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# Associations of urinary orosomuroid, N-acetyl- $\beta$ -D-glucosaminidase, and albumin with blood pressure and hypertension after 7 years. The Tromsø Study

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## ABSTRACT

**Purpose:** Subclinical chronic kidney disease is known to exacerbate hypertension and progression of kidney damage. In order to initiate timely interventions, early biomarkers for this vicious circle are needed. Our aim was to describe the cross-sectional associations of urinary orosomuroid and urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG) with blood pressure and the longitudinal associations of urinary orosomuroid and NAG to hypertension after 7 years, and to compare the strength of these associations to the urinary albumin excretion (UAE).

**Material and methods:** The Tromsø Study is a population-based, prospective study of inhabitants of the municipality of Tromsø, Northern Norway. Morning spot urine samples were collected on three consecutive days in the Tromsø 6 survey (2007–2008). We assessed the cross-sectional associations of urinary orosomuroid, NAG and UAE with blood pressure in Tromsø 6. In a cohort of participants attending Tromsø 6 and Tromsø 7 (2015–2016), we studied whether urinary biomarkers were longitudinally associated with hypertension.

**Results:** A total of 7197 participants with a mean age of 63.5 years (*SD* 9.2), and a mean blood pressure of 141/78 mmHg (*SD* 23.0/10.6), were included in the study. Orosomuroid and UAE, but not NAG, was significantly associated with systolic and diastolic blood pressure in all the crude and multivariable cross-sectional analyses. Orosomuroid had consistently, although marginally, stronger associations with blood pressure. Incident hypertension at follow-up (Tromsø 7) was consistently significantly associated with urinary orosomuroid, but not urinary NAG or UAE. However, the standardized regression coefficients for orosomuroid were only marginally stronger than the standardized regression coefficients for ACR.

**Conclusion:** In a cohort from the general population urine orosomuroid had a stronger cross-sectional association with blood pressure than UAE. After 7 years, urine orosomuroid showed the strongest association with incident hypertension. There were varying and weak associations between U-NAG, blood pressure and hypertension.

## PLAIN LANGUAGE SUMMARY

### • What is the context?

There is a relationship between high blood pressure and cardiovascular and kidney disease. Hypertension is defined as the level of blood pressure at which the benefits of treatment outweigh the risks of treatment.

Hypertension is a risk factor for developing kidney disease, and kidney disease is a risk factor for developing hypertension.

Today, kidney function is assessed by blood and urine samples (estimated glomerular filtration rate and urinary albumin excretion). However, today's blood and urine samples are not sensitive enough to capture kidney damage due to hypertension at a stage when prevention may be most effective.

### • What is new?

In this study, we assessed if urine orosomuroid and N-acetyl- $\beta$ -D-glucosaminidase (NAG) are more strongly associated with blood pressure and hypertension than urinary albumin excretion.

In the population-based study of residents in Tromsø, Northern Norway, we assessed the relationship between the urine biomarkers and blood pressure, and the development of hypertension after 7 years.

In the general population urine orosomuroid had a stronger relationship with blood pressure

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Hypertension; blood pressure; urinary albumin excretion; N-acetyl- $\beta$ -D-glucosaminidase; orosomuroid;  $\alpha$ -1-acid glycoprotein

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than urinary albumin excretion.

After 7 years, urine orosomuroid had the strongest relationship with the development of hypertension.

There were only varying and weak relationships between NAG, blood pressure and hypertension.

- **What is the impact?**

Orosomuroid showed a stronger relationship with blood pressure and the development of hypertension than urinary albumin excretion. Urine orosomuroid may aid targeted prevention and treatment in hypertension, but further prospective clinical studies are needed to assess if orosomuroid is a clinically useful biomarker in hypertension.

## Introduction

According to the World Health Organization, hypertension is the most significant single contributor to overall morbidity and mortality [1]. An estimated 1.4 billion people worldwide have high blood pressure [2]. Subclinical kidney disease is associated with developing hypertension in the general population [3,4]. Therefore, the development of subclinical kidney damage can initiate a vicious circle with exacerbated hypertension and further progression of kidney damage. The underlying mechanism leading to kidney damage in hypertension is not settled, but endothelial dysfunction, afferent arteriopathy, damage to the glomerular filtration barrier and tubular ischemia/dysfunction may be important and early steps [4].

Many observational studies have shown that a reduced GFR and albuminuria are independently and multiplicatively associated with an increased risk of cardiovascular events [5]. However, since a significant reduction in kidney function is needed to detect a decrease in estimated GFR (eGFR) [6], it is considered an insensitive marker of kidney damage. Furthermore, only a minority of patients with hypertension have elevated UAE to a level detected by most assays [7–10]. Overall, both eGFR and UAE are insensitive biomarkers of early renal damage in hypertension, at a stage when prevention may be most effective [4,11,12]. Further, lowering of UAE per se in hypertensive patients has not been shown to reduce cardiovascular disease (CVD) risk [13], making UAE a deficient surrogate therapeutic target in hypertension [14]. Finding a panel of biomarkers related to hypertension and hypertension-mediated organ damage at an early stage, that varies with modifiable risk factors, may aid targeted prevention and treatment [12,15].

Similar to UAE, urinary orosomuroid ( $\alpha$ -1-acid glycoprotein) is considered a marker of general endothelial dysfunction and a damaged glomerular filtration barrier [16–18]. Orosomuroid is a constituent of the endothelial surface layer and maintains permselectivity [18,19]. Damage to this layer is considered a

cause for atherosclerosis [20]. As tubular dysfunction and damage may increase UAE [21], urinary orosomuroid may be a more specific marker of endothelial dysfunction and glomerular damage in hypertension [18,19,22,23].

N-acetyl- $\beta$ -D-glucosaminidase (NAG) is a lysosomal enzyme that, in the kidney, is found predominantly in lysosomes of proximal tubular cells [11]. The NAG present in the urine is secreted from proximal tubular cells by exocytosis [24]. NAG is thus exclusively a marker of tubular cell function [25]. Myocardial infarction, ischemic stroke, and mortality in the general population have been associated with NAG levels [26]. A small study of patients with newly diagnosed hypertension without microalbuminuria found a significantly higher NAG activity in hypertensive subjects compared to healthy controls [27]. Whereas UAE is dependent on alterations in the glomerular filtration barrier and impaired proximal tubular function [21], NAG may be an earlier and more specific marker of proximal tubular dysfunction in hypertension [27,28].

In this study, we aimed to assess if urinary orosomuroid and NAG have stronger associations cross-sectionally to blood pressure and longitudinally to hypertension than UAE.

