European Journal of Cardiovascular Prevention & Rehabilitation
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European Journal of Cardiovascular Prevention & Rehabilitation 2011 18: 656 originally published online 28 February 2011
DOI: 10.1177/1741826710389419

The online version of this article can be found at:
http://cpr.sagepub.com/content/18/4/656

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Original scientific paper

European Journal of Cardiovascular Prevention & Rehabilitation

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European Journal of Cardiovascular Prevention & Rehabilitation
18(4) 656–663

DOI: 10.1177/1741826710389419
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Urinary nitric oxide metabolites and individual blood pressure progression to overt hypertension

Marcus Baumann[1,2,*], Christoph Schmaderer[1,*], Tatiana Kuznetsova[3], Roger Bartholome[2], Jos FM Smits[2], Tom Richart[4], Harry Struijker-Boudier[2] and Jan A Staessen[3]

Abstract

Baseline blood pressure (BP) is the strongest known determinant of progression to hypertension, but for an individualized prediction of the incidence of hypertension, the identification of additional biomarkers is crucial. In animal models of hypertension, renal nitric oxide (NO) handling modifies the systemic BP responses prior to the development of hypertension. This study aimed to evaluate whether urinary NO metabolites (NOx) predict the progression of hypertension in normotensive subjects. Among 62 participants enrolled in the Flemish Study on Environment, Genes and Health Outcomes, we assessed progression to hypertension over 4.6 years. In a case-control design, 49 normotensive subjects including 10 subjects with high-normal blood pressure were enrolled of whom 25 remained normotensive (controls), whereas 24 'progressed' to hypertension (progressors). Thirteen hypertensive patients served as negative controls. Urinary NOx concentration, renal function and the urinary excretion of electrolytes were assessed at baseline and follow-up. At baseline, progressors showed higher BP values than controls and urinary NOx concentration was significantly lower in progressors as compared to the normotensive controls ($p < 0.01$). In all initially normotensive subjects baseline urinary NOx concentration was associated with follow-up BP ($r = -0.55$, $p < 0.001$) and the relative increase of BP over time ($r = -0.47$, $p < 0.001$). In progressors baseline urinary NOx was associated with follow-up BP ($r = -0.52$, $p < 0.009$) and the relative increase of BP over time ($r = -0.44$, $p = 0.033$). Baseline urinary NOx and BP were independent predictors for the relative BP increase. A urinary NOx threshold of <130.5 mg/L predicted 75% of all progressors. In context with high-normal baseline BP, 87.5% of all progressors were identified. These findings indicate that urinary NO metabolites are associated with BP development in normotensive subjects. Moreover, urinary NOx predicts the progression to hypertension independent of baseline BP suggesting urinary NOx as a biomarker for individual new-onset hypertension.

Keywords

Normotension, progression, hypertension, nitric oxide

Received 11 March 2010; accepted 27 September 2010

Introduction

The risk of cardiovascular complications already starts to rise with increasing blood pressure at levels as low as 115 mmHg systolic and 75 mmHg diastolic.[1,2] Among the non-hypertensive individuals, who entered the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) at an age below 50, the 4-year progression rates to hypertension associated with optimal, normal and high-normal blood pressure were 7.4%, 17.9% and 24.5%. The corresponding 4-year rates of progression for individuals aged 50 years or older were 16.4%, 26.3%, and 54.0%.

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In multivariate Cox regression analysis, the blood pressure category at baseline was the strongest predictor of hypertension.[3] Transition to hypertension from these blood pressure categories was similar in the Framingham Heart Study.[4]

Based on the longitudinal cohort of the Framingham Heart Study, a risk score for predicting the incidence of hypertension has been developed.[5] In addition to the blood pressure, this score includes sex, body mass index, smoking status and parental hypertension. This allows the estimation of an individual's absolute risk for hypertension on follow-up. This development is of practical relevance as recent studies focused on the feasibility of prehypertensive treatment with the aim to reduce the incidence of hypertension.[6–9] However, the individualized risk stratification remains largely associated with the blood pressure at baseline, which is the predominant predictor of hypertension.

