and the Rancho Bernardo Study, BP trajectories were not superior to average systolic BP in predicting CVD and all-cause mortality. In the Minnesota Study, however, the associations of BP trajectories with mortality outcomes were twice as strong as for average BP. This may be explained by the higher accuracy of BP assessment in Minnesota than in the other studies. BP was recorded three times at each occasion with intervals of five minutes in Minnesota, compared to two recordings at shorter intervals in

Zutphen and Rancho Bernardo. Consequently, BP trajectories were probably better characterized in the Minnesota Study, with less misclassification of individuals.

In all three cohort studies, the optimal number of BP trajectories obtained by statistical modeling was four. This was based on the model fit (using the Bayesian Information Criterion), on the significance of the trajectory parameters (intercept and slope), and on the number of individuals per trajectory. By characterizing BP trajectories, individuals are forced into subgroups that are assumed to follow a similar pattern of BP over time. BP trajectories were identified in relatively small cohorts, consisting of 261 (Minnesota), 632 (Zutphen) and 762 (Rancho Bernardo) individuals. These sample sizes of the cohort studies could have limited the differentiation of BP trajectories. Few individuals were assigned to the highest-level BP trajectories, which warrants cautious interpretation for these specific findings. If samples sizes would have been larger, probably more BP trajectories could be identified. This leads to more precision and consequently, a higher predictive value for CVD and all-cause mortality.

Public health implications

BP trajectories in epidemiological studies provide information on the development of BP over time in population subgroups. Findings of this thesis, however, suggest that systolic BP trajectories have little added value in predicting mortality. Average and usual BP (defined as single BP adjusted for regression dilution) were both strong predictors of CVD and all-cause mortality and superior to single BP. These two measures can be easily obtained in a study in which repeated BP measurements in all or part of the subjects are available over time. Although the number of BP measurements differed between studies, similar associations of average systolic BP with CVD and all-cause mortality were observed. These findings suggest that a few BP measurements during a specified time interval (for example 3-4 BP measurements per person as observed in the Rancho Bernardo Study) are sufficient to provide a valid estimate of average BP. Therefore, if repeated measurements of BP are available in prospective cohort studies, usual BP and average BP are the preferred predictors. Of these two predictors, average BP is the most practical tool, because computing usual BP requires extra statistical modeling. If repeated BP measurements are available for only subgroup of the population, usual BP is the preferred predictor.

Findings of this thesis underline the importance of taking age-related BP change into account. In individuals younger than 60 years, the current recommendation is to start pharmacologic treatment at BP levels of ≥140 mmHg systolic or ≥90 mmHg diastolic[27]. Findings from the trajectory analyses demonstrated again that increases in BP are important before reaching the treatment cut-off level of 140/90 mmHg. In the cohorts of Minnesota and Zutphen, an estimated 1 to 3 life years were lost among those with a systolic BP level of ~130 mmHg at age 50, which increased to ~140 mmHg at age 60. Therefore, these findings emphasize the importance of treating BP in the pre-hypertensive range by means of healthy diet and lifestyle.

In the Rancho Bernardo Study, average BP was a strong predictor of CVD and all-cause mortality, irrespective of antihypertensive medication use. However, these findings refer to a period when the first-generation antihypertensive drugs were used. Future research is needed on BP as a predictor of CVD and all-cause mortality in current settings, with individuals receiving state-of-the-art antihypertensive treatment.

CONCLUSIONS

In this thesis, various approaches were used to study the relation between protein intake and BP. Findings from individual studies and a meta-analysis suggest that dietary protein *per se* does not affect BP within the range of intake generally consumed in the Netherlands. Replacing carbohydrates by protein, however, has a beneficial effect on BP.

Moreover, this thesis showed that BP trajectories are not superior to average BP in predicting CVD and all-cause mortality. A few repeated BP measurements, e.g. three or four, are likely to be sufficient for obtaining a reliable average BP and had a similar predictive value for mortality compared to BP trajectories. Therefore, average BP can be considered the most practical tool for estimating mortality risk.

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ENGLISH SUMMARY

Cardiovascular diseases (CVD) are the main cause of death worldwide. In 2012, about 17.5 million people died from CVD, accounting for 30% of all deaths. High blood pressure (BP) is a major cardiovascular risk factor, which was responsible for 10.4 million deaths in 2013. Diet and lifestyle play an important role in the etiology of hypertension. Maintenance of a desirable body weight, physical activity, and low intake of alcohol and salt are well-known measures to avoid high BP. Whether dietary protein, or more specifically plant and animal protein, could contribute to maintaining a healthy BP is less clear. The association between BP and CVD mortality has been extensively investigated. BP in prospective studies can be analyzed using different approaches, such as single BP (measured at one moment in time), single BP adjusted for regression dilution, average BP, and trajectories of BP. It is not yet clear which of these approaches is to be preferred for CVD risk prediction.