## Study population

The Tromsø Study is a population-based, prospective study of residents of the municipality of Tromsø, Northern Norway. Since 1974, seven surveys (Tromsø 1–7) have been conducted. The participants in Tromsø 6 (2007–2008) were recruited from four invited groups: Those who took part in the special study in the Tromsø 4, a random 10% sample of residents aged 30–39, everyone aged 40–42 or 60–87 and a random 40% sample of residents aged 43–59 years. A total of 12,984 attended (65.7% of the invited population) [29]. Common CVD risk factors were mapped in the first visit.

Further, 7955 of the participants were invited to undergo an extensive examination. The population eligible for the second visit included first visit participants aged 50–62 and 75–84 years, a 20% random sample aged 63–74, and finally all subjects who had also attended the second visit of Tromsø 4 (1994–1995). The attendance rate for this second visit was 91.8% ( $n = 7306$ ). Morning spot urine samples from 7197 participants on three consecutive days were collected. UAE was available in 7195 participants (3090 men and 4105 women), urinary orosomucoid was available in 7181 participants (3086 men and 4095 women), and urinary NAG measurements were available in 7170 participants (3082 men and 4088 women).

The invited population in Tromsø 7 (2015–2016) consisted of all individuals aged 40 and older. A total of 21,083 attended (64.7% of the invited population) [30].

A total of 5114 attended both the second visit of the Tromsø 6 and the Tromsø 7 surveys (Figure 1). UAE was available in 5046 participants, urinary orosomucoid was available in 5033 participants and urinary NAG measurements were available in 5025 participants of the Tromsø 7.

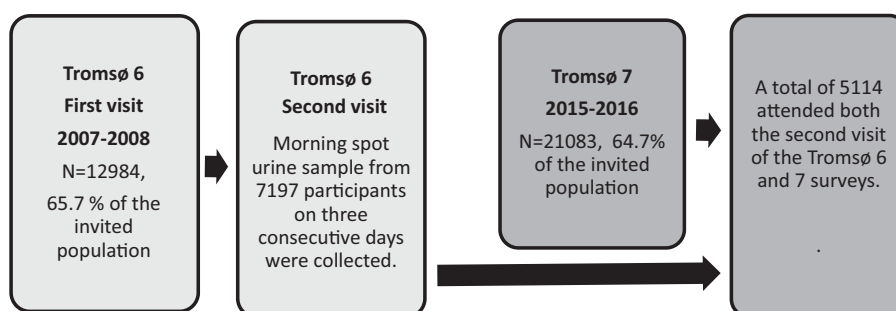
The UiT, The Arctic University of Norway conducted the survey in cooperation with The National Health Screening Service. The Regional Committee for Medical and Health Research Ethics approved the study, and all participants gave their written consent [29,30].

## Methods

In both Tromsø 6 and 7, the participants returned a self-administered questionnaire, including information

about current medication, diabetes, CVD, and smoking habits. Tobacco use was dichotomized into current smokers or not (all others). Blood samples and time since the last meal were obtained. We defined fasting as time since the previous meal of at least eight hours. We defined the presence of diabetes as self-reported diabetes, self-reported use of glucose-lowering drugs, HbA1c  $\geq 6.5\%$ , non-fasting glucose  $\geq 11$  mmol/L, or a fasting blood glucose  $> 7.0$  mmol/L. We calculated the body mass index (BMI; kg/m<sup>2</sup>) from height and weight measured by study personnel.

Blood pressure was recorded in triplet by trained personnel, using an automatic device (the Dinamap Pro care 300 Monitor (GE Healthcare)). The cuff was chosen after the circumference of the upper arm was measured. After a 2-min rest, three attended readings on the upper right arm were taken in a sitting position, separated by a 1-min interval. We used the mean of the second and third readings in the analyses [31]. Several antihypertensive agents may affect the levels of urinary biomarkers independently from their blood pressure lowering effect [32], and in the cross-sectional design the cohort was dichotomized into those with and without treated hypertension. We defined treated hypertension as using one or more blood pressure-lowering drugs. In the longitudinal analyses, we defined incident hypertension as being normotensive in Tromsø 6 and having started with one or more blood pressure-lowering drugs or an increase in blood pressure  $\geq 140/90$  mmHg at follow-up (Tromsø 7). In the longitudinal analysis, we further defined controlled hypertension at baseline (Tromsø 6) as blood pressure  $< 140/90$  mmHg while using one or more blood pressure-lowering drugs. Among those with controlled hypertension at baseline, we defined progressive hypertension as an increase in the number of blood



**Figure 1.** Selection of study participants from the Tromsø Study, 6th and 7th wave. Figure 1 depicts a range of change in predicted probabilities. A standardized coefficient of 0.01 represents 1% of the maximum change possible in predicted probability of incident hypertension. NAG: (Log urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio per SD unit); standardized beta coefficients at different probability reference value; ACR: (Log urinary albumin to creatinine ratio per SD unit); standardized beta coefficients at different probability reference value; OCR: (Log urinary orosomucoid to creatinine ratio per SD unit); standardized beta coefficients at different probability reference value. Fully Adjusted Model: Adjusted for CVD risk factors and NAG and ACR and OCR. CVD risk factors: Sex, Age, BMI, Current smoker, Diabetes, Prior cardiovascular event (stroke or heart attack).

pressure-lowering drugs or blood pressure  $\geq 140/90$  mmHg while on the same blood pressure-lowering drugs at follow-up (Tromsø 7).

We used morning urine samples collected on three consecutive days at baseline (Tromsø 6). We measured creatinine, albumin, and NAG in fresh urine samples at the time of collection. Urine creatinine was measured using colorimetric methods (Jaffes reaction) and an autoanalyser (ABX PENTRA, Horiba ABX, Montpellier, France). We measured the urine albumin concentration by an immunoturbidimetric method with the ABX Pentra Micro-albumin CP (Horiba ABX, Montpellier, France). A colorimetric method (with 3-cresolsulfonylthaleinyl-N-acetyl- $\beta$ -D-glucosaminide; Boehringer Mannheim, Germany) was applied for the NAG measurements. Urine samples were frozen at  $-20^{\circ}\text{C}$ , shipped to the Department of Quality and Research, Regional Hospital of Randers, University of Aarhus, Denmark and analyzed for orosomuroid by a highly sensitive EU-labelled fluorometric immunoassay (DELFLIA) [33]. We added Tween 20, stored the urine samples at  $-20^{\circ}\text{C}$  and analyzed thawed samples at  $37^{\circ}\text{C}$  based on a self-developed application in the AutoDELFLIA system [33,34]. For each urine sample, we calculated the ratios of albumin, NAG, and orosomuroid concentrations with the urine creatinine concentration (NAG-creatinine ratio [NAG-Cr], albumin-creatinine ratio [ACR], orosomuroid-creatinine ratio [OCR]), respectively). The median values of these biomarkers assessed on days 1, 2 and 3 were used in the analyses.

