



Published in final edited form as:

Hypertension. 2009 October ; 54(4): 751–755. doi:10.1161/HYPERTENSIONAHA.109.135582.

DIET-DEPENDENT NET ACID LOAD AND RISK OF INCIDENT HYPERTENSION IN US WOMEN

Luxia Zhang^{1,2,3}, Gary C. Curhan^{1,2}, and John P. Forman^{1,2}

¹Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

²Renal Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

³Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China

Abstract

Animal and human studies suggest a potential link between acid-base status and blood pressure. Contemporary Western diets yield a daily systemic acid load of varying amounts, yet the association with hypertension has never been explored. We prospectively examined the association between the diet-dependent net acid load (also known as the estimated net endogenous acid production) and the risk of incident hypertension among 87 293 women without a history of hypertension in Nurses' Health Study II. We also used the ratio of animal protein intake to potassium intake as an alternative evaluation of diet-dependent net acid load. We identified 15 385 incident cases of hypertension during 995 239 person-years of follow-up. After adjusting for potential confounders, women in the top decile of estimated diet-dependent net acid load had an increased risk of hypertension (relative risk 1.14, 95% confidence interval 1.05–1.24; P for trend = 0.01) compared with women in the bottom decile. In order to test whether the association between estimated diet-dependent net acid load and hypertension is independent of its individual components, additional adjustment for intakes of protein and potassium was made and resulted in a relative risk of 1.23 (95% confidence interval 1.08–1.41, P for trend=0.003) for the top decile of estimated diet-dependent net acid load. Results of the ratio of animal protein intake to potassium intake were similar with those of estimated diet-depend net acid load. In conclusion, a high diet-dependent net acid load is independently associated with a higher risk of incident hypertension.

Keywords

Acid-base equilibrium; hypertension; risk factors; epidemiology; human

Hypertension is the most important modifiable cardiovascular risk factor, contributing to one half of the coronary heart disease and approximately two thirds of the cerebrovascular disease burdens¹. Accordingly, there has been a growing emphasis in national practice guidelines on the importance of preventing hypertension to reduce the public health burden of cardiovascular disease². The National Health and Nutrition Examination Survey (NHANES) 1999–2000 data estimated that 65 million individuals in the United States had hypertension, and that number

Corresponding author Luxia Zhang M.D. Channing Laboratory/Renal Division Brigham and Women's Hospital 181 Longwood Avenue Boston, MA 02115 Telephone number: 617-525-2037 Fax number: 617-525-2008 nhlzh@channing.harvard.edu.

Conflict of Interest None

was increasing³. Therefore, identifying risk factors for hypertension could lead to specific preventive interventions that may favorably affect public health.

Animal and human studies suggest a potential link between acid-base status and blood pressure (BP)^{4–8}. For example, spontaneously hypertensive rats have lower serum bicarbonate concentrations and lower blood pH compared to control normotensive rats; these acid-base abnormalities precede the development of hypertension^{5, 6}. This perturbation of acid-base status may arise from increased metabolic acid production⁷. Lower renal bicarbonate excretion after administration of sodium citrate was also observed in salt-sensitive men, if compared with salt-resistant men⁴. More recent population-based studies are also consistent with the hypothesis that increased endogenous acid production results in higher BP^{9–11}.

Contemporary Western diets contain acid precursors in excess of base precursors, which leads to chronic, low-grade metabolic acidosis¹². This diet-dependent net acid load, which is called “estimated net endogenous acid production (NEAP)”¹³, is known to be associated with diseases of bone mineralization¹⁴. However, its association with hypertension has never been explored. We prospectively examined the association between NEAP and the risk of incident hypertension among 87 293 women without history of hypertension in the Nurses' Health Study II (NHS II).

Methods

Study Population

The NHS II is an ongoing prospective cohort study of 116 430 female registered nurses that began in 1989. Participants are followed via biennial questionnaires that gather information on health-related behaviors and medical events. The follow-up for the cohort exceeded 90% through 2005. This study was approved by the institutional review board at Brigham and Women's Hospital. Receipt of each questionnaire implies participant's consent.

Women were excluded from this analysis if they died before 1991, had prevalent hypertension at baseline, were using blood pressure-lowering medications at baseline but did not report a history of hypertension, or had cancer (except for non-melanoma skin cancer) at baseline. The final study sample included 87 293 women.