The kidneys play a central part in the pathogenesis of essential hypertension[10,11] and hypertension itself is associated with micro- and macroalbuminuria.[12,13] Blood pressure starts to rise when they require higher than usual blood pressure to maintain extracellular fluid volume within normal limits. Lahera and coworkers[14] first observed that the inhibition of the generation of nitric oxide (NO) in rats reduced urinary volume, sodium excretion, renal blood flow and glomerular filtration, even before blood pressure started to rise, probably via intrarenal modulation of the effects of angiotensin II. In spontaneously hypertensive (SH) rats, vascular and renal NO excretion was significantly reduced in diabetic hypertensive rats.[15]

To reach the goal of an individualized risk stratification, the crucial question is whether biomarkers exist which have additive value to predict progression from normotension to overt hypertension, in addition to baseline blood pressure values. Therefore this study aimed to evaluate whether the measurement of urinary NO metabolites (NOx), as a marker of increased oxidative stress, may predict the progression to hypertension in normotensive subjects. This may open the perspective of a more directed early preventive or therapeutic intervention. As a pilot study, we mounted a small case-control study nested within the prospective FLEMENGHO survey.

Methods

Case-control study

The Ethics Committee of the University of Leuven approved the FLEMENGHO study. From August 1985 until December 2005, we randomly recruited a family-based population sample from a geographically defined area in northern Belgium. The study population included 3108 subjects. The participants gave informed written consent and the participation rate among the subjects contacted averaged 64.3%.

For this case-control study, we searched in the database for subjects, who were untreated at baseline and whose blood pressure had been measured at an interval of approximately 4 years. From these cases, we primarily selected participants progressing from normotension to hypertension ('progressors', NT→HT) with a 24-hour urine sampling at baseline and follow-up. Furthermore, we designed an amount of 20–25% high-normal blood pressure cases in the normotensive group to achieve a similar distribution as in epidemiological studies.[16] According to the progressor group ($n = 24$), we considered subjects who at baseline and follow-up belonged to the same blood pressure category with comparable age, sex and BMI: subjects with sustained normotension (NT) or sustained hypertension (HT).

At the enrollment home visit, trained nurses measured anthropometric characteristics and blood pressure in the sitting position at the individuals' homes. They also administered a questionnaire to collect information about each subject's medical history, smoking and drinking habits, and intake of non-steroid anti-inflammatory drugs (NSAIDs). Blood pressure was the average of five consecutive readings. Body mass index was weight in kilograms divided by the square of height in metres (BMI). The participants donated a venous blood sample and collected 24-hour urine samples in a wide-neck polyethylene container for measurement of sodium, potassium, and creatinine.

We applied the criteria of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC VI),[17] and the European Societies of Cardiology and Hypertension (ESC/ESH)[18] to classify subjects untreated at baseline according to their blood pressure. Normotension was a blood pressure lower than 140 mmHg systolic and 90 mmHg diastolic; high-normal blood pressure from 130 to 139 mmHg systolic or from 85 to 89 mmHg diastolic. If systolic and diastolic blood pressures belonged to different categories, the higher level was used for classification. Hypertension was defined as having a blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic or using antihypertensive drugs.

Measurement of urinary nitrite and nitrate and renal sodium handling

The urinary concentrations of nitrite and nitrate were measured as an index of the endogenous production of nitric oxide. We applied a previously described HPLC method, which has been validated by mass

spectrometry.[19] Briefly, we used a 250 × 4.6 mm × ¼ inch IonoSPer 5A column (Varian Inc., Lake Forrest, CA) at a wavelength of 210 nm and 0.1M phosphate buffer at pH 4 as the HPLC eluent. We represented summed results for nitrite and nitrate as NOx.

