RESEARCH

Serum uromodulin is inversely associated with the metabolic syndrome in the KORA F4 study

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Abstract

Objective: Metabolic syndrome and obesity are risk factors for chronic kidney disease. However, early kidney alterations may escape diagnosis in these conditions due to glomerular hyperfiltration. Uromodulin, a glycoprotein exclusively synthesized in tubular cells of the thick ascending limb of Henle´s loop, is a novel tissue-specific biomarker for kidney function. In contrast to the commonly used markers creatinine and cystatin C, serum uromodulin does not primarily depend on glomerular filtration. We hypothesized that serum uromodulin is a marker for metabolic syndrome and related components.

Design: The analyses included 1088 participants of the population-based KORA F4 study aged 62–81 years. Metabolic syndrome was present in 554 participants. After a mean follow-up time of 6.5 years, 621 participants were reevaluated, of which 92 had developed incident metabolic syndrome.

Methods: The association of serum uromodulin with metabolic syndrome and its components were assessed using multivariable logistic regression models.

Results: Serum uromodulin was inversely associated with metabolic syndrome after adjustment for sex, age, estimated glomerular filtration rate, physical activity, smoking, alcohol consumption and high-sensitivity C-reactive protein (OR 0.65; 95% CI 0.56–0.76 per standard deviation uromodulin; $P < 0.001$). Serum uromodulin was inversely associated with all single components of metabolic syndrome. However, serum uromodulin was not associated with new-onset metabolic syndrome after the follow-up period of 6.5 ± 0.3 years (OR 1.18; 95% CI 0.86–1.60).

Conclusions: Serum uromodulin is independently associated with prevalent, but not with incident metabolic syndrome. Low serum uromodulin may indicate a decreased renal reserve in the metabolic syndrome.

Key Words

▶ serum uromodulin
▶ uromodulin
▶ sUmod
▶ metabolic syndrome
▶ obesity
▶ kidney function

Introduction

The metabolic syndrome is associated with an increased risk for cardiovascular and renal complications, and chronic renal failure in metabolic syndrome may progress to end-stage renal disease (1, 2). Kidney pathophysiological alterations reveal a type of ischemic nephropathy with predominant tubular atrophy in addition to interstitial fibrosis, microvascular sclerosis and segmental glomerulosclerosis (3, 4). However, kidney alterations in metabolic syndrome may escape early clinical diagnosis, since obesity and other components of the metabolic syndrome, such as elevated fasting glucose and elevated blood pressure, are associated with glomerular hyperfiltration. The currently available kidney function markers (creatinine, cystatin C and urea) all reflect the glomerular filtration rate (GFR) and may thus deliver falsely inconspicuous results in case of glomerular hyperfiltration. Additionally, obesity may be associated with variable degrees of skeletal muscle loss, including sarcopenic obesity, which is especially frequent in elderly individuals and can further influence the estimated GFR (eGFR) values based on creatinine (5). Therefore, additional kidney function markers not directly depending on glomerular filtration would be useful to unravel early kidney disease in elderly individuals with metabolic syndrome.

The currently most promising alternate marker of kidney function is uromodulin. Uromodulin is a tissue-specific glycoprotein synthesized in tubular cells of the thick ascending limb of Henle´s loop. The larger proportion of uromodulin is secreted into the urinary tract, where it is the most abundant protein under physiological conditions (50–100 mg/24 h) and exerts anti-infective, anti-lithogen and immunomodulatory functions (6, 7, 8, 9, 10). In addition to the apical secretion, a small amount of uromodulin is secreted basolaterally into the interstitial space and transferred into the blood stream (11, 12, 13). The physiological function of serum uromodulin (sUmod) is elusive to date, but sUmod emerged as a highly interesting marker of kidney function (12, 13). As a tubular protein, sUmod does not directly depend on glomerular filtration, but mirrors tubular integrity and indirectly nephron mass and thus the renal reserve (14).

In the current study, we used sUmod as a novel marker of kidney integrity in elderly participants of the population-based KORA F4 cohort. We hypothesized that sUmod was inversely associated with metabolic syndrome independently of the eGFR, indicating a decreased renal tubular reserve in metabolic syndrome, obesity and related conditions.