This thesis is centered on BP as a major cardiovascular risk factor. In the first part (Chapter 2, 3 and 4), the relation of dietary protein intake with BP level and change was examined. In the second part (Chapter 5 and 6), various approaches for analyzing repeated BP measurements were compared in relation to CVD and all-cause mortality risk. The final chapter discusses the main findings and their implications.

Chapter 2 describes the association of 24-h urinary urea excretion, as a biomarker of total protein intake, with 9-year incidence of hypertension. We analyzed data of ~4000 men and women aged 28–75 years, who participated in the Prevention of Renal and Vascular Endstage Disease (PREVEND) study, a prospective cohort study. BP was measured four times during 1997–2009 and participants were followed for hypertension incidence, defined as BP ≥140/90 mmHg or use of antihypertensive medication. Urea excretion was assessed in two consecutive 24-h urine collections at baseline and approximately 4 years later, from which total protein intake was estimated. Protein intake based on 24-h urinary urea excretion was not associated with incident hypertension.

Chapter 3 presents findings for long-term total, animal and plant protein intake in relation to 5-year BP change. Analyses were based on 702 observations of 272 men who participated in the Zutphen Elderly Study. Participants did not use

antihypertensive medication and were initially free of CVD. Physical and dietary examinations were performed in 1985, 1990, 1995, and 2000. BP was measured twice at each examination and protein intake was assessed using the cross-check dietary history method. The upper tertiles of plant protein intake were associated with a mean 5-year change in systolic BP of -2.9 mmHg (95% CI: -5.6, -0.2), compared with the bottom tertile. Total and animal protein intake was not associated with BP.

Chapter 4 describes a meta-analysis of 12 observational studies and 17 randomized controlled trials (RCTs) of dietary protein, including animal and plant protein, in relation to BP. Protein intake in prospective cohort studies was not associated with incident hypertension. For RCTs that used carbohydrate as a control treatment, the pooled BP effect was -2.1 mmHg systolic (95% CI: -2.9, -1.4) for a weighted mean contrast in protein intake of 41 grams per day. There was no differential effect of animal and plant protein on BP.

Chapter 5 describes repeated BP measures and their association with CVD and all-cause mortality and life years lost in two prospective and nearly extinct cohorts of middle-aged men, the Minnesota Business and Professional Men Study (n=261) and the Zutphen Study (n=632). BP was measured annually during 1947–1957 in Minnesota and 1960–1970 in Zutphen. After 10 years of BP measurements, men were followed until death on average 20 years later. Each 25-mmHg increase in average SBP was associated with a 49% to 72% greater CVD mortality risk, 34% to 46% greater all-cause mortality risk and 3 to 4 life years lost. Four systolic BP trajectories were identified, in which mean systolic BP increased by 5 to 49 mmHg in Minnesota and 5 to 20 mmHg in Zutphen between age 50 and 60. In Zutphen, a 2-times greater CVD and all-cause mortality risk and 4 life years lost were observed when comparing trajectories. In Minnesota, associations were twice as strong. BP trajectories were the strongest predictors of CVD mortality and life years lost in Minnesota men, whereas in Zutphen men, the average BP was superior to other measures.

Chapter 6 presents findings for average BP and BP trajectories in relation to CVD and all-cause mortality, taking into account antihypertensive medication. A total

of 762 participants aged ≥50 years of the Rancho Bernardo Study were examined five times from 1984 to 2002 and monitored for causespecific mortality from 2002 to 2013. Each 20-mmHg increment in average systolic BP was associated with 35% greater CVD mortality and 25% greater all-cause mortality risk. We identified four trajectories for systolic BP for which BP increases ranged from 5 to 12 mmHg between age 60 and 70. In individuals who belonged to the higher trajectories, 2-3-times greater CVD mortality and 1.5-times greater all-cause mortality risks were observed, compared to those who belonged to the lowest trajectory. Long-term systolic BP trajectories and average systolic BP were both significant predictors of CVD and all-cause mortality. The associations were not modified by antihypertensive medication.