We measured the sodium concentration in serum and urine by flame photometry, serum and urinary creatinine by an automated enzymatic method. Clearances (C) were calculated as $C_x = U_x \times V/P_x$, where U_x and P_x are the urinary and plasma concentrations of the solute x, and V is the volume of the urine sample. We computed the fractional excretion of sodium (FE_{NA}) by dividing the sodium clearance by the creatinine clearance. We expressed these ratios as a percentage.

Statistical analysis

For database management and statistical analysis, we used the SPSS software package, version 14. We reported normally and nonnormally distributed data as mean ± SD and as median with interquartile range, respectively. To compare means, medians and proportions, we used analysis of variance with Dunnett's test, Wilcoxon's test, χ^2 test and Fisher's exact test, with Bonferroni's correction of the significance levels, if appropriate. We used multiple linear regression analysis and Cox regression analysis to investigate the association between the biomarkers and the follow-up blood pressure and the relative blood pressure increase, adjusting for the baseline variables of age, sex, body mass index, and NSAID. All tests were two-sided and significance was accepted at a *p*-value of 0.05 or less.

Results

Baseline characteristics

At enrollment, the 62 participants had a mean (±SD) age of 41.4 ± 12.8 (range, 18 to 71 years) and included 28 women (45.1%). The study sample included at baseline 49 normotensive subjects of which 10 (20.4%) had high-normal blood pressure and a further 13 hypertensive cases.

Table 1 shows the characteristics of the participants divided into normotensive subjects and hypertensive cases at both time points, and progressors. The differences between the three groups were significant for systolic and diastolic blood pressure as well as urinary NOx concentration ($p < 0.001$). Body mass index was only elevated in hypertensive cases ($p < 0.05$). Gender was similarly distributed between the groups.

Table 1. Characteristics of cases and controls at baseline

Label Baseline blood pressure category Blood pressure category at follow-up	Controls normotensive normotensive	 hypertensive hypertensive	Cases normotensive hypertensive
Number	25	13	24
Age (years)	39.0 ± 2.4	44.4 ± 2.1	42.6 ± 3.0
Body mass index (kg/m^2)	25.3 ± 0.8	28.2 ± 1.0*	26.1 ± 0.8
Systolic pressure (mmHg)†	114.8 ± 1.6	145.3 ± 3.2**	125.3 ± 1.2**
Diastolic pressure (mmHg)†	73.0 ± 1.7	97.1 ± 2.7**	77.4 ± 1.6**
Serum biochemistry			
Serum sodium (mmol/L)	140.0 ± 0.5	140.4 ± 0.5	140.2 ± 0.5
Serum creatinine (μmol/L)	89.3 ± 4.2	94.7 ± 3.6	96.3 ± 2.1
Urinary excretion rate			
Sodium excretion (mmol/h)	7.7 ± 0.5	7.2 ± 0.5	6.5 ± 0.5
Nitric oxide concentration in urine (mg/L)	199.5 ± 20.6	126.0 ± 22.9**	109.4 ± 10.6**
Renal function			
Creatinine clearance (mL/min)	116.7 ± 4.6	112.8 ± 8.4	101.8 ± 6.2
Fractional excretion of sodium (%)	0.62 ± 0.06	0.85 ± 0.10	0.78 ± 0.10
Number (%) with characteristic			
Women	14 (56.0)	5 (41. 7)	9 (37.5)
Smoker	5 (20.0)	3 (25.0)	5 (20.8)
Non-steroidal anti-rheumatics	3 (12.0)	4 (30.8)	6 (25.0)
Antihypertensive drug treatment	0 (0.0)	6 (46.1%)	0 (0.0)

Follow-ups were at 4.6 ± 0.1 years. Values are mean ± SE or No. (%) of subjects.† Based on the average of five blood pressure readings at the subjects' homes. *$p < 0.05$ and **$p < 0.01$ versus 'stable' normotensive.