Materials and methods

Study participants

The KORA (Cooperative Health Research in the Region of Augsburg) F4 (2006-2008) and FF4 (2013-2014) cohort studies are follow-up examinations of the population-based KORA S4 study (1999-2001). Recruitment and eligibility criteria for the KORA studies have been described previously (15). The study design, standardized sampling methods and data collection (medical history, medication, anthropometric measurements, blood pressure) have been described in detail elsewhere (16, 17). All study participants gave written informed consent. The study was approved by the Ethics Committees of the Bavarian Medical Association in adherence to the Declaration of Helsinki. sUmod was measured in the 1119 participants aged 62–81 years of the KORA F4 study with available serum samples (from a total of 1161 participants in this age group). All variables necessary for the cross-sectional analyses were available in 1088 participants. Of these 1088 participants, 116 died and 341 declined participation in the FF4 survey or could not be contacted. Thus, the study sample in the longitudinal F4/FF4 examination comprised 631 participants, of which ten were excluded due to missing covariables, leaving 621 participants for the longitudinal analyses (Supplementary Fig. 1, see section on supplementary data given at the end of this article). The follow-up time was 6.5 ± 0.3 years. Clinical characteristics of the participants included in the follow-up analysis are available in Supplementary Table 1. Metabolic syndrome was defined according to the International Diabetes Federation definition as presence of at least three of the following five criteria: (1) elevated waist circumference (waist circumference ≥94 cm in men and ≥80 cm in women); (2) fasting triglycerides ≥1.7 mmol/L and/or use of fibrates or nicotinic acid; (3) high-density lipoprotein (HDL) cholesterol <1.0 mmol/L in men or <1.3 mmol/L in women and/or use of fibrates or nicotinic acid; (4) fasting glucose ≥5.6 mmol/l and/or use of glucose-lowering medication; (5) elevated blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive) (18). No participant took omega 3 fatty acid in a dose ≥2 g/day. Leisure time physical activity was assessed with two separate questions concerning leisure time sport activity in winter and in summer (cycling included). Possible answers were (1) >2 h, (2) 1–2 h, (3) <1 h and (4) none per week. Participants who had a total score <5, obtained by summing the numbers

(1)–(4) relating to winter and summer, were classified to be 'physically active'. Alcohol consumption was categorized in three groups defined as no (0 g/day), moderate (men 0.1–39.9 g/day and women 0.1–19.9 g/day), and high (men ≥40 g/day and women ≥20 g/day) alcohol consumption. Smoking behavior was categorized into three groups (active smoker, former smoker, never smoker).

Laboratory measurements

Blood was collected after an overnight fast of at least 8 h. The samples were kept at room temperature until centrifugation. Plasma and serum samples were assayed immediately or stored at −80°C. Measurements of serum creatinine (fresh serum), high-sensitivity C-reactive protein (hsCRP) (frozen plasma), glucose (fresh serum), total cholesterol, low-density lipoprotein (LDL), HDL and triglycerides (all fresh serum) were performed as described previously (19, 20). sUmod (frozen serum) was measured with a commercially available enzyme-linked immunosorbent assay kit (Euroimmun AG, Lübeck, Germany) with a lower detection limit of 2 ng/mL, an intra-assay coefficient of variation of 2.3% and inter-assay coefficients of variation of 4.4% and 9.5% for sUmod target values of 24.9 and 142.2 ng/mL, respectively. The measurement procedure was performed as described by Steubl *et al.* (12). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (2009) based on serum creatinine (21).

Statistical analyses

Characteristics of the study participants were compared between participants with and without metabolic syndrome using *t*-tests in case of approximately normally distributed variables. Mann–Whitney *U* tests were performed for variables with skewed distributions. Binomial proportions were compared with chi-square tests. The associations of sUmod with the outcomes of interest were assessed in logistic regression models in case of categorical dependent variables and in linear regression models in case of continuous dependent variables. sUmod was analyzed as log-transformed continuous independent variable per standard deviation. In multinomial logistic and linear regression analyses, the associations of sUmod with the respective dependent variables were adjusted for covariates in different models: Model 1: age (continuous) and sex; Model 2: Model 1 plus eGFR (log-transformed,

continuous); Model 3: Model 2 plus physical activity (active/inactive), smoking status (never/former/current), and alcohol consumption (no/moderate/high); Model 4: Model 3 plus hsCRP (log-transformed, continuous); Model 5: Model 4 plus arterial hypertension (yes/no) and fasting glucose (continuous); Model 6: Model 5 plus BMI (continuous) and treatment with statins and fibrates (yes/no, respectively). Model 1 and Model 2 were calculated for all outcome variables; Model 3 and Model 4 for the metabolic syndrome and its components; Model 5 for anthropometric measures and Model 6 for lipid parameters as outcome variables. Preexisting cases were excluded from the longitudinal incidence analyses. The level of statistical significance was set at 5% (two-sided). All calculations were performed using the statistical environment R, version 3.5.2.