Assessment of Diet-Dependent Net Acid Load

Semi-quantitative food frequency questionnaires (FFQ) were used to measure dietary intake and were completed in 1991, 1995, 1999 and 2003. Nutrient intakes were calculated by multiplying the frequency of intake by the nutrient content of the specified portion. Nutrient contents were obtained from the Harvard University food consumption database, which was derived from US Department of Agriculture, manufacturers, and published reports. The reproducibility and validity of the questionnaire by women in a similar cohort (Nurses' Health Study I) has been documented^{15, 16}, and a similar questionnaire has been shown to be valid and reproducible in men¹⁷.

The diet-dependent net acid load was estimated by the formula described by Frassetto et al¹⁸: Estimated NEAP (mEq/day) = [54.5×protein (g/day)/potassium (mEq/day)] – 10.2

Based on work by Zwart et al¹⁹, we also used the ratio of animal protein intake to potassium intake (AP/K) as an alternative evaluation of diet-dependent net acid load:

AP/K = animal protein (g/day)/potassium (g/day).

The intake of protein, animal protein and potassium for the calculation were all adjusted for total energy intake by the residual method²⁰. Potassium from supplements was not included in the calculation, because the vast majority was potassium chloride which does not contribute to dietary-dependant acid load. Information on dietary intake of protein, animal protein and potassium was updated every 4 years as participants returned subsequent FFQs.

Assessment of Other Covariates

Body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), physical activity (metabolic equivalent tasks), smoking status and information on oral contraceptive use were ascertained on the 1991 questionnaire and updated every 4 years. Intakes of alcohol, sodium, calcium, magnesium, folate and vegetable protein were ascertained and updated from the FFQ. Except for intake of alcohol, nutrient values were adjusted for total energy intake by the residual method²⁰.

Assessment of Hypertension

The baseline and biennial follow-up questionnaires inquired about physician-diagnosed hypertension and the year of diagnosis. Self-reported hypertension was found to be highly reliable in a similar cohort of nurses²¹. To validate hypertension self-report in NHS II, we obtained relevant medical records from a sub-set of randomly selected NHS II participants who self-reported a new diagnosis of hypertension on the 2005 biennial questionnaire, as well as randomly selected participants who denied this diagnosis in 2005 and in every prior year. The sensitivity of self-reported hypertension was 94%. The specificity of a nurse reporting no diagnosis of hypertension was 85% (unpublished data). Additionally, self-reported hypertension was predictive of subsequent cardiovascular events²¹. A participant was considered to have prevalent hypertension if she reported this diagnosis on any questionnaire up to and including the 1991 questionnaire, and therefore was excluded from the study. Incident cases included individuals who first reported hypertension on subsequent questionnaires and whose year of diagnosis was after the return of the 1991 questionnaires.

Statistical Analyses

Participants who did not return the 1991 questionnaire for this study were allowed to contribute person-time for later time intervals. Person-time was truncated at the date of hypertension diagnosis, at the date of death, at the date of cancer diagnosis (except for non-melanoma skin cancer), at the first date of anti-hypertensive medication initiation in the absence of hypertension, or June 2005, whichever came first.

The estimated NEAP and AP/K were analyzed in deciles; deciles 2–3, deciles 4–5, deciles 6–7 and deciles 8–9 were merged because the distribution ranges were very narrow. Cox proportional hazards regression models²² were used to estimate relative risks (RRs) and 95% confidence intervals (CIs). Multivariable models were constructed to adjust for potential confounding variables that have been previously associated with incident hypertension (age [continuous], BMI [6 categories], smoking status [past, current, never], family history of hypertension [yes/no], current oral contraceptive use [yes/no], physical activity [quintiles], and intakes of alcohol [6 categories], sodium, calcium, magnesium and folate [quintiles]). Intake of vegetable protein (quintiles) was added to multivariable models of AP/K. To test whether estimated NEAP and AP/K (which are essentially interaction terms of dietary protein and potassium intake) influence hypertension risk independent of their individual components, we also adjusted our estimated NEAP-hypertension models for protein and potassium (both in quintiles), and our AP/K-hypertension models for animal protein and potassium (both in quintiles). We determined P values for trend for each of the exposures of interest by using the median for each category.