Table 2. Characteristics of cases and controls at follow-up

Label	Controls		Cases
Baseline blood pressure category	normotensive	hypertensive	normotensive
Blood pressure category at follow-up	normotensive	hypertensive	hypertensive
Number	25	13	24
Age (years)	43.6 ± 2.4	49.0 ± 2.1	47.2 ± 3.0
Body mass index (kg/m^2)	26.3 ± 0.7	28.8 ± 1.1*	27.2 ± 0.9
Systolic pressure (mm Hg)†	118.3 ± 2.1	130.4 ± 3.2**	144.3 ± 1.6**
Diastolic pressure (mm Hg)†	77.1 ± 1.8	85.0 ± 2.6**	88.5 ± 1.4**
Serum biochemistry			
Serum sodium (mmol/L)	140.1 ± 0.5	140.3 ± 0.4	140.3 ± 0.4
Serum creatinine (μmol/L)	80.8 ± 4.1	81.3 ± 5.2	92.8 ± 6.3
Urinary excretion rate			
Sodium excretion (mmol/h)	8.9 ± 0.6	8.3 ± 0.4	8.0 ± 0.5
Nitric oxide concentration in urine (mg/L)	141.2 ± 18.8	102.6 ± 17.0**	79.3 ± 8.5**
Renal function			
Creatinine clearance (mL/min)	101.1 ± 14.9	103.1 ± 11.0	91.7 ± 19.0
Fractional excretion of sodium (%)	0.71 ± 0.12	0.80 ± 0.19	0.88 ± 0.18

Follow-ups were at 4.6 ± 0.1 years. Values are mean ± SE or No. (%) of subjects. †Based on the average of five blood pressure readings at the subjects' homes. *$p < 0.05$ and **$p < 0.01$ versus 'stable' normotensive.

Progression to hypertension

Twenty-four progressors were investigated during a follow-up period of 4.6 ± 0.1 years (Table 2). In these progressors, hypertension was determined on the basis of an increase in SBP alone in two participants (8.3%), DBP alone in seven participants (29.2%), and as a result of crossing both the SBP and the DBP thresholds in 15 (62.5%).

Sixteen cases with optimal or normal blood pressure values were progressors (41%) out of a group of 39 subjects with optimal or normal blood pressure values at baseline. Ten cases with high-normal blood pressure were included in the study. Eight of them were progressors, while two cases remained normotensive. This results in a positive predictive value of 80% for progression in high-normal blood pressure cases. Therefore, only 8 out of 24 progressors were predicted by baseline high-normal blood pressure (33.3%).

At baseline, urinary NOx concentration was significantly lower in progressors as compared to the normotensive controls ($p < 0.01$). In all 49 initial normotensive subjects, baseline urinary NOx concentration was associated with follow-up blood pressure ($r = -0.55$, $p < 0.001$) and the relative increase of blood pressure over time ($r = -0.47$, $p < 0.001$). In progressors, urinary NOx baseline concentration was associated with follow-up blood pressure ($r = -0.52$, $p < 0.009$) and the relative increase of blood pressure over time ($r = -0.44$, $p = 0.033$) (Figure 1).

In exploratory analyses of progressors and normotensive controls, adjustment for baseline variables of age, sex, body mass index and use of NSAID were performed. Baseline SBP, urinary NOx concentration and creatinine clearance were associated with the absolute blood pressure value at follow-up ($r = 0.58$, -0.43 and -0.37, respectively). They were determinants explaining the absolute follow-up blood pressure values (SBP, $R^2 = 0.37$; SBP and NOx, $R^2 = 0.51$; SBP, NOx and CrCl, $R^2 = 0.60$) and the relative blood pressure increase in stepwise regression (NOx, $R^2 = 0.45$; NOx and CrCl, $R^2 = 0.58$; Table 3). Longitudinal changes of body weight and BMI did not show relevance for follow-up blood pressure or relative blood pressure increase.