Results

Study population characteristics

Table 1 displays the baseline characteristics of the total study population and stratified by metabolic syndrome. Metabolic syndrome was present in 43% of the participating women and in 56% of the men, corresponding to 51% of the total cohort. Participants with metabolic syndrome had significantly lower sUmod concentrations compared to participants without metabolic syndrome (*P*<0.001).

Inverse association of sUmod with the metabolic syndrome

sUmod displayed an inverse association with the metabolic syndrome (odds ratio (OR) 0.58; 95% confidence interval (CI) 0.50–0.67) after adjustment for sex and age (Table 2). Additional correction for eGFR modestly attenuated the association (OR 0.61; 95% CI 0.53–0.70). Further adjustment for physical activity, smoking and alcohol consumption had hardly any influence (OR 0.62; 95% CI 0.53–0.72) and additional adjustment for hsCRP did not substantially attenuate the associations of sUmod with the metabolic syndrome (OR 0.65; 95% CI 0.56–0.76).

Inverse association of sUmod with single components of the metabolic syndrome

sUmod was inversely associated with each single component of the metabolic syndrome (Table 2). After adjustment for age, sex, eGFR, physical activity,

Table 1 Characteristics of the study participants.[a]

	Total study cohort	No metabolic syndrome	Metabolic syndrome	*P* value
n	1088	534	554	
Female sex, *n* (%)	537 (49)	301 (56)	236 (43)	<0.001[k]
Age (years)	70.3 ± 5.5	70.1 ± 5.6	70.5 ± 5.4	0.32[i]
Elevated waist circumference *n* (%)[b]	913 (84)	375 (70)	538 (97)	<0.001[k]
Elevated triglycerides *n* (%)[c]	308 (28)	31 (6)	277 (50)	<0.001[k]
Reduced HDL cholesterol *n* (%)[d]	206 (19)	17 (3)	189 (34)	<0.001[k]
Elevated fasting glucose *n* (%)[e]	528 (49)	80 (15)	448 (81)	<0.001[k]
Elevated blood pressure *n* (%)[f]	797 (73)	288 (54)	509 (92)	<0.001[k]
BMI (kg/m^2)	28.7 ± 4.3	26.9 ± 3.9	30.4 ± 4.3	<0.001[i]
Waist circumference (cm)	98.1 ± 12.1	92.5 ± 10.9	103.4 ± 10.6	<0.001[i]
Waist-to-hip ratio	0.91 ± 0.08	0.88 ± 0.08	0.94 ± 0.07	<0.001[i]
Total cholesterol (mmo/L)	5.77 ± 1.04	5.85 ± 1.04	5.69 ± 1.07	0.007[i]
HDL cholesterol (mmol/L)	1.45 ± 0.37	1.61 ± 0.34	1.30 ± 0.31	<0.001[i]
Ratio total cholesterol/HDL cholesterol	4.18 ± 1.13	3.75 ± 0.87	4.59 ± 1.19	<0.001[i]
LDL cholesterol (mmol/L)	3.65 ± 0.93	3.67 ± 0.94	3.61 ± 0.93	0.24[i]
Triglycerides (mmol/L)	1.28 (0.93; 1.78)	1.06 (0.82; 1.35)	1.70 (1.20; 2.33)	<0.001[j]
hsCRP (mg/dL)	1.52 (0.78; 3.11)	1.29 (0.64; 2.48)	1.98 (0.98; 3.82)	<0.001[j]
eGFR (mL/min/1.73 m^2)	77.9 (67.3; 87.7)	80.1 (67.3; 87.8)	75.4 (64.2; 86.2)	<0.001[j]
Physically inactive *n* (%)[g]	537 (49)	236 (44)	301 (54)	0.001[k]
Smoker never/former/current (%)	49/45/6	51/41/7	47/48/5	0.07[k]
Alcohol consumption no/moderate/high (%)[h]	32/56/12	30/59/11	33/54/13	0.23[k]
Serum uromodulin (ng/mL)	153 (111; 208)	176 (130; 227)	132 (99; 186)	<0.001[j]

[a]Mean ± standard deviation, median (first quartile; third quartile), or number of participants (proportion in %). [b]Defined as ≥80 cm in women and ≥94 cm in men. [c]Defined as ≥1.7 mmol/L and/or intake of fibrates or nicotinic acid. [d]Defined as <1.0 mmol/L in men and <1.3 mmol/L in women and/or intake of fibrates or nicotinic acid. [e]Defined as ≥5.6 mmol/L and/or intake of anti-diabetic medication. [f]Defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive. [g]Physically inactive: <1-h sports/week in winter and summer. [h]Alcohol consumption: no (0 g/day), moderate (men 0.1–39.9 g/day and women 0.1–19.9 g/day), high (men ≥40 g/day and women ≥20 g/day). [i]*T*-test; [j]Mann–Whitney *U* test; [k]chi-square test.