Serum creatinine was measured with an enzymatic method (Modular P; Roche Diagnostics). Cystatin C was measured with a particle-enhanced turbidimetric immunoassay using reagents from Gentian on a Modular P800 analyzer (Roche Diagnostics). We calculated creatinine-cystatin C based eGFR by applying the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [35,36]. Serum glucose, triglycerides, and cholesterol were measured on a Modular P800 (Roche Diagnostics). HbA1c was measured with a liquid chromatographic method (Variant II instrument, Bio-Rad Laboratories, Hercules, CA).

### Statistical analyses

Baseline characteristics were summarized for the entire cohort and subgroups with or without treated hypertension and men and women with a mean (*SD*) for normally distributed variables, median for skewed variables, and *n* (percentage) for categorical variables. Student's *t*-

test, Mann-Whitney tests, Wilcoxon-signed-rank test and chi-square test were used for comparison, as appropriate.

In the cohort of participants with morning spot urine samples from Tromsø 6, second visit, we used univariable and multivariable linear regression analyses to assess the associations between the urinary biomarkers and the dependent variables systolic and diastolic blood pressure in the whole cohort and subgroups with and without treated hypertension. The creatinine adjusted urinary biomarkers were non-normally distributed and were log-transformed. As standardized regression coefficients are context dependent, we did the crude analysis to address which biomarker explains the most variance, ignoring the effect of the other independent variables. We did multivariable and fully adjusted models to address which biomarker had the most unique variance (i.e. the largest beta weight) when the other independent variables were included in the regression models. In crude linear models, we added one urinary biomarker ([NAG-Cr] or [ACR] or [OCR]). In multivariable models, we added CVD risk factors (sex, age, BMI, current smoker, diabetes and prior cardiovascular event (stroke or heart attack)) and one urinary biomarker ([NAG-Cr] or [ACR] or [OCR]). In fully adjusted models, we added CVD risk factors and all urinary biomarkers ([NAG-Cr] and [ACR] and [OCR]). We included eGFR in all multivariable models but excluded this variable from the models if it had no impact on the standardized regression coefficients. In the same crude and multivariable linear models, we also used the log-transformed median concentration of the urinary biomarkers without the urine creatinine concentration ratio. The strengths of the associations were compared and presented as standardized *b*-coefficients (std beta) in the whole cohort and in those with and without treated hypertension.

In a cohort of participants who delivered urine samples in Tromsø 6 and later participated in Tromsø 7, we assessed the association between the biomarkers and the dependent variable incident hypertension in the normotension cohort in Tromsø 6. We further evaluated the association between the biomarkers and the dependent variable progressive hypertension in the cohort with controlled hypertension in Tromsø 6. We used univariable and multivariable logistic regression analyses and the same regression models described in the cross-sectional analyses. The logistic regression analysis reports unstandardized regression coefficients, *p* values, the odds ratio with corresponding 95% confidence intervals and the semi-standardized regression



coefficients using the Kaufman formula to compare the strength of the associations [37,38]. The semi-standardized coefficient given by the Kaufman formula measures the change in predicted probability associated with a one standard deviation change in the predictor and is restricted to the interval  $-1$  to  $1$  [38]. The semi-standardized regression coefficients were calculated at the mean predicted probability for the outcome in the population for the two longitudinal analyses (mean predicted probability 0.32 for incident hypertension and mean predicted probability 0.55 for progressive hypertension). The range of estimated semi-standardized regression coefficients for different probability reference values was calculated for the urine biomarkers and displayed graphically for the fully adjusted models.

Participants with missing data were excluded only for analyses for which the case had missing data.

The urine albumin assay's limit of detection in Tromsø 6 is given at 4 mg/L, but the PENTRA instrument reports albumin concentrations as low as 1 mg/L. These results were published in previous publications from the Tromsø Study [39–41] and in the main analyses of the present study. The limit of detection for the albumin concentration almost corresponds to the median albumin concentration in Tromsø 6. Given a large number of observations below the limit, we further treated all observations of albumin concentration lower than 4 mg/L as left-censored and repeated the linear and logistic regressions for the association with blood pressure and incident hypertension using the deletion method [7,42].

We explored interaction effects between the urinary biomarkers, age, and sex. We present the most pronounced interaction effects in a supplementary table and display the mean associations with standardized beta coefficients in the main tables. Significant interactions with sex are presented as standardized beta coefficients for men and women. Significant interactions with age are presented as centered scores expressed as *SD* (i.e. standardized regression coefficients) corresponding to the mean age  $\pm 1$  *SD*.

Statistical significance was defined as two-sided  $p < 0.05$  in all analyses. We used SPSS Statistics for Windows, Version 27.0 (IBM SPSS Statistics for Windows, Version 27 .0. Armonk, NY: IBM Corp).

## Results

### Baseline characteristics

A total of 7197 participants with a mean age of 63.5 years (*SD* 9.2), a mean blood pressure of 141/78 mmHg (*SD* 23.0/10.6), and a mean BMI of 27.3 kg/

m<sup>2</sup> (*SD* 4.2) were included in the cross-sectional study (Figure 1).

Of the 7197 participants, 2326 (32%) used one or more blood pressure-lowering drugs. Subjects using blood pressure-lowering drugs were older and had higher blood pressure, BMI, and more frequently diabetes and CVD compared to participants without antihypertensive treatment. On the other hand, current smoking was observed more frequently among those not using blood pressure-lowering drugs (Table 1). The participants using blood pressure-lowering drugs had lower eGFR and higher OCR, NAG-Cr, and ACR (Table 2). The average number of blood pressure-lowering drugs in the treated cohort was 2.

Women had lower eGFR and OCR but higher NAG-Cr. Women not using blood pressure-lowering drugs had higher ACR compared to men (Supplementary Table S1).

The correlation coefficient between the log-transformed median ACR and OCR was 0.55. The correlation coefficient between the log-transformed median ACR and NAG-Cr was 0.13. The corresponding correlations between the urinary biomarker concentrations (not adjusted for the urine creatinine concentration) were essentially the same ( $p < 0.001$  for all correlations).