We also investigated whether the association between diet-dependent net acid load and hypertension varied according to age (<36 yrs or ≥36 yrs, the cohort median at baseline) and BMI (<25 kg/m² or ≥25 kg/m²). Stratified multivariable analyses were performed, and appropriate interaction terms were generated to test whether interactions were statistically significant.

All P values are 2-tailed. Statistical tests were performed using SAS version 9.1 for UNIX statistical software package (SAS Institute Inc, Gary, NC).

Results

Over the 14 years (995 239 person-years of follow-up), 15 385 incident cases of physician-diagnosed hypertension were reported (15.5 cases per 1000 person-years). Participant characteristics by categories of estimated NEAP are presented in Table 1. Women with higher estimated NEAP had higher BMI, were less physically active, and had lower intakes of alcohol, folate and magnesium. Past and current smoking were more common in lower estimated NEAP categories. Oral contraceptive use was more frequent among the higher categories of estimated NEAP.

After controlling for age, BMI, physical activity and potassium intake, NEAP is positively correlated with protein intake (correlation coefficient=0.96, P<0.001). And NEAP is negatively correlated with potassium intake (correlation coefficient=-0.96, P<0.001) after controlling for age, BMI, physical activity and protein intake.

Estimated NEAP was positively associated with the risk of incident hypertension in age-adjusted and multivariable-adjusted analyses (Table 2). Compared with those in the lowest decile of estimated NEAP, the multivariable RR of incident hypertension for those in the highest decile of estimated NEAP was 1.14 (95% CI 1.05–1.24; P for trend = 0.01). Additional adjustment for intakes of protein and potassium resulted in a RR of 1.23 (95% CI 1.08–1.41, P for trend=0.003) for the top decile of estimated NEAP.

The Cox proportional hazards models for AP/K showed similar results (Table 3). The multivariable RR comparing those in the top decile with those in the bottom decile was 1.15 (95% CI 1.06–1.25; P for trend = 0.003). After adding intakes of animal protein and potassium to the model, the RR was 1.27 (95% CI 1.09–1.47, P for trend <0.001) for the top decile of AP/K.

The association between estimated NEAP and risk of incident hypertension was greater among women with a BMI less than 25 kg/m² (P value for interaction <0.001). The multivariable RR for the top decile of estimated NEAP was 1.27 (95% CI 1.00–1.62) among those with BMI < 25 kg/m², and was 1.21 (95% CI 1.02–1.43) among those with BMI ≥ 25 kg/m². We did not observe effect modification by age (P value for interaction = 0.88).

Discussion

In our prospective study of 87 293 women followed for 14 years, we found that a higher diet-dependent net acid load was independently associated with the risk of incident hypertension. The association remained significant after controlling for dietary factors such as sodium, magnesium, calcium, folate, protein and potassium. To the best of our knowledge, this is the first prospective study to report an association between diet-dependent net acid load and the risk of incident hypertension.

Evidence from several rat models of hypertension and salt-sensitive humans indicate an association between acid-base status and hypertension. For example, plasma pH and

bicarbonate were lower in spontaneously hypertensive rats than in normotensive rats, and these acid-base abnormalities preceded the development of hypertension^{5, 6}. Metabolic studies demonstrated increased renal net acid excretion in Dahl/Rapp salt-sensitive rats compared with their salt-resistant counterparts, which might be explained by increased endogenous acid production⁷. A metabolic study of 24 normotensive men by Sharma et al.⁴ demonstrated similar findings in humans. The renal bicarbonate excretion after administration of sodium citrate was markedly lower in the salt-sensitive than in the salt-resistant men during both the low-salt and high-salt diets (reduced by 46% and 32%, respectively). Because those men had normal renal function, and because the salt-sensitive men had comparatively lower arterial plasma pH and bicarbonate levels than the salt-resistant men⁸, the authors hypothesized that the decreased renal bicarbonate excretion was a compensation for increased endogenous acid production.