smoking and alcohol consumption, sUmod was significantly inversely associated with an elevated waist circumference, elevated triglycerides, reduced HDL cholesterol, elevated fasting glucose and elevated blood pressure. As for the metabolic syndrome itself, the major influencing factor for the associations of sUmod with the single metabolic syndrome components was eGFR, except for the association of sUmod with elevated waist circumference, which was hardly influenced by adjustment for eGFR. Additional adjustment for hsCRP attenuated most of the associations of sUmod with the metabolic syndrome and its components moderately, which still remained significant, except for the association of sUmod with elevated waist circumference that lost significance.

Table 2 Odds ratios (95% confidence interval) for metabolic syndrome and single components of the metabolic syndrome as dependent variables and sUmod as independent variable (per standard deviation): results of logistic regression models.

Metabolic syndrome (yes: *n* = 554; no: ***n*** = 534)	Elevated waist circumference[a] (yes: *n* = 913; no: ***n*** = 175)	Elevated triglycerides[b] (yes: *n* = 308; no: ***n*** = 780)	Reduced HDL cholesterol[c] (yes: *n* = 206; no: ***n*** = 882)	Elevated fasting glucose[d] (yes: *n* = 528; no: ***n*** = 560)	Elevated blood pressure[e] (yes: *n* = 797; no: ***n*** = 291)
Adjustment for sex and age					
0.58 (0.50–0.67)[f]	0.71 (0.59–0.86)[f]	0.69 (0.60–0.80)[f]	0.70 (0.60–0.81)[f]	0.69 (0.60–0.79)[f]	0.64 (0.54–0.75)[f]
Adjustment for sex, age and eGFR					
0.61 (0.53–0.70)[f]	0.71 (0.59–0.87)[f]	0.74 (0.64–0.86)[f]	0.76 (0.64–0.90)[f]	0.71 (0.62–0.81)[f]	0.67 (0.56–0.79)[f]
Adjustment for sex, age, eGFR, physical activity, smoking and alcohol consumption					
0.62 (0.53–0.72)[f]	0.73 (0.60–0.89)[g]	0.75 (0.64–0.87)[f]	0.78 (0.66–0.93)[g]	0.72 (0.62–0.82)[f]	0.67 (0.56–0.79)[f]
Adjustment for sex, age, eGFR, physical activity, smoking, alcohol consumption and hsCRP					
0.65 (0.56–0.76)[f]	0.82 (0.67–1.01)	0.75 (0.65–0.88)[f]	0.81 (0.68–0.97)[h]	0.74 (0.64–0.86)[f]	0.70 (0.59–0.83)[f]

[f]*P* < 0.001; [g]*P* < 0.01; [h]*P* < 0.05.
[a]Defined as ≥80 cm in women and ≥94 cm in men. [b]Defined as ≥1.7 mmol/l and/or intake of fibrates or nicotinic acid. [c]Defined as <1.0 mmol/l in men and <1.3 mmol/l in women and/or intake of fibrates or nicotinic acid. [d]Defined as ≥5.6 mmol/l and/or intake of anti-diabetic medication. [e]Defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive.

Table 3 Association estimates between sUmod and anthropometric outcome variables: β coefficient ± standard error from linear regression models are given per standard deviation sUmod ($n = 1088$).

BMI (continuous)	Waist circumference (continuous)	Waist-to-hip ratio (continuous)
Adjustment for sex and age		
-0.21 ± 0.03[a]	-0.18 ± 0.02[a]	-0.10 ± 0.02[a]
Adjustment for sex, age and eGFR		
-0.19 ± 0.03[a]	-0.17 ± 0.03[a]	-0.09 ± 0.02[a]
Adjustment for sex, age, eGFR, physical activity, smoking, alcohol consumption, hsCRP, arterial hypertension, fasting glucose		
-0.08 ± 0.03[b]	-0.08 ± 0.02[b]	-0.04 ± 0.02

[a]$P < 0.001$; [b]$P < 0.01$.

In addition to waist circumference, BMI and waist-to-hip ratio were also inversely associated with sUmod after adjustment for sex and age ($P < 0.001$; Table 3). The association of sUmod with BMI remained significant in the multivariable regression models ($P < 0.01$), whereas its association with the waist-to-hip ratio lost significance after multivariable adjustment.