As described in **Chapter 7**, various approaches were used to study the relation between protein intake and BP. Findings from individual studies and a meta-analysis suggest that dietary protein *per se* does not affect BP within the range of intake generally consumed in the Netherlands. Replacing carbohydrates by protein, however, has a beneficial effect on BP.

Moreover, this thesis showed that BP trajectories are not superior to average BP in predicting CVD and all-cause mortality. A few repeated BP measurements, e.g. three or four, are likely to be sufficient for obtaining a reliable average BP and had a similar predictive value for mortality compared to BP trajectories. Therefore, average BP can be considered the most practical tool for estimating mortality risk.

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ABOUT THE AUTHOR

CURRICULUM VITAE

Susanne Tielemans was born on August 5, 1986 in Veghel, the Netherlands. After completing secondary school at Zwijsen College in Veghel in 2004, she started studying Nutrition and Dietetics at the Hogeschool van Arnhem en Nijmegen in 2005. She obtained her Bachelor's degree in 2009 and continued her education in Nutrition and Health at Wageningen University. In 2011, Susanne completed her MSc thesis entitled: "Evaluation of the performance of a food frequency questionnaire and urinary biomarkers as measures of protein and amino acid intake". Thereafter, she was an intern at the Department of Epidemiology and Public Health of University College London where she investigated the association between physical activity and cardiovascular disease and all-cause mortality in patients with type 1 diabetes mellitus. In 2011, Susanne obtained her Master's degree with a specialization in Nutritional Epidemiology and Public Health. After graduating, she started working as a junior researcher at the Division of Human Nutrition of Wageningen University. In 2012, she started her PhD training in dietary protein, blood pressure and mortality at the Division of Human Nutrition of Wageningen University. During her PhD training, Susanne was involved in teaching at the BSc and MSc level and chaired the PhD committee of the Division of Human Nutrition. She attended several (international) conferences and courses within the educational programme of VLAG. Moreover, she was selected as a participant in the 45th Ten-Day International Teaching Seminar on Cardiovascular Disease Epidemiology and Prevention in Incheon, South Korea. Currently, Susanne is working as a postdoctoral researcher at the Centre for Infectious Diseases, Epidemiology and Surveillance of the National Institute for Public Health and the Environment (RIVM).

LIST OF PUBLICATIONS

Tielemans SMAJ, Geleijnse JM, Laughlin GA, Boshuizen HC, Barrett-Connor E, Kromhout D. Repeated blood pressure measurements in relation to cardiovascular and all-cause mortality in individuals with and without antihypertensive medication: the Rancho Bernardo Study. Submitted

van Hees NJM, Giltay EJ, **Tielemans SMAJ**, Geleijnse JM, Puvill T, Janssen N, van der Does W. Essential amino acids in the gluten-free diet and serum in relation to depression in patients with celiac disease. PLoS ONE 10(4): e0122619

Tielemans SMAJ, Geleijnse JM, Menotti A, Boshuizen HC, Soedamah-Muthu SS, Jacobs Jr DR, Blackburn H, Kromhout D. Ten-year blood pressure trajectories, cardiovascular mortality and life years lost in two extinction cohorts: the Minnesota Business and Professional Men and the Zutphen Study. J Am Heart Assoc;2015:4:e001378

Tielemans SMAJ, Kromhout D, Altorf–van der Kuil W, Geleijnse JM. Associations of plant and animal protein with 5-year changes in blood pressure: the Zutphen Elderly Study. Nutr Metab Cardiovasc Dis 2014;24:1228-1233

Tielemans SMAJ, Geleijnse JM, van Baak MA, Engberink MF, Brink EJ, de Jong PE, Gansevoort RT, Bakker SJL. Twenty-four hour urinary urea excretion and 9-year risk of hypertension: the PREVEND study. J Hypertens 2013; 31:1564-1569

Soedamah-Muthu SS, De Neve M, Shelton NJ, **Tielemans SMAJ**, Stamatakis E. Joint associations of alcohol consumption and physical activity with all-cause and cardiovascular mortality. Am J Cardiol 2013;112:380-386

Tielemans SMAJ, Altorf–van der Kuil W, Engberink MF, Brink EJ, van Baak MA, Bakker SJL, Geleijnse JM. Intake of total, plant and animal protein in relation to blood pressure: a meta-analysis of observational and intervention studies. J Hum Hypertens 2013;27:564-571