The cohort of participants who delivered urine samples in Tromsø 6 and later participated in Tromsø 7 consisted of 5114 participants (Figure 1). During this time period mean serum creatinine increased from 69.2  $\mu\text{mol/L}$  (*SD* 15.1) to 76.2  $\mu\text{mol/L}$  (*SD* 24.0) and accordingly eGFR decreased from 93.8 mL/min/1.73m<sup>2</sup> (*SD* 14.6) to 80.1 mL/min/1.73 m<sup>2</sup> (*SD* 18.9) (Supplementary Table S2).

### Cross-sectional associations

OCR and ACR, but not NAG-Cr, was significantly associated with systolic and diastolic blood pressure in all the crude and multivariable cross-sectional analyses. OCR had a consistently, although marginally, stronger association with blood pressure than ACR in crude and multivariable models. In fully adjusted models with cardiovascular risk factors and all urinary biomarkers included, OCR was the only urine biomarker significantly associated with blood pressure in all groups (i.e. the groups with and without treated hypertension and the entire cohort) (Table 3). Adjustment for eGFR did not affect the standardized regression coefficients in the multivariable models and was therefore excluded from the analyses. OCR exhibited the most pronounced significant

**Table 1.** Characteristics. Individuals with and without treated HT in the Tromsø 6–2 study.

	Without treated HT		With treated HT		<i>p</i> Value
	<i>(n</i> = 4871)		<i>(n</i> = 2326)		
Female sex, <i>n</i> (%)	2810	(57.6)	1297	(55.9)	0.12
Age, years	61.7	(8.8)	67.5	(8.7)	<0.001
Systolic blood pressure, mmHg	138	(22.3)	147	(22.9)	<0.001
Diastolic blood pressure, mmHg	78	(10.6)	79	(10.6)	<0.001
Body mass index, kg/m <sup>2</sup>	26.4	(3.9)	28.5	(4.5)	<0.001
No. blood pressure lowering drugs, <i>n</i>	0		2	(1.0–2.0)	
Cholesterol, mmol/L	5.9	(1.1)	5.4	(1.1)	<0.001
HDL cholesterol, mmol/L	1.6	(0.5)	1.5	(0.4)	<0.001
LDL cholesterol, mmol/L	3.8	(0.9)	3.4	(1.0)	<0.001
Triglycerides, mmol/L	1.2	(0.9–1.7)	1.4	(1.1–2.0)	<0.001
HbA1c, %	5.6	(5.4–5.8)	5.8	(5.5–6.1)	<0.001
Current smoking, <i>n</i> (%)	976	(20.4)	340	(14.9)	<0.001
Diabetes, <i>n</i> (%)	251	(5.4)	397	(18.5)	<0.001
Physically active, <i>n</i> (%)	1475	(31.4)	479	(22.0)	<0.001
Self-reported previous cardiovascular event, <i>n</i> (%)	129	(2.7)	493	(22.4)	<0.001

(*n* = 7197).

Data are mean (*SD*), median (interquartile range) or number (%) as appropriate. Treated HT was defined as use of one or more blood pressure lowering drug. Blood pressure lowering drugs: Drugs containing beta blocking agents, calcium channel blockers, ACE inhibitors, angiotensin II antagonists or diuretics, self-reported. Smoking habits were defined as current smoking or no smoking; the nonsmoking group consisted of never smokers and previous smokers. Diabetes: self-reported diabetes, self-reported use of glucose lowering drugs, HbA1c  $\geq 6.5\%$ , non-fasting glucose  $\geq 11$  mmol/L, fasting blood glucose  $>7.0$ . Physically active: at least 2 times per week with exercise duration  $>30$  min with sweating and/or breathlessness, self-reported. Cardiovascular event: prior stroke or heart attack.

interactions with sex and age. The age interaction was most prominent for OCR in men, and OCR showed a stronger association with blood pressure in the youngest category (men  $\leq 60$  years) (Supplementary Table S3).

The associations of urinary albumin and orosomuroid concentrations with blood pressure did not differ substantially from the corresponding associations with ACR and OCR, respectively (Supplementary Table S4). Orosomuroid had the highest unique variance with blood pressure in the cohort with treated hypertension. Although NAG concentration showed a stronger association with blood pressure than NAG-Cr, urinary NAG concentration was not significantly associated with blood pressure in fully adjusted models and explained the least variance in crude and multivariable models. (Supplementary Table S4).

### Incident hypertension in longitudinal analysis

The number of participants with normotension in Tromsø 6 was 2744, among these, 681 participants were classified with incident hypertension in Tromsø 7. OCR and ACR, but not NAG-Cr, were significantly associated with incident hypertension after seven years in crude and multivariable models. The semi-standardized regression coefficients showed that OCR had the marginally strongest association with incident hypertension. In fully adjusted models, OCR was the only urine biomarker with a significant association with incident hypertension. In these models, the difference between the semi-standardized regression

coefficients for ACR and OCR increased due to the correlation between these biomarkers and displaying a higher unique variance between OCR and incident hypertension (Table 4 and Figure 2). Adjustment for baseline eGFR did not affect the semi-standardized regression coefficients, and there were no interactions between the biomarker-creatinine ratios and age or sex.

The associations with incident hypertension did not change for albumin or orosomuroid in analysis without adjustment for the urine creatinine concentration, but NAG showed a stronger association with incident hypertension than NAG-Cr. Orosomuroid was the only significant urine biomarker in the fully adjusted model. The difference between the semi-standardized regression coefficients in the fully adjusted model increased, displaying a higher unique variance between orosomuroid and incident hypertension (Supplementary Table S5). Inclusion of baseline eGFR in these models did not alter the standardized regression coefficients.

### Progressive hypertension in longitudinal analysis

The number of participants with controlled hypertension in Tromsø 6 was 353, and 193 were classified as having progressive hypertension in Tromsø 7. In the longitudinal analyses of progressive hypertension, only OCR and ACR, but not the NAG-Cr, yielded significant or borderline significant results in crude and multivariable models. OCR and ACR showed equally strong associations with progressive

**Table 2.** Markers of kidney damage.

	Without treated HT		With treated HT		<i>p</i> Value
	<i>(n = 4871)</i>		<i>(n = 2326)</i>		
Serum creatinine ( $\mu\text{mol/L}$ )	68.1	(13.3)	74.5	(22.2)	<0.001
Cystatin C, mg/L	0.82	(0.15)	0.94	(0.25)	<0.001
Estimated GFR ( $\text{mL/min/1.73 m}^2$ )	94.7	(13.9)	83.7	(18.9)	<0.001
ACR, mg/mmol	0.32	(0.18–0.65)	0.47	(0.23–1.10)	<0.001
OCR, g/g	0.38	(0.21–0.80)	0.62	(0.30–1.7)	<0.001
NAG/Cr, U/g	1.76	(1.05–2.78)	2.02	(1.20–3.12)	<0.001

Individuals with and without treated HT in the Tromsø 6–2 study ( $n = 7197$ ).