Analysis of further serum lipid parameters revealed an association between sUmod and increased total cholesterol, LDL and HDL cholesterol, and the ratio of total cholesterol to HDL cholesterol after adjustment for sex, age and eGFR. However, these associations did not remain significant in the fully adjusted model (Table 4). Thus, among the serum lipid parameters, only two major components of the metabolic syndrome, HDL cholesterol (β: 0.10 ± 0.03) and triglycerides (β: -0.10 ± 0.03), displayed a significant association with sUmod after multivariable adjustment.

Lack of an association of sUmod with incident metabolic syndrome in the longitudinal analysis

Baseline sUmod was not associated with new-onset metabolic syndrome or incidence of any component of the metabolic syndrome after the follow-up time of 6.5 ± 0.3 years (Table 5). Furthermore, sUmod was not associated

with changes of BMI or waist-to-hip ratio after adjustment for sex, age, eGFR and the respective baseline parameter (data not shown). After multivariable adjustment including sex, age, eGFR, physical activity, smoking, alcohol consumption, hsCRP, arterial hypertension, fasting glucose, BMI, the respective baseline parameter and intake of statins and fibrates in the follow-up examination, there was no association of sUmod with total cholesterol, LDL, HDL, the ratio of total cholesterol to HDL cholesterol and triglycerides in the follow-up examination (data not shown).

Discussion

The key finding of the current study is the strong and independent inverse relationship between sUmod and the metabolic syndrome in a large population-based elderly cohort. Low sUmod levels were associated with single components of the metabolic syndrome (elevated triglycerides, reduced HDL cholesterol, elevated fasting glucose and elevated blood pressure), indicating that sUmod is widely connected with metabolic alterations. To our knowledge, a detailed examination of the association of uromodulin with the metabolic syndrome has not been published before.

Table 4 Association estimates between sUmod and parameters of lipid metabolism: β coefficient ± standard error from linear regression models are given per standard deviation sUmod ($n = 1088$).

Total cholesterol	LDL cholesterol	HDL cholesterol	Ratio total cholesterol to HDL cholesterol	Triglycerides
Adjustment for sex and age				
0.12 ± 0.03[a]	0.10 ± 0.03[b]	0.21 ± 0.03[a]	-0.12 ± 0.03[a]	-0.18 ± 0.03[a]
Adjustment for sex, age and eGFR				
0.11 ± 0.03[b]	0.09 ± 0.03[c]	0.19 ± 0.03[a]	-0.10 ± 0.03[b]	-0.16 ± 0.03[a]
Adjustment for sex, age, eGFR, physical activity, smoking, alcohol consumption, hsCRP, arterial hypertension, fasting glucose, BMI, treatment with statins and fibrates				
0.06 ± 0.03	0.04 ± 0.03	0.10 ± 0.03[a]	-0.54 ± 0.03	-0.10 ± 0.03[a]

[a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$.

Table 5 Odds ratios (95% confidence interval) for incident metabolic syndrome and incident single components of the metabolic syndrome as dependent variables and sUmod as independent variable (per standard deviation): results of logistic regression models (*n* = 621). The numbers of participants with and without event are given in parentheses.

Metabolic syndrome (yes: *n* = 92; no: **n** = 233)	Elevated waist circumference[a] (yes: *n* = 60; no: **n** = 56)	Elevated triglycerides[b] (yes: *n* = 46; no: **n** = 409)	Reduced HDL cholesterol[c] (yes: *n* = 9; no: **n** = 508)	Elevated fasting glucose[d] (yes: *n* = 121; no: **n** = 209)	Elevated blood pressure[e] (yes: *n* = 62; no: **n** = 126)
Adjustment for sex and age					
1.05 (0.79–1.39)	0.69 (0.45–1.07)	1.05 (0.74–1.50)	0.77 (0.40–1.50)	0.92 (0.71–1.19)	0.99 (0.70–1.40)
Adjustment for age, sex and eGFR					
1.13 (0.84–1.52)	0.69 (0.45–1.08)	1.03 (0.72–1.48)	0.79 (0.40–1.56)	0.96 (0.74–1.26)	0.99 (0.70–1.40)
Adjustment for sex, age, eGFR, physical activity, smoking, alcohol consumption and hsCRP					
1.18 (0.86–1.60)	0.75 (0.47–1.21)	1.03 (0.71–1.48)	0.68 (0.34–1.36)	1.00 (0.76–1.32)	1.07 (0.74–1.55)

[a]Defined as ≥80 cm in women and ≥94 cm in men. [b]Defined as ≥1.7 mmol/L and/or intake of fibrates. [b]Defined as ≥1.7 mmol/L and/or intake of fibrates. [c]Defined as <1.0 mmol/L in men and <1.3 mmol/l in women and/or intake of fibrates. [d]Defined as ≥5.6 mmol/L and/or intake of anti-diabetic medication. [e]Defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive. [f]Number of participants with/without event.