By contrast, investigating the controls, only baseline SBP was predictive for the follow-up SBP ($R^2 = 0.46$). In Cox regression analysis urinary NOx (HR, 0.967 (0.941–0.995)) and baseline SBP (1.368 (1.045–1.789)) predicted the new-onset of hypertension.

In the following, normotensive subjects ($n = 49$) were stratified to the median of the baseline urinary NOx excretion (130.5 mg/L (84.5–204.2)). Of the 22 normotensive subjects with baseline values below the threshold, 18 were progressors (82%). Of these progressors, six cases had high-normal blood pressure and twelve optimal or normal blood pressure. Four cases out of 22 with urinary NOx below the threshold remained normotensive at baseline and follow-up. Thus, the NOx treshold (<130.5 mg/L) predicted 18 (75%) out of the group of 24 progressors. False

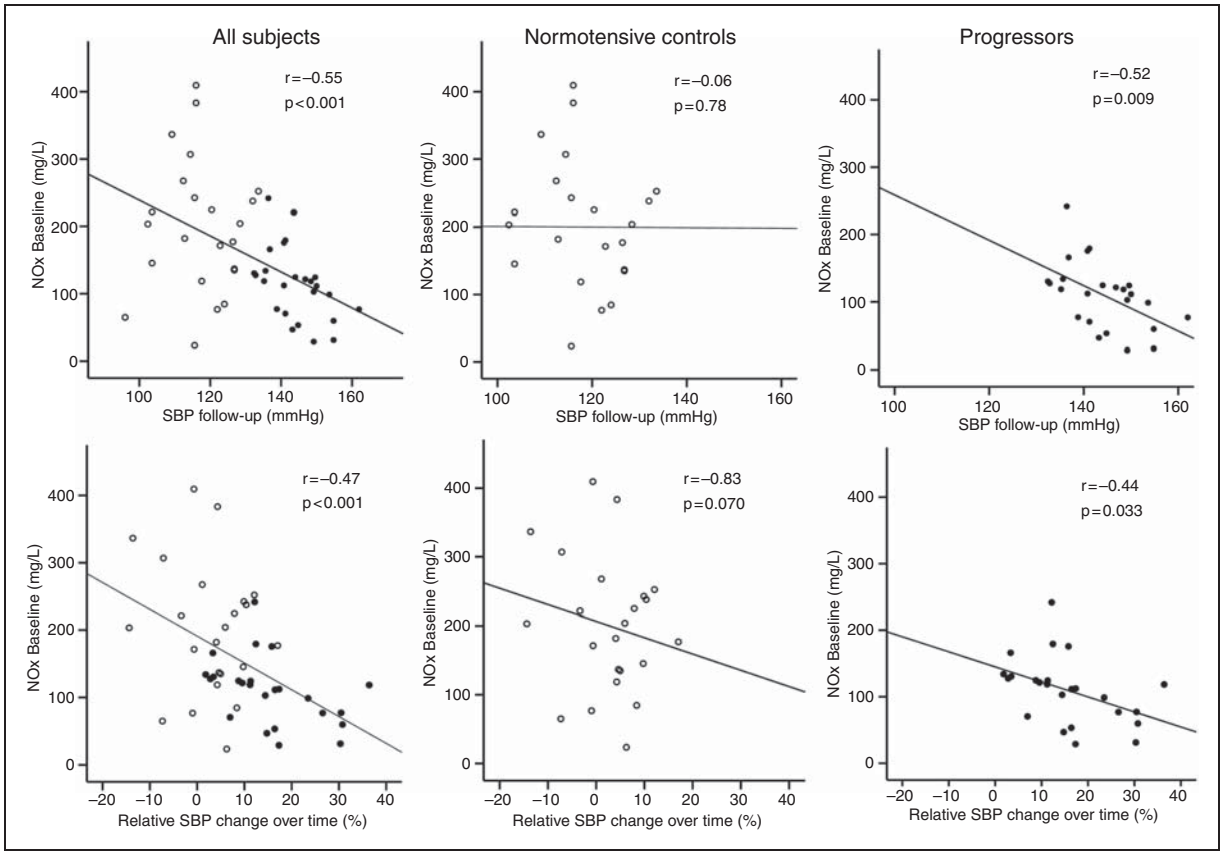

Figure 1. Urinary NOx concentration and blood pressure development in normotensive subjects. Association of baseline urinary NOx concentration (mg/L) with follow-up systolic blood pressure (SBP) and relative increase of blood pressure (%) in normotensive subjects. Subgroups are characterized by normotensive controls (NT: ○) and progressors (NT→HT: ●).

Table 3. Linear regression analysis

	All baseline normotensive subjects		Normotensive controls		Progressors	
Dependent variable: follow-up blood pressure						
Independent variables	Coefficient ± SE	P_{linear}	Coefficient ± SE	P_{linear}	Coefficient ± SE	P_{linear}
Baseline SBP (mmHg)	0.89 ± 0.19	< 0.001	0.84 ± 0.23	0.002	0.02 ± 0.18	n.s.
Baseline NOx (mg/L)	−0.07 ± 0.02	0.001	−0.02 ± 0.03	n.s	−0.08 ± 0.02	0.006
Baseline CrCl (mL/min)	−0.20 ± 0.07	0.01	−0.14 ± 0.14	n.s	−0.12 ± 0.25	n.s.