Tielemans SMAJ, Soedamah-Muthu SS, De Neve M, Toeller M, Chaturvedi N, Fuller JH, Stamatakis E. Association of physical activity with all-cause mortality and incident and prevalent cardiovascular disease among patients with type 1 diabetes: the EURODIAB Prospective Complications Study. Diabetologia 2013;56(1):82-91

Published abstracts

Geleijnse JM, **Tielemans SMAJ**, GA Laughlin, HC Boshuizen, E Barrett-Connor, D Kromhout. Repeated Blood Pressure Measurements in Relation to Cardiovascular and All-Cause Mortality in Individuals with and without Antihypertensive Medication: The Rancho Bernardo Study. Circulation 2016; 133 (Suppl 1): AP188

Mertens E, **Tielemans SMAJ**, Soedamah-Muthu SS, Geleijnse JM. Pulse pressure trajectories in relation to cardiovascular mortality and dietary protein intake: the Zutphen Study. Proceedings of the Nutrition Societ 2015; 74 (OCE5): E346

Tielemans SMAJ, Geleijnse JM, Boshuizen HC, Soedamah-Muthu SS, Menotti A, Jacobs Jr DR, Blackburn H, Kromhout D. Ten-year blood pressure trajectories and long-term risk of cardiovascular mortality: the Minnesota Business and Professional Men Study. Circulation 2014; 129 (Suppl 1): AP044.

Tielemans SMAJ, Geleijnse JM, van Baak MA, Engberink MF, Brink EJ, de Jong PE, Gansevoort RT, Bakker SJL. Twenty-four hour urinary urea excretion and 9-year risk of hypertension: the PREVEND study. J Hypertens 2013; 31: e-Supplement A, ESH 2013 Abstract Book, e10.

Tielemans SMAJ, Kromhout D, Altorf-van der Kuil W, Geleijnse JM. Animal and plant protein and change in blood pressure during 15 years of follow-up: the Zutphen Elderly Study. Circulation 2013; 127: AP403.

Tielemans SMAJ, Altorf-van der Kuil W, Engberink MF, Brink EJ, van Baak MA, Bakker SJL, Geleijnse JM. Abstract P089: Total, plant and animal protein intake and blood pressure: a meta-analysis of observational and intervention studies. Circulation 2013; 127: AP089.

Soedamah-Muthu SS, **Tielemans SMAJ**, Stamatakis E. The association between physical activity and cardiovascular disease and all-cause mortality in patients with type 1 diabetes mellitus: The EURODIAB Prospective Complications Study. Nederlands Tijdschrift voor Diabetologie 2011; 9 (3).

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities

- European Congress of Epidemiology, The Netherlands Epidemiology Society (in collaboration with the International Epidemiological Association & European Epidemiology Federation), Maastricht, The Netherlands, 2015
- Epidemiology and Prevention | Nutrition, Physical Activity and Metabolism – Scientific Sessions, American Heart Association, San Francisco, USA, 2014
- 45th Ten-Day International Teaching Seminar on Cardiovascular Disease Epidemiology and Prevention, International Society of Cardiovascular Disease Epidemiology and Prevention, Incheon, South Korea, 2013
- 23rd European Meeting on Hypertension & Cardiovascular Protection, European Society of Hypertension, Milan, Italy, 2013
- Epidemiology and Prevention | Nutrition, Physical Activity and Metabolism – Scientific Sessions, American Heart Association, New Orleans, USA, 2013
- Master Class in Longitudinal Data Analysis (Mixed Models), Graduate School VLAG, Wageningen, The Netherlands, 2013
- Annual meeting of NWO Nutrition, Deurne, The Netherlands, 2012
- Survival Analysis, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2012
- Regression Analysis, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2012
- Introduction to Global Public Health, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2012
- Topics in Meta-Analysis, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2012

General courses

- Career Orientation, Wageningen Graduate Schools, Wageningen, The Netherlands, 2014
- Philosophy and Ethics of Food Science and Technology, Graduate Schools, Wageningen, The Netherlands, 2014
- Techniques for Writing and Presenting Scientific Papers, Wageningen Graduate Schools, Wageningen, The Netherlands, 2013
- Scientific Writing, Wageningen Graduate Schools, Wageningen, The Netherlands, 2013

Optionals

- Epi-research meetings, methodology club meetings and staff seminars, Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands, 2012 – 2015
- PhD tour, Division of Human Nutrition, Melbourne/Sydney, Australia, 2013
- Preparation of PhD research proposal, Wageningen University. Wageningen, The Netherlands, 2012

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