In the urinary tract, uromodulin exerts immunomodulatory functions (9, 10). Whether immunomodulatory uromodulin properties also play a role in the circulation is not yet known. Nevertheless, uromodulin is capable of binding immunoglobulins and complement and to activate immune cells in experimental settings, indicating the presence of relevant systemic effects (22, 23, 24). Since obesity and metabolic syndrome are associated with low-grade inflammation, immunomodulatory sUmod properties might positively influence metabolism in this condition. This may serve as one explanation of an inverse connection of uromodulin and metabolic disturbances. In our cohort, sUmod was inversely associated with hsCRP (25). However, adjustment for hsCRP only moderately attenuated the association of sUmod with the metabolic syndrome and its components, indicating that chronic subclinical inflammation as assessed by hsCRP is not the only factor explaining the interplay of the metabolic syndrome and sUmod. Only the association of sUmod with elevated waist circumference was more strongly attenuated and lost significance after additional adjustment for hsCRP, possibly reflecting a stronger impact of chronic subclinical inflammation related to increased visceral adipose tissue on this association.

Despite the strong inverse associations in the cross-sectional analysis, there was no evidence for an association between sUmod and the development of metabolic syndrome, nor any of its components. Thus, metabolic factors may negatively influence sUmod levels, indicating that the metabolic syndrome may impair tubular integrity and protein synthesis. Recently, Scheurlen *et al.* described an increase of sUmod following Roux-en-Y-gastric bypass in patients with severe obesity, indicating improved 'kidney health' or renal reserve after bariatric surgery, which was

not detectable by traditional kidney function markers (26). We (25) and others (27) previously showed that sUmod was inversely associated with type 2 diabetes independently of the eGFR. Thus, sUmod possibly represents a tissue-specific marker for early diabetic nephropathy that escapes GFR estimates due to diabetes-related glomerular hyperfiltration. The inverse association of sUmod with BMI and waist circumference independently of fasting glucose in the current study indicates that sUmod may also unravel other early kidney alterations related to hyperfiltration independently of hyperglycemia-related hyperfiltration. An inverse relation of sUmod with BMI was also described by Steubl *et al.* (12) and is possibly due to a relatively lower nephron mass in obesity, which is characteristically not detected by eGFR due to obesity-related hyperfiltration, but represents a risk factor for chronic kidney disease (2). However, in another cohort comprising 200 participants, of which 170 suffered from chronic kidney disease, sUmod was not associated with BMI (28). These discrepancies supposedly originate from differences in the study populations, since in overt preexisting chronic kidney disease, uromodulin values are substantially reduced as a sequelae of tubular atrophy, interstitial fibrosis and an overall loss of nephrons unrelated to metabolic alterations. The discrepancies between the studies emphasize the need to further elucidate the impact of sUmod on human health and disease in the general community.

The strong association of sUmod with HDL and triglycerides is a remarkable finding. In order to determine the independence of the correlation of sUmod with HDL and triglycerides from obesity and other metabolic traits, we performed a thorough adjustment for possible confounders. The associations still remained significant, indicating an intrinsic interaction of sUmod with HDL

and triglycerides. In the urinary tract, uromodulin is able to bind various components including leukocytes, fimbriated bacteria and calcium phosphate nanocrystals (6). It is not yet clarified, whether sUmod also has similar properties in the circulation and can bind, modulate, neutralize or eliminate serum components as well. Intrinsic systemic effects of sUmod are very probable. For example, sUmod is inversely associated with incident cardiovascular events independently of the eGFR and well-known traditional cardiovascular risk factors, indicating an intrinsic vasoprotective effect of the glycoprotein that has not yet been specified (29, 30). However, the weak or missing associations of sUmod with total cholesterol and LDL, and the lack of an association of sUmod with lipid levels in the longitudinal analysis argue against a broad implication of sUmod in lipid metabolism.

Study strengths and limitations

Strengths of our study are the population-based design with a large, well-characterized community-based cohort, and the follow-up time of 6.5 years. Unlike most previous studies using urinary uromodulin, we measured sUmod with a sensitive and robust ELISA. In contrast to uromodulin of urine origin, which forms various polymers with changing episodes and different antigenic sites (31), sUmod is a stable antigen lacking such important pre-analytical disadvantages (13). To our knowledge, this is the first study elucidating the association of sUmod with the metabolic syndrome. However, only participants aged 62–81 years were included, so that the relation of sUmod with metabolic disturbances remains to be confirmed in a younger population. Further, the incidence of some single components of the metabolic syndrome, namely elevated triglycerides and reduced HDL cholesterol was relatively low, limiting the power of the study in these longitudinal analyses.