Dependent variable: relative blood pressure increase over time						
Independent variables	Coefficient ± SE	P_{linear}	Coefficient ± SE	P_{linear}	Coefficient ± SE	P_{linear}
Baseline SBP (mmHg)	−0.21 ± 0.16	n.s.	−0.18 ± 0.28	n.s	−0.96 ± 0.14	<0.001
Baseline NOx (mg/L)	−0.06 ± 0.02	0.002	−0.02 ± 0.06	n.s	−0.06 ± 0.02	0.004
Baseline CrCl (mL/min)	−0.17 ± 0.06	0.01	−0.18 ± 0.19	n.s	−0.15 ± 0.21	n.s.

The model was adjusted for age, gender, body mass index, NSAID. SBP, systolic blood pressure; NOx, nitric oxide metabolites; CrCl, creatinine-clearance; n.s., not significant.

negative cases out of the group with NOx above the threshold (n = 27) but progressing to hypertension, were present in six subjects (22%). False positive cases, thus subjects with NOx below the threshold but not progressing to hypertension, were four (18%) out of 22 subjects.

Out of the eight progressors with high-normal blood pressure, six cases showed NOx values below the median (75%). By contrast, the two cases with high-normal blood pressure that remained normotensive had NOx values ≥130.5 mg/L. Only three out of 24 progressors were not predicted by either baseline

high-normal blood pressure or urinary NOx excretion <130.5 mg/L (specificity, 87.5%).

Discussion

The key finding of our study is that urinary NOx concentration is an independent predictor for the individual progression to hypertension in normotensive subjects. Baseline blood pressure combined with urinary NOx and creatinine-clearance predicted the follow-up blood pressure. An urinary NOx threshold <130.5 mg/L predicted 75%, and in combination with high-normal blood pressure, 87.5% of new-onset hypertension in this study.