Data are mean (*SD*) or median (interquartile range) as appropriate. Treated HT was defined as use of one or more blood pressure lowering drug. Estimated GFR: glomerular filtration rate, calculated using the creatinine-cystatin C based CKD-EPI equation. ACR: urinary albumin to creatinine ratio; OCR: urinary orosomucoid to creatinine ratio; NAG-Cr: urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio.

**Table 3.** Urine biomarkers as ratios with urine creatinine concentration cross sectional analysis of the Tromsø 6–2 Study.

Groups		Crude			Multivariable model			Fully adjusted model		
		Std beta	<i>p</i>	95% CI	Std beta	<i>p</i>	95% CI	Std beta	<i>p</i>	CI
Systolic BP as dependent variable										
All	NAG-Cr	0.01	0.60	(−0.02–0.03)	−0.01	0.23	(−0.04–0.01)	−0.03	0.006	(−0.06 to −0.01)
	ACR	0.18	<0.001	(0.16–0.20)	0.10	<0.001	(0.07–0.12)	0.06	<0.001	(0.04–0.09)
	OCR	0.21	<0.001	(0.18–0.23)	0.11	<0.001	(0.08–0.13)	0.08	<0.001	(0.05–0.11)
Without treated HT	NAG-Cr	−0.01	0.60	(−0.04–0.02)	−0.01	0.47	(−0.04–0.02)	−0.03	0.04	(−0.06 to −0.00)
	ACR	0.18	<0.001	(0.15–0.21)	0.11	<0.001	(0.08–0.14)	0.08	<0.001	(0.05–0.11)
	OCR	0.21	<0.001	(0.18–0.23)	0.11	<0.001	(0.08–0.14)	0.08	<0.001	(0.05–0.11)
With treated HT	NAG-Cr	0.003	0.87	(−0.03–0.04)	−0.02	0.42	(−0.06–0.02)	−0.03	0.12	(−0.07–0.01)
	ACR	0.10	<0.001	(0.06–0.14)	0.08	<0.001	(0.04–0.12)	0.05	0.09	(−0.01–0.10)
	OCR	0.11	<0.001	(0.07–0.15)	0.08	<0.001	(0.04–0.13)	0.06	0.03	(0.01–0.12)
Diastolic BP as dependent variable										
All	NAG-Cr	−0.05	<0.001	(−0.08 to −0.03)	−0.01	0.38	(−0.03–0.01)	−0.03	0.02	(−0.05 to −0.01)
	ACR	0.07	<0.001	(0.05–0.10)	0.10	<0.001	(0.07–0.12)	0.06	<0.001	(0.03–0.09)
	OCR	0.11	<0.001	(0.08–0.13)	0.11	<0.001	(0.08–0.13)	0.08	<0.001	(0.05–0.11)
Without treated HT	NAG-Cr	−0.06	<0.001	(−0.09 to −0.03)	−0.01	0.51	(−0.04–0.02)	−0.03	0.04	(−0.06–0.00)
	ACR	0.08	<0.001	(0.05–0.11)	0.11	<0.001	(0.09–0.14)	0.08	<0.001	(0.05–0.11)
	OCR	0.13	<0.001	(0.10–0.16)	0.11	<0.001	(0.08–0.14)	0.08	<0.001	(0.05–0.11)
With treated HT	NAG-Cr	−0.04	0.04	(−0.08 to −0.001)	−0.01	0.69	(−0.05–0.04)	−0.02	0.27	(−0.07–0.02)
	ACR	0.04	0.07	(−0.01 to −0.08)	0.07	0.002	(0.03–0.11)	0.03	0.22	(−0.02–0.09)
	OCR	0.05	0.03	(0.01–0.09)	0.08	<0.001	(0.04–0.13)	0.07	0.01	(0.02–0.13)

Linear regression with standardized beta coefficients for urine biomarkers ( $n = 7197$ ).

Systolic and diastolic blood pressure (BP) as dependent variable (mmHg).

Treated HT: defined as use of one or more blood pressure lowering drug.

CVD risk factors: Sex. Age. BMI. Current smoker. Diabetes. Prior cardiovascular event (stroke or heart attack).

Crude Model: NAG or ACR or OCR.

Multivariable Model: Adjusted for CVD risk factors and NAG, or ACR, or OCR.

Fully Adjusted Model: Adjusted for CVD risk factors and NAG and ACR and OCR.

NAG-Cr: (Log urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio per *SD* unit): standardized beta coefficients. *p*-Value and 95% CI.

ACR: (Log urinary albumin to creatinine ratio per *SD* unit): standardized beta coefficients. *p*-Value and 95% CI.

OCR: (Log urinary orosomucoid to creatinine ratio per *SD* unit): standardized beta coefficients. *p*-Value and 95% CI.

hypertension in the multivariable models. However, none of the biomarkers were significantly associated with progressive hypertension in the fully adjusted model (Table 5, Figure 3). Adjustment for baseline eGFR affected the standardized regression coefficients, and eGFR was included in these multivariable models (Supplementary Table S6). There were no interactions between the biomarkers-creatinine ratios and age or sex.

The analyses with progressive hypertension and biomarkers without the urine creatinine ratios yielded no significant associations.

### Left censored analysis for ACR

The analyses with ACR left-censored, including participants with urine albumin concentration  $\geq 4$  mg/L only, comprised 2553 participants in the cross-sectional analysis with blood pressure, and 752 participants in the longitudinal analysis with incident hypertension, which affected the regression coefficients for ACR in the univariable and multivariable models. The analysis reinforced the previously described pattern with the strongest associations between OCR, blood pressure and incident hypertension (Supplementary Table S7, S8).



**Table 4.** Urine biomarkers as ratios with urine creatinine concentration.Longitudinal analysis: Incident hypertension ( $n = 2744$ ).