Conclusions

We demonstrate an inverse association of prevalent metabolic syndrome with sUmod in elderly study participants from the general community. Further studies are required to assess whether a decrease of sUmod levels in metabolic syndrome, indicating tubular injury, reflects a reduced renal reserve due to a relatively lower nephron mass in this condition or whether obesity and other risk factors in concert with the metabolic syndrome influence uromodulin biosynthesis.

References

1 Galassi A, Reynolds K & He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. *American Journal of Medicine* 2006 **119** 812–819. (https://doi.org/10.1016/j.amjmed.2006.02.031)

2 Garofalo C, Borrelli S, Minutolo R, Chiodini P, De Nicola L & Conte G. A systematic review and meta-analysis suggests obesity

predicts onset of chronic kidney disease in the general population. *Kidney International* 2017 **91** 1224–1235. (https://doi.org/10.1016/j.kint.2016.12.013)

3 Alexander MP, Patel TV, Farag YM, Florez A, Rennke HG & Singh AK. Kidney pathological changes in metabolic syndrome: a cross-sectional study. *American Journal of Kidney Diseases* 2009 **53** 751–759. (https://doi.org/10.1053/j.ajkd.2009.01.255)

4 Ohashi Y, Thomas G, Nurko S, Stephany B, Fatica R, Chiesa A, Rule AD, Srinivas T, Schold JD, Navaneethan SD, *et al.* Association of metabolic syndrome with kidney function and histology in living kidney donors. *American Journal of Transplantation* 2013 **13** 2342–2351. (https://doi.org/10.1111/ajt.12369)

5 Stangl MK, Böcker W, Chubanov V, Ferrari U, Fischereder M, Gudermann T, Hesse E, Meinke P, Reincke M, Reisch N, *et al.* Sarcopenia – endocrinological and neurological aspects. *Experimental and Clinical Endocrinology and Diabetes* 2019 **127** 8–22. (https://doi.org/10.1055/a-0672-1007)

6 Devuyst O, Olinger E & Rampoldi L. Uromodulin: From physiology to rare and complex kidney disorders. *Nature Reviews. Nephrology* 2017 **13** 525–544. (https://doi.org/10.1038/nrneph.2017.101)

7 Raffi HS, Bates JM, Laszik Z & Kumar S. Tamm-Horsfall protein protects against urinary tract infection by proteus mirabilis. *Journal of Urology* 2009 **181** 2332–2338. (https://doi.org/10.1016/j.juro.2009.01.014)

8 Mo L, Huang HY, Zhu XH, Shapiro E, Hasty DL & Wu XR. Tamm-Horsfall protein is a critical renal defense factor protecting against calcium oxalate crystal formation. *Kidney International* 2004 **66** 1159–1166. (https://doi.org/10.1111/j.1523-1755.2004.00867.x)

9 Kreft B, Jabs WJ, Laskay T, Klinger M, Solbach W, Kumar S & Van Zandbergen G. Polarized expression of tamm-Horsfall protein by renal tubular epithelial cells activates human granulocytes. *Infection and Immunity* 2002 **70** 2650–2656. (https://doi.org/10.1128/iai.70.5.2650-2656.2002)

10 Darisipudi MN, Thomasova D, Mulay SR, Brech D, Noessner E, Liapis H & Anders HJ. Uromodulin triggers IL-1β-dependent innate immunity via the NLRP3 inflammasome. *Journal of the American Society of Nephrology* 2012 **23** 1783–1789. (https://doi.org/10.1681/ASN.2012040338)

11 El-Achkar TM, McCracken R, Liu Y, Heitmeier MR, Bourgeois S, Ryerse J & Wu XR. Tamm-Horsfall protein translocates to the basolateral domain of thick ascending limbs, interstitium, and circulation during recovery from acute kidney injury. *American Journal of Physiology. Renal Physiology* 2013 **304** F1066–F1075. (https://doi.org/10.1152/ajprenal.00543.2012)

12 Steubl D, Block M, Herbst V, Nockher WA, Schlumberger, Satanovskij R, Angermann S, Hasenau A-L, Stecher L, Heemann U, *et al.* Plasma uromodulin correlates with kidney function and identifies early stages in chronic kidney disease patients. *Medico* 2016 **95** e3011. (https://doi.org/10.1097/MD.0000000000003011)