To our knowledge, no previous study addressed the influence of changes in NO handling on the progression to hypertension. So far several authors demonstrated that reduced NO metabolites are associated with higher blood pressure values in animal models[20–23] and humans.[24–26] These observations are based on the results of Lahera and coworkers.[14] They observed that the acute and progressive inhibition of NO in rats obtained by the successive infusion of 0.1, 1 and 10 mg of the NO synthesis inhibitor, L-NAME, produced significant decrements in urinary volume, sodium excretion, renal blood flow (RBF) and glomerular filtration rate (GFR) before blood pressure started to rise. The most sensitive index affected during systemic inhibition of NO was the decrease of sodium and water excretion and the elevation of intrarenal vascular resistance.[27] The infusion of small doses of angiotensin II in experimental animal models also produced an increase in intrarenal vascular resistance and a fall in water and sodium excretion before peripheral systemic resistance and blood pressure started to rise.[28–30] By and large, these observations are consistent with the idea that the effects of NO on the renal microvasculature and tubular sodium handling continuously counterbalance those of angiotensin II.[14,27–30] Thus, a reduced renal NO bioavailability leads to vasoconstriction resulting in increased glomerular vascular resistance and altered tubulo-glomerular feedback.[29–30] These results are in line with the modern pathomechanistic view on hypertension as a tubulo-interstitial disease.[31–32]

In this context, our data shows reduced urinary NOx in progressors as compared to controls. Additionally, the urinary NOx is a blood pressure independent predictor for the follow-up blood pressure and the extent of blood pressure increase. Beyond correlation, the chosen urinary NOx concentration threshold (<130.5 mg/L) predicted in 75% the new-onset of hypertension in this study. Thus, our data suggest that a reduced NO bioavailability results in increased risk for development of hypertension.

In context with early renal dysfunction, others indicated that cystatin C levels[33] and higher albumin/creatinine ratios – even within the normal range – are associated with increased risk for development of hypertension.[34] However, in this cohort creatinine-clearance and sodium excretion were no predictor for progressors.

High-normal blood pressure represents a strong predictor for new-onset hypertension. We demonstrate that the chosen urinary NOx threshold, independently of baseline blood pressure, predicts blood pressure progression with a high specificity and across the ranges of optimal, normal and high-normal blood pressure. Therefore, urinary NOx adds further information to the individual blood pressure progression.[3] This is confirmed by the increased predictive value for progressors if high-normal blood pressure and low urinary NOx values are combined.

The origin of reduced urinary NOx is multifactorial and includes some dietary[35] or pharmaceutical (NSAID) aspects,[36] which were not the aim of the study. We cannot state whether factors decreasing urinary NOx cause future blood pressure elevation.[37] A limitation of the study is the small number of participants. Large epidemiological studies further detected a number of factors such as elevated baseline BMI, fast heart rate, parental hypertension and renal dysfunction as forerunners of hypertension.[5,33] We detected none of these factors in this study, which may be related to the small number and young age of the chosen cohort. Large epidemiological studies have to detect its clinical relevance.

In conclusion, this study demonstrates that urinary NOx concentration independently predicts blood pressure development in normotensive subjects. Moreover, urinary NOx predicts new-onset hypertension in this cohort and adds more information than high-normal blood pressure alone. This makes urinary NOx a potential biomarker to detect normotensives overt to hypertension and is suggestive for a role of NO handling in blood pressure development. However, the predictive value of the urinary NOx concentration has to be confirmed in large-scaled prospective studies.

Acknowledgements

FLEMENGHO would not have been possible without the voluntary collaboration of participants and their general practitioners. The municipality Hechtel-Eksel (Belgium) gave logistic support. The authors gratefully acknowledge the expert technical assistance of Sandra Covens, Linda Custers, Marie-Jeanne Jehoul, Katrien Staessen, Hanne Truyens, and Renilde Wolfs (Studies Coordinating Centre, University of Leuven, Belgium).

Funding

This work was supported by the European Union (grant numbers IC15-CT98–0329-EPOGH, QLGI-CT-2000-01137-

EURNETGEN, and LSHM-CT-2006-037093 InGenious HyperCare), the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (grant numbers G.0424.03 and G.0575.06), and the Katholieke Universiteit Leuven, Belgium (grant numbers OT/99/28, OT/00/25 and OT/05/49).

Conflict of interest

None of the authors has a conflict of interest with regard to the data presented in this paper.

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