	Unstandardized beta coefficient ( $p$ -value)	95% CI for odds ratio			Standardized regression coefficient at mean predicted probability 0.32
		Lower	Odds ratio	Upper	
<b>Crude</b>					
NAG-Cr	0.07 ( $p$ 0.48)	0.89	1.07	1.29	0.007
ACR	0.53 ( $p < 0.001$ )	1.32	1.69	2.16	0.044
OCR	0.52 ( $p < 0.001$ )	1.35	1.68	2.08	0.050
<b>Multivariable Model</b>					
NAG-Cr	0.08 ( $p$ 0.45)	0.88	1.08	1.33	0.009
ACR	0.39 ( $p$ 0.005)	1.13	1.48	1.95	0.033
OCR	0.36 ( $p$ 0.003)	1.13	1.43	1.81	0.034
<b>Fully Adjusted Model</b>					
NAG-Cr	0.03 ( $p$ 0.80)	0.84	1.03	1.26	0.003
ACR	0.26 ( $p$ 0.08)	0.97	1.29	1.73	0.022
OCR	0.28 ( $p$ 0.03)	1.02	1.32	1.7	0.027

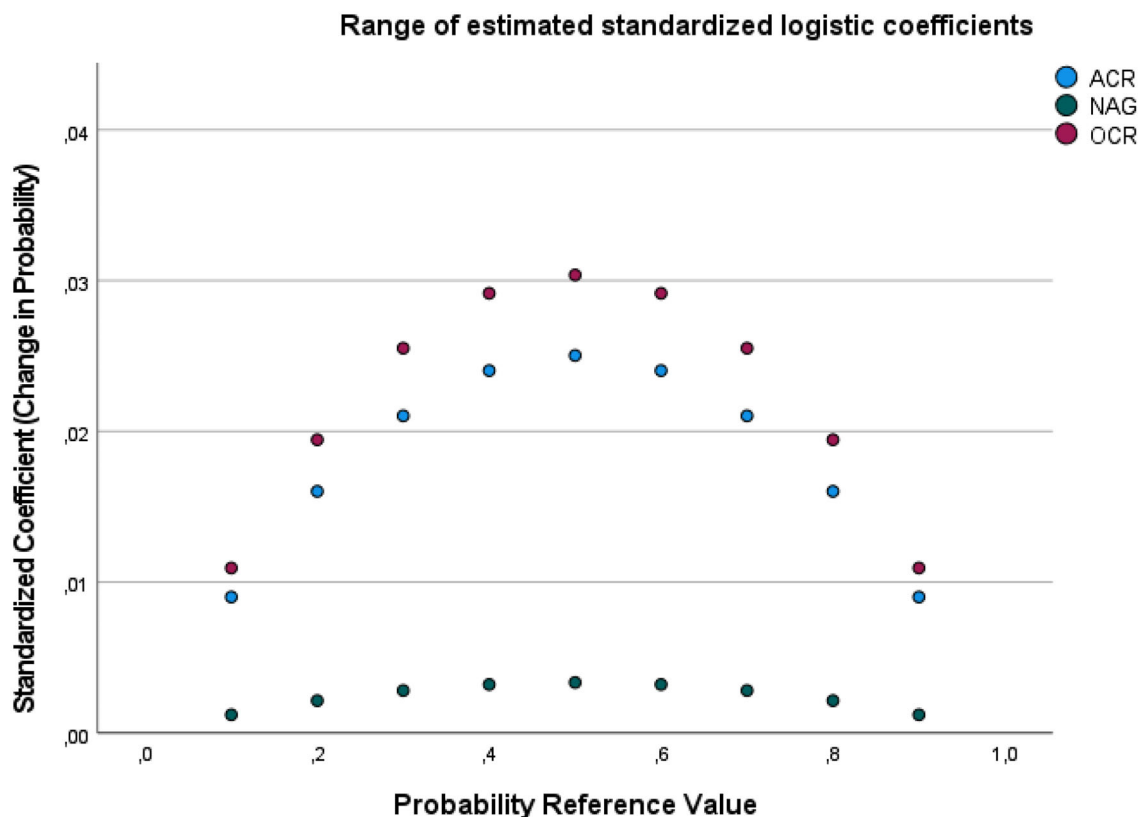
Baseline population Tromsø Study 6\_2 without blood pressure lowering drug and blood pressure  $< 140/90$  (mmHg).Dependent variable: Incident Hypertension defined as use of one or more blood pressure lowering drug or blood pressure  $\geq 140/90$  (mmHg) at T7.

CVD risk factors: Sex, Age, BMI, Current smoker, Diabetes, Prior cardiovascular event (stroke or heart attack).

Crude model: NAG or ACR or OCR.

Multivariable model: Adjusted for CVD risk factors and NAG, or ACR, or OCR.

Fully adjusted model: Adjusted for CVD risk factors and NAG and ACR and OCR.

NAG-Cr: (Log urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio per  $SD$  unit): unstandardized beta coefficients ( $p$ -value), odds ratio with 95% CI and standardized regression coefficient.ACR: (Log urinary albumin to creatinine ratio per  $SD$  unit): unstandardized beta coefficients ( $p$ -value), odds ratio with 95% CI and standardized regression coefficient.OCR (Log urinary orosomuroid to creatinine ratio per  $SD$  unit): unstandardized beta coefficients ( $p$ -value), odds ratio with 95% CI and standardized regression coefficient.

**Figure 2.** Fully adjusted model without eGFR. Range of change in predicted probabilities in incident hypertension. Figure 2 depicts a range of change in predicted probabilities. A standardized coefficient of 0.01 represents 1% of the maximum change possible in predicted probability of incident hypertension. NAG: (Log urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio per  $SD$  unit): standardized beta coefficients at different probability reference value; ACR: (Log urinary albumin to creatinine ratio per  $SD$  unit): standardized beta coefficients at different probability reference value; OCR: (Log urinary orosomuroid to creatinine ratio per  $SD$  unit): standardized beta coefficients at different probability reference value. Fully Adjusted Model: Adjusted for CVD risk factors and NAG and ACR and OCR. CVD risk factors: Sex, Age, BMI, Current smoker, Diabetes, Prior cardiovascular event (stroke or heart attack).

**Table 5.** Urine biomarkers as ratios with urine creatinine concentration.Longitudinal analysis: progressive hypertension ( $n = 353$ ).