More recent data from population-based studies show that several metabolic indicators of “sub-clinical” metabolic acidosis are associated with increased BP. First, among more than 3000 participants of three large cohort studies, participants with hypocitraturia (<320 mg/day) were 2.5 times more likely to have prevalent hypertension than those without hypocitraturia¹¹. Acid-base status is the dominant regulator of urinary citrate excretion. The lower urinary citrate could result from systemic acidosis, and the associated decrease in the intracellular pH of renal proximal tubular cells leads to increased re-absorption of the filtered citrate²³. Second, a study of 5043 participants from NHANES revealed that every 1 mEq/L increase in the serum anion gap was associated with a significant 0.48 mm Hg higher systolic BP independent of age, gender, BMI and renal function¹⁰. This positive association between serum anion gap and higher blood pressure has also been confirmed in a distinct cohort⁹.

Our study suggests that NEAP estimated from diet is an independent risk factor of incident hypertension. Under ordinary physiological circumstances, composition of the diet is a major determinant of the daily NEAP, which in turn determines the degree of perturbation in systemic acid-base equilibrium¹⁸. The rate of sulfuric acid production from protein metabolism and the rate of bicarbonate generation from metabolism of intestinally absorbed potassium salts of organic acids are major components of the NEAP¹⁸. Thus the diet-dependent net acid load could be predicted from the dietary protein and potassium^{13, 18}, and NEAP is already known to adversely influence bone health in several studies¹⁴. Because the sulfur content of vegetable proteins is much more variable than that of animal proteins²⁴, and because a recent study showed that the ratio of animal protein intake to potassium intake more consistently predicted directional changes in bone markers than the ratio of total protein intake to potassium intake¹⁹, we also used AP/K as an estimation of the diet-dependent net acid load. The results from AP/K were not materially different from results of estimated NEAP. The reason why estimated NEAP and AP/K showed similar results may stem from the lower between-person variability in vegetable protein intake compared with the higher variability in animal protein intake. Furthermore, because animal protein intake is a much larger contributor to total protein than vegetable protein, the estimated NEAP and AP/K were highly correlated ($r=0.95$, $P<0.001$). Along those lines, in the study of Frassetto et al¹⁸, the correlation of urinary sulfate excretion with animal protein content was only marginally stronger than with total protein content ($r = 0.88$ compared with 0.84).

Dietary intake of protein itself has been reported to be associated with blood pressure, and the direction of the association may depend on the amount and the type of protein consumed²⁵. Higher potassium intake has also been associated with reduced BP in some studies^{26, 27}. Since NEAP is essentially an interaction term of dietary protein and potassium intake, we further adjusted for intakes of protein and potassium in our models. The RRs were still significant, suggesting NEAP is associated with risk of hypertension even after controlling for intakes of protein and potassium. The estimated NEAP of the Dietary Approaches to Stop Hypertension

(DASH) diet²⁸ was 34.8 mEq/day, which is close to the median of lowest category of estimated NEAP in present study. Hence, low NEAP might be an alternative mechanism of the BP-lowering effect of the DASH diet.

In addition to Frassetto's equation used in the present study, there are also several other algorithms for estimating NEAP from the composition of the diet¹³. Detailed data of diet composition and/or anthropometric data are needed for those algorithms, which limits their use in population-based studies, and there are no data suggesting the superiority of these algorithms. The Frassetto's equation has been validated by renal net acid excretion, which is one of the best available estimates of NEAP¹³. Importantly, the concept of Frassetto's equation makes it possible to develop simple dietary guidelines for regulating NEAP.

Our study has limitations. First, we relied on self-reported hypertension and did not directly measure the blood pressure of our participants; however, all participants are registered nurses, and we demonstrated that hypertension reporting by participants of this cohort is highly sensitive²¹. Nonetheless, the specificity of hypertension reporting may have resulted in the misclassification of a few truly hypertensive individuals as being non-hypertensive controls; however, such misclassification would tend to diminish the magnitude of the odds ratio. Therefore, our findings may indeed be an underestimate of the true association. Second, despite the validity of our FFQs to compute dietary intake, the instrument is imperfect and may result in some misclassification of estimated NEAP and AP/K. However, this type of misclassification is likely to be random resulting in a RR closer to 1.0; thus, the true RR may in fact be larger than what we report. Third, our population was almost entirely white and exclusively female; thus our results may not be generalizable to other populations. Fourth, we do not have information on renal function, which plays a central role in acid base homeostasis. However, a study in a subset of these participants indicated that the prevalence of renal dysfunction was vanishingly small²⁹. Additionally, we excluded women from our analysis who had hypertension at baseline. For these reasons, we doubt that renal dysfunction was sufficiently prevalent in this population to influence our results. Fifth, we lack data regarding potential metabolic indicators of increased NEAP, such as serum anion gap and bicarbonate. Finally, our study was observational; thus we cannot exclude the possibility of residual confounding.