	Unstandardized beta coefficient ( $p$ -value)	95% CI for odds ratio			Standardized regression coefficient. at mean Predicted Probability 0.55
		Lower	Odds ratio	Upper	
Crude					
NAG-Cr	-0.02 ( $p$ 0.94)	0.62	0.98	1.56	-0.002
ACR	0.45 ( $p$ 0.024)	1.06	1.57	2.32	0.06
OCR	0.38 ( $p$ 0.058)	0.99	1.47	2.18	0.05
Multivariable model with eGFR					
NAG-Cr	-0.13 ( $p$ 0.06)	0.53	0.88	1.46	-0.014
ACR	0.49 ( $p$ 0.048)	1.00	1.63	2.65	0.069
OCR	0.51 ( $p$ 0.044)	1.01	1.67	2.74	0.070
Fully adjusted model with eGFR					
NAG-Cr	-0.29 ( $p$ 0.28)	0.44	0.75	1.27	-0.033
ACR	0.36 ( $p$ 0.22)	0.81	1.43	2.53	0.051
OCR	0.39 ( $p$ 0.20)	0.82	1.47	2.64	0.053

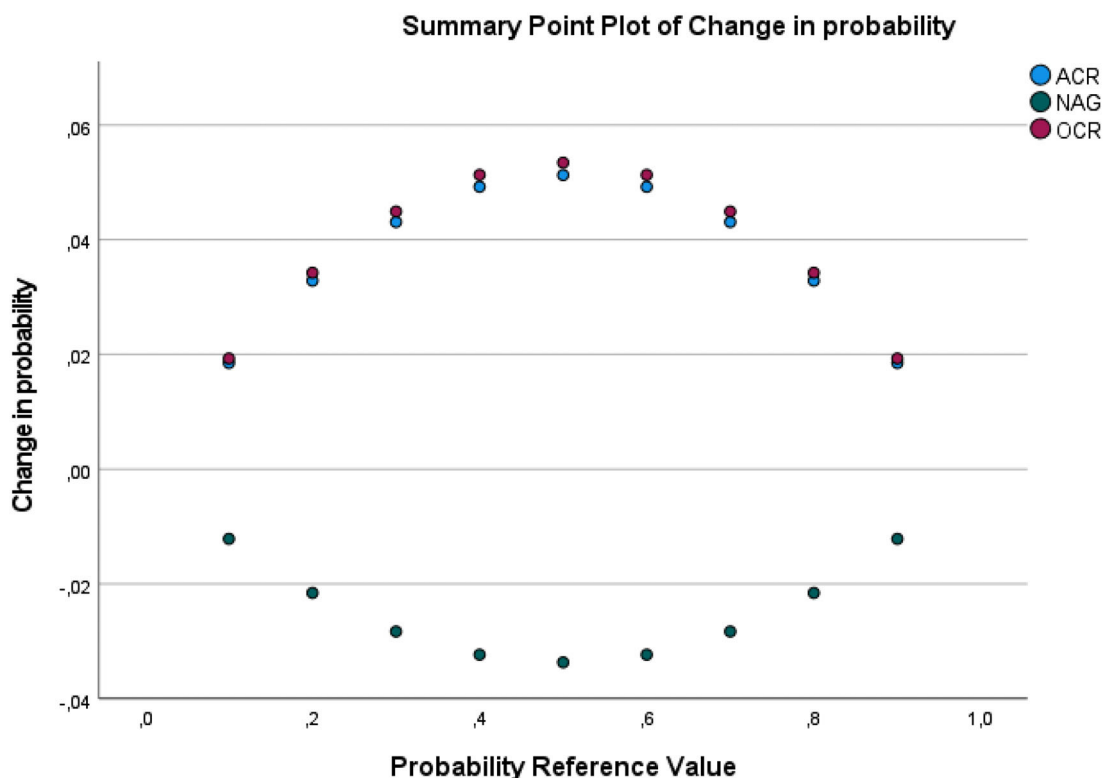
Baseline population Tromsø Study 6–2 with  $\geq 1$  blood pressure lowering drugs and blood pressure  $<140/90$  (mmHg).Dependent variable: Progressive Hypertension defined as an increase in one or more blood pressure lowering drug and/ or blood pressure  $\geq 140/90$  (mmHg) at T7.

CVD risk factors: Sex, Age, BMI, Current smoker, Diabetes, Prior cardiovascular event (stroke or heart attack), eGFR calculated using the creatinine-Cystatin C based CKD-EPI equation.

Crude Model: NAG or ACR or OCR.

Multivariable Model: Adjusted for CVD risk factors and NAG, or ACR, or OCR.

Fully Adjusted Model: Adjusted for CVD risk factors and NAG and ACR and OCR.

NAG-Cr: (Log urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio per  $SD$  unit): unstandardized beta coefficients ( $p$ -value), odds ratio with 95% CI and standardized regression coefficient.ACR: (Log urinary albumin to creatinine ratio per  $SD$  unit): unstandardized beta coefficients ( $p$ -value), odds ratio with 95% CI and standardized regression coefficient.OCR: (Log urinary orosomucoid to creatinine ratio per  $SD$  unit): unstandardized beta coefficients ( $p$ -value), odds ratio with 95% CI and standardized regression coefficient.

**Figure 3.** Fully adjusted model with eGFR. Range of change in predicted probabilities in progressive hypertension. Figure 3 depicts a range of change in predicted probabilities. A standardized coefficient of 0.01 represents 1% of the maximum change possible in predicted probability of progressive hypertension. NAG: (Log urinary N-acetyl- $\beta$ -D-glucosaminidase to creatinine ratio per  $SD$  unit): standardized beta coefficients at different probability reference value; ACR: (Log urinary albumin to creatinine ratio per  $SD$  unit): standardized beta coefficients at different probability reference value; OCR: (Log urinary orosomucoid to creatinine ratio per  $SD$  unit): standardized beta coefficients at different probability reference value. Fully Adjusted Model: Adjusted for CVD risk factors and NAG and ACR and OCR. CVD risk factors: Sex, Age, BMI, Current smoker, Diabetes, Prior cardiovascular event (stroke or heart attack), eGFR calculated using the creatinine-Cystatin C based CKD-EPI equation.

## Discussion

The present population-based, cross-sectional and prospective observational study is, according to our knowledge, the first study that aims to compare UAE, urinary orosomuroid and NAG in relation to blood pressure and the development and progression of hypertension. Associations between urinary orosomuroid and urinary NAG with kidney function decline, CKD, and CVD have been described [26,43–48]. Mechanisms for these associations are unclear, and correlation coefficients between UAE and these biomarkers are small to moderate, between 0.1 and 0.5 [26,49,50], and traditional cardiovascular risk factors such as hypertension may modify the associations. In our study, urine orosomuroid excretion had a marginally stronger association than UAE with blood pressure in the cross-sectional analysis and with incident hypertension in the longitudinal analysis. These findings may be of clinical implication because both eGFR and ACR are insensitive biomarkers of early kidney and endothelial damage in hypertension at a stage when prevention may be most effective [4,11,12].