Perspectives

Our prospective analysis suggests that a higher diet-dependent net acid load is independently associated with an increased risk of incident hypertension. Women eating “typical” American diets are already at increased risk for incident hypertension, and thus these results have substantial public health implications. Our findings should be tested in randomized trials to determine whether dietary interventions to reduce diet-dependent net acid load (e.g. increase intake of foods which supply alkali, such fruits and vegetables; decrease intake of foods which have a high acid load, such as meat and cheeses; and increasing the ratio of potassium to protein in diets) or perhaps treatment with alkalizing supplements could reduce the risk of hypertension.

Acknowledgments

None

Sources of Funding This study was funded by the American Heart Association grant 0535401T, NIH grants HL079929-01 and CA50385, and the Beijing NOVA program from the Beijing Municipal Science and Technology Commission. Additionally this work has been made possible through the International Society of Nephrology-funded Fellowship (LZh).

References

1. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 2003;21:1983–1992. [PubMed: 14597836]
2. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr. Jones DW, Materson BJ, Oparil S, Wright JT Jr. Roccella EJ. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003;42:1206–1252. [PubMed: 14656957]
3. Fields LE, Burt VL, Cutler JA, Hughes J, Roccella EJ, Sorlie P. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. *Hypertension* 2004;44:398–404. [PubMed: 15326093]
4. Sharma AM, Cetto C, Schorr U, Spies KP, Distler A. Renal acid-base excretion in normotensive salt-sensitive humans. *Hypertension* 1993;22:884–890. [PubMed: 8244521]
5. Lucas PA, Lacour B, Comte L, McCarron DA, Druke T. Abnormal parameters of acid-base balance in genetic hypertension. *Kidney Int Suppl* 1988;25:S19–22. [PubMed: 3184610]
6. Lucas PA, Lacour B, McCarron DA, Druke T. Disturbance of acid-base balance in the young spontaneously hypertensive rat. *Clin Sci (Lond)* 1987;73:211–215. [PubMed: 3652625]
7. Battle DC, Sharma AM, Alsheikha MW, Sobrero M, Saleh A, Gutterman C. Renal acid excretion and intracellular pH in salt-sensitive genetic hypertension. *J Clin Invest* 1993;91:2178–2184. [PubMed: 8486783]
8. Sharma AM, Kribben A, Schattenfroh S, Cetto C, Distler A. Salt sensitivity in humans is associated with abnormal acid-base regulation. *Hypertension* 1990;16:407–413. [PubMed: 2210808]
9. Forman JP, Rifas-Shiman SL, Taylor EN, Lane K, Gillman MW. Association between the serum anion gap and blood pressure among patients at Harvard Vanguard Medical Associates. *J Hum Hypertens* 2008;22:122–125. [PubMed: 17855799]
10. Taylor EN, Forman JP, Farwell WR. Serum anion gap and blood pressure in the national health and nutrition examination survey. *Hypertension* 2007;50:320–324. [PubMed: 17562979]
11. Taylor EN, Mount DB, Forman JP, Curhan GC. Association of prevalent hypertension with 24-hour urinary excretion of calcium, citrate, and other factors. *Am J Kidney Dis* 2006;47:780–789. [PubMed: 16632016]
12. Kurtz I, Maher T, Hulter HN, Schambelan M, Sebastian A. Effect of diet on plasma acid-base composition in normal humans. *Kidney Int* 1983;24:670–680. [PubMed: 6663989]
13. Frassetto LA, Lanham-New SA, Macdonald HM, Remer T, Sebastian A, Tucker KL, Tyllavsky FA. Standardizing terminology for estimating the diet-dependent net acid load to the metabolic system. *J Nutr* 2007;137:1491–1492. [PubMed: 17513412]
14. Macdonald HM, New SA, Fraser WD, Campbell MK, Reid DM. Low dietary potassium intakes and high dietary estimates of net endogenous acid production are associated with low bone mineral density in premenopausal women and increased markers of bone resorption in postmenopausal women. *Am J Clin Nutr* 2005;81:923–933. [PubMed: 15817873]
15. Willett, WC. *Nutritional Epidemiology*. Vol. 2nd ed.. Oxford University Press; New York, NY: 1998.
16. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51–65. [PubMed: 4014201]
17. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* 1992;135:1114–1126. [PubMed: 1632423]discussion 1127-1136
18. Frassetto LA, Todd KM, Morris RC Jr. Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr* 1998;68:576–583. [PubMed: 9734733]
19. Zwart SR, Hargens AR, Smith SM. The ratio of animal protein intake to potassium intake is a predictor of bone resorption in space flight analogues and in ambulatory subjects. *Am J Clin Nutr* 2004;80:1058–1065. [PubMed: 15447920]
20. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986;124:17–27. [PubMed: 3521261]

21. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, Hennekens CH, Speizer FE. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol* 1986;123:894–900. [PubMed: 3962971]
22. Cox DR. Regression models and life tables. *Journal of the Royal statistical Society* 1972;34:187–220.
23. Simpson DP. Citrate excretion: a window on renal metabolism. *Am J Physiol* 1983;244:F223–234. [PubMed: 6338740]
24. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc* 1995;95:791–797. [PubMed: 7797810]
25. Obarzanek E, Velletri PA, Cutler JA. Dietary protein and blood pressure. *JAMA* 1996;275:1598–1603. [PubMed: 8622252]
26. Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM. Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension* 2006;47:296–308. [PubMed: 16434724]
27. Adroge HJ, Madias NE. Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med* 2007;356:1966–1978. [PubMed: 17494929]
28. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* 1997;336:1117–1124. [PubMed: 9099655]
29. Forman JP, Fisher ND, Schopick EL, Curhan GC. Higher levels of albuminuria within the normal range predict incident hypertension. *J Am Soc Nephrol* 2008;19:1983–1988. [PubMed: 18579639]

Table 1

Baseline characteristics of the cohort in 1991 by categories of estimated net endogenous acid production*.

Variables	Deciles of estimated NEAP, mEq/d						
	D1	D2-D3	D4-D5	D6-D7	D8-D9	D10	
Age (yrs)	37 (34-41)	37 (33-40)	36 (33-40)	36 (32-39)	35 (32-39)	35 (31-39)	
Body mass index (kg/m ²)	22.1 (20.4-24.6)	22.7 (20.8-25.6)	22.9 (21.0-25.8)	23.2 (21.0-26.5)	23.4 (21.3-27.0)	23.8 (21.4-28.0)	
Alcohol consumption (g/d)	1.1 (0.0-4.8)	1.1 (0.0-4.7)	1.1 (0.0-3.8)	0.9 (0.0-3.1)	0.9 (0.0-2.8)	0.0 (0.0-2.0)	
Physical activity (METs/w)	17.7 (7.0-37.2)	15.0 (6.1-30.4)	13.2 (5.5-27.0)	12.0 (5.0-25.4)	11.0 (4.2-23.2)	9.2 (3.5-20.9)	
Family history of HTN (%)	50.4	49.9	49.9	50.0	50.5	50.3	
Current smoker (%)	16.0	14.1	12.3	11.2	10.0	10.4	
Past smoker (%)	26.6	24.8	23.3	21.1	19.4	16.8	
Current OC use (%)	9.9	9.5	10.5	11.1	12.6	12.8	
Dietary factors (per day)							
Folate (µg)	467 (343-715)	409 (312-650)	380 (293-620)	358 (276-595)	329 (256-564)	286 (221-504)	
Sodium (mg)	2066 (1810-2340)	2137 (1915-2368)	2152 (1946-2372)	2163 (1954-2380)	2160 (1942-2380)	2125 (1879-2380)	
Magnesium (mg)	352 (305-409)	327 (287-374)	314 (276-358)	301 (265-343)	286 (252-327)	262 (228-303)	
Calcium (mg)	826 (659-1022)	851 (685-1072)	861 (690-1104)	848 (677-1092)	823 (651-1063)	750 (593-960)	
Total protein (g)	70.9 (62.4-78.7)	79.9 (72.4-87.4)	85.1 (77.3-92.9)	88.8 (80.6-97.1)	92.8 (84.0-101.7)	98.5 (88.3-109.4)	
Animal protein (g)	45.4 (36.2-53.6)	56.2 (48.5-64.0)	62.3 (54.4-70.3)	66.7 (58.3-75.3)	71.5 (62.6-80.9)	78.5 (68.0-90.1)	
Vegetable protein(g)	25.3 (21.8-29.7)	23.3 (20.5-26.3)	22.4 (19.8-25.1)	21.7 (19.2-24.4)	20.9 (18.3-23.6)	19.5 (16.8-22.4)	
Potassium (mg)	3476 (3118-3853)	3177 (2883-3476)	2999 (2729-3277)	2848 (2583-3109)	2659 (2404-2914)	2384 (2119-2655)	
Estimated NEAP (mEq/d)	34.4 (30.6-36.7)	43.5 (41.3-45.3)	50.1 (48.5-51.6)	56.1 (54.6-57.7)	63.7 (61.5-66.4)	76.0 (72.6-81.6)	