Orosomuroid is a 41–43 kDa acute phase glycoprotein, synthesized mainly by hepatocytes [51], but also produced in endothelial cells [16]. Similar to albumin, it is negatively charged due to the presence of sialic acids [51]. In addition to functioning as a serum transport protein [52,53], it modulates immune and inflammatory responses [54,55], and it is a constituent of the endothelial surface layer and maintains permselectivity [18,19]. A study of plasma orosomuroid, and other inflammation-sensitive plasma proteins, showed a cross-sectional association with blood pressure and a longitudinal association with increased stroke risk [45]. In patients with type 2 diabetes and normal UAE, increased urinary orosomuroid independently predicted cardiovascular mortality [50,56,57]. This relationship is proposed to be caused by inflammation and early endothelial dysfunction [58]. Sun et al found an association between established markers of kidney dysfunction, orosomuroid and hypertension in a prospective study on people with prediabetes [48]. Urine, but not serum orosomuroid, may also be an early and predictive marker for lupus nephritis [22,59], indicating that urine orosomuroid may reflect a pathophysiological process in the kidneys, and not just systemic inflammation. Levels of urinary orosomuroid was significantly higher with active compared to inactive lupus nephritis, both for absolute levels and levels standardized by urine creatinine. Urine orosomuroid was however not significantly different

between lupus nephritis groups when corrected for nonselective proteinuria [22]. Due to its smaller molecular weight and possibly less efficient tubular reabsorption [60,61], urinary orosomuroid excretion may be a more specific marker of endothelial dysfunction and glomerular damage than UAE [18,19,22]. The pathophysiological mechanisms of orosomuroid in hypertension has not been clarified and we cannot make inferences about causality from observational studies. A cross sectional study of participants in Tromsø 6 second visit showed an association between urinary orosomuroid and diastolic dysfunction and carotid arteriopathy [62]. UAE was not significantly associated with diastolic dysfunction, but the associations with carotid arteriopathy were comparable to UOCR. In our study, urine orosomuroid showed a stronger relationship with blood pressure and the development of hypertension than UAE, but further prospective clinical studies are needed to assess if orosomuroid is a clinically useful biomarker in hypertension.

The proximal tubules are responsible for reabsorbing approximately 65% of filtered load and most low molecular weight proteins [63]. It is hypothesized that subclinical kidney injury, with the development of afferent arteriopathy and tubulointerstitial disease, especially in the outer medulla, leads to kidney ischemia, inflammation and oxidative stress, which is associated with a decrease in sodium filtration and an increase in blood pressure [4]. Urinary NAG is a marker of proximal tubular cell function and is considered a sensitive marker related to inflammation and oxidative stress [25]. Earlier studies of urinary NAG in blood pressure and hypertension are limited in sample size or in the urine collection method [12,64–67]. A small study of patients with newly diagnosed hypertension without microalbuminuria found a significantly higher NAG activity in hypertensives compared to healthy controls [28]. Further, NAG, but not ACR, was significantly associated with incident hypertension among the HIV-uninfected women in the Women's Interagency HIV Study [64]. In this general population cohort, consisting of participants with overall normal kidney function, urinary NAG yielded only weak or non-significant associations with blood pressure and hypertension in our multivariable models with CVD risk factors.

Further, the limit of detection for the albumin concentration assay corresponded almost to the population's median albumin concentration, similarly to other studies [8–11]. Orosomuroid was analyzed by a highly sensitive immunoassay with a limit of detection

below the lowest measured value in our population [35]. Additional recommended analyses with ACR left-censored [8,35] reinforced the described pattern of associations between biomarkers, blood pressure and incident hypertension. In the left-censored analyses, OCR had the strongest association with blood pressure in treated and untreated subjects. Further, only OCR had significant associations with incident hypertension in the additional analysis.

The best measure of biomarker excretions in urine is likely to vary for glomerular and tubular biomarkers [26,68]. We used morning urine samples collected on three consecutive days. We reduced the impact of a day-to-day variation using the median values of the three specimens. There is a high correlation between 24-h urine albumin excretion and ACR [69,70]. Whether this correlation also applies to OCR is unknown. Timed urine collections may be problematic due to imprecision in timing and volume of the collections, especially in outpatients settings. But tubular biomarker excretion in urine may be more accurately estimated with timed collections than in spot urine samples. The excretion of a tubular biomarker may not correlate with the amount of filtered creatinine. The timed collections may further be expressed with ratios of urine creatinine to adjust for varying urine concentration and dilution [71]. To study the effect of the urine creatinine concentration, we analyzed the relationship between the biomarkers, blood pressure and hypertension both with and without adjustment for urine creatinine. The associations of urinary albumin and orosomucoid concentrations with blood pressure and incident hypertension did not differ substantially from the corresponding associations with ACR and OCR. NAG showed a stronger association with blood pressure and incident hypertension than NAG-Cr. The analysis without adjustment for urine creatinine did not affect the assessment of the strength of these associations compared to urinary albumin excretion (UAE). The functioning nephron mass may be another factor altering the urine biomarker excretion [72]. However, in our study, adjusting for eGFR did not change associations with blood pressure and hypertension for most analyses. The relatively healthy study population with a narrow distribution of eGFR may explain this.

Participants with the highest blood pressure at baseline, or the largest increase in blood pressure during follow-up, would be most likely to increase their antihypertensive treatment. To take this into account, we dichotomized blood pressure into incident

hypertension and progressive hypertension in the longitudinal analysis, which led to a loss of statistical power.

The strengths of the present study includes the combination of a cross-sectional and prospective design, the inclusion of a large, well described cohort with a high attendance rate, and an extended follow-up. Moreover, using three urinary samples per participants made the estimation of the biomarkers more robust. However, there are several limitations. The population included in the Tromsø Study consists almost exclusively of North-European middle-aged and elderly persons, limiting broad generalizations. Information about drug dosing or adherence to prescribed hypertension treatment was lacking, which may bias the results, particularly in the analysis assessing progressive hypertension. We were unable to obtain information regarding detection limits for the NAG measurements. Finally, this study did not address the associations between the urine biomarkers and clinically relevant endpoints in hypertension.

## Conclusions

In a cohort from the general population, urine orosomucoid excretion showed a stronger cross-sectional association with blood pressure in treated and untreated individuals and a stronger association with incident hypertension after 7 years than UAE. Urine orosomucoid excretion may be an earlier biomarker than UAE for kidney and endothelial damage in hypertension. However, further studies are needed to determine the pathophysiological mechanisms and the clinical value of orosomucoid as a biomarker in hypertension. The tubulointerstitial function is essential in blood pressure regulation, and tubulointerstitial damage appears early in CKD. However, we found only varying and weak associations between U-NAG, blood pressure and hypertension. The observational design limits suggestions about cause and effect and further prospective studies should be undertaken.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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