* Data are presented as median (inter-quartile range) or percentages; MET, metabolic equivalent; HTN, hypertension; OC, oral contraceptives; NEAP, estimated net endogenous acid production.

Estimated net endogenous acid production (NEAP) and risk of incident hypertension.

Table 2

Characteristics	Deciles of estimated NEAP							P value for trend
	D1	D2-D3	D4-D5	D6-D7	D8-D9	D10		
Person years	91481	182950	206953	208465	205620	99772		
Number of cases	1056	2406	3071	3340	3631	1881		
Age-adjusted relative risk (95% CI)	1.00 reference	1.19 (1.10-1.27)	1.27 (1.18-1.36)	1.40 (1.30-1.50)	1.59 (1.48-1.71)	1.73 (1.60-1.86)	<0.001	
Multivariable relative risk* (95% CI)	1.00 reference	1.07 (1.00-1.15)	1.08 (1.00-1.16)	1.10 (1.03-1.19)	1.16 (1.08-1.25)	1.14 (1.05-1.24)	0.01	
Multivariable relative risk† (95% CI)	1.00 reference	1.09 (1.01-1.18)	1.12 (1.03-1.21)	1.16 (1.05-1.27)	1.23 (1.10-1.38)	1.23 (1.08-1.41)	0.003	

CI, confidence interval.

* adjusted for age, body mass index (BMI), physical activity, family history of hypertension, smoking status (current/past), current oral contraceptive use, intake of alcohol, intake of sodium, magnesium, calcium and folate.

† adjusted for age, BMI, physical activity, family history of hypertension, smoking status (current/past), current oral contraceptive use, intakes of alcohol, sodium, magnesium, calcium and folate, plus intakes of protein and potassium.

Table 3

The ratio of animal protein intake to potassium intake (AP/K) and risk of incident hypertension.

Characteristics	Deciles of AP/K										P value for trend
	D1 (0.0–15.1)	D2–D3 (15.1–19.2)	D4–D5 (19.2–22.0)	D6–D7 (22.0–25.0)	D8–D9 (25.0–30.0)	D10 (30.0–78.1)					
Person years	101742	202094	200636	198948	196705	95114					
Number of cases	1197	2790	3029	3173	3375	1821					
Age-adjusted relative risk (95% CI)	1.00 reference	1.21 (1.13–1.29)	1.36 (1.27–1.45)	1.47 (1.37–1.57)	1.62 (1.52–1.73)	1.83 (1.70–1.96)					
Multivariable relative risk (95% CI)	1.00 reference	1.06 (0.99–1.13)	1.10 (1.02–1.18)	1.11 (1.03–1.19)	1.13 (1.05–1.22)	1.15 (1.06–1.25)					
Multivariable relative risk [†] (95% CI)	1.00 reference	1.08 (1.00–1.17)	1.14 (1.04–1.25)	1.17 (1.06–1.31)	1.22 (1.08–1.38)	1.27 (1.09–1.47)					

CI, confidence interval.

* adjusted for age, BMI, physical activity, family history of hypertension, smoking status (current/past), current oral contraceptive use, intakes of alcohol, sodium, magnesium, calcium, folate and vegetable protein.

[†] adjusted for age, BMI, physical activity, family history of hypertension, smoking status (current/past), current oral contraceptive use, intake of alcohol, intake of sodium, magnesium, calcium, folate and vegetable protein, plus intake of animal protein and potassium.