13 Scherberich JE, Gruber R, Nockher WA, Christensen EI, Schmitt H, Herbst V, Block M, Kaden J & Schlumberger W. Serum uromodulin-a marker of kidney function and renal parenchymal integrity. *Nephrology, Dialysis, Transplantation* 2018 **33** 284–295. (https://doi.org/10.1093/ndt/gfw422)

14 Pivin E, Ponte B, de Seigneux S, Ackermann D, Guessous I, Ehret G, Pechère-Bertschi A, Olinger E, Mohaupt M, Vogt B, *et al.* Uromodulin and nephron mass. *Clinical Journal of the American Society of Nephrology* 2018 **13** 1556–1557. (https://doi.org/10.2215/CJN.03600318)

15 Holle R, Happich M, Löwel H, Wichmann HE & KORA. KORA – a research platform for population based health research. *Gesundheitswesen* 2005 **67** (Supplement 1) S19–S25. (https://doi.org/10.1055/s-2005-858235)

16 Rathmann W, Strassburger K, Heier M, Holle R, Thorand B, Giani G & Meisinger C. Incidence of Type 2 diabetes in the elderly German population and the effect of clinical and lifestyle risk factors: KORA

S4/F4 cohort study. *Diabetic Medicine* 2009 **26** 1212–1219. (https://doi.org/10.1111/j.1464-5491.2009.02863.x)

17 Meisinger C, Rückert IM, Rathmann W, Döring A, Thorand B, Huth C, Kowall B & Koenig W. Retinol-binding protein 4 is associated with prediabetes in adults from the general population: the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study. *Diabetes Care* 2011 **34** 1648–1650. (https://doi.org/10.2337/dc11-0118)

18 Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr, *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009 **120** 1640–1645. (https://doi.org/10.1161/CIRCULATIONAHA.109.192644)

19 Seissler J, Feghelm N, Then C, Meisinger C, Herder C, Koenig W, Peters A, Roden M, Lechner A, Kowall B, *et al.* Vasoregulatory peptides pro-endothelin-1 and pro-adrenomedullin are associated with metabolic syndrome in the population-based KORA F4 study. *European Journal of Endocrinology* 2012 **167** 847–853. (https://doi.org/10.1530/EJE-12-0472)

20 Laxy M, Knoll G, Schunk M, Meisinger C, Huth C & Holle R. Quality of diabetes care in Germany improved from 2000 to 2007 to 2014, but improvements diminished since 2007. Evidence from the population-based KORA studies. *PLoS ONE* 2016 **11** e0164704. (https://doi.org/10.1371/journal.pone.0164704)

21 Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, *et al.* A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine* 2009 **150** 604–612. (https://doi.org/10.7326/0003-4819-150-9-200905050-00006)

22 Rhodes DCJ, Hinsman EJ & Rhodes JA. Tamm-Horsfall glycoprotein binds IgG with high affinity. *Kidney International* 1993 **44** 1014–1021. (https://doi.org/10.1038/ki.1993.343)

23 Rhodes DCJ. Binding of tamm-Horsfall protein to complement 1q measured by ELISA and resonant mirror biosensor techniques under various ionic-strength conditions. *Immunology and Cell Biology* 2000 **78** 474–482. (https://doi.org/10.1111/j.1440-1711.2000.t01-3-.x)

24 Säemann MD, Weichhart T, Zeyda M, Staffler G, Schunn M, Stuhlmeier KM, Sobanov Y, Stulnig TM, Akira S, von Gabain A, *et al.* Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a Toll-like receptor-4–dependent mechanism. *Journal of Clinical Investigation* 2005 **115** 468–475. (https://doi.org/10.1172/JCI22720)

25 Then C, Then H, Meisinger C, Heier M, Peters A, Koenig W, Rathmann W, Scherberich J & Seissler J. Serum uromodulin is associated with but does not predict type 2 diabetes in elderly KORA F4/FF4 study participants. *Journal of Clinical Endocrinology and Metabolism* 2019 [epub]. (https://doi.org/10.1210/jc.2018-02557)

26 Scheurlen KM, Billeter AT, Kopf S, Herbst V, Block M, Nawroth PP, Zeier M, Scherberich JE & Müller-Stich BP. Serum uromodulin and Roux-en-Y gastric bypass – improvement of a marker reflecting nephron mass. *Surgery for Obesity and Related Diseases* 2019 **1** 1319–1325. (https://doi.org/10.1016/j.soard.2019.05.002)

27 Leiherer A, Muendlein A, Saely CH, Kinz E, Brandtner EM, Fraunberger P & Drexel H. Serum uromodulin is associated with impaired glucose metabolism. *Medicine* 2017 **96** e5798. (https://doi.org/10.1097/MD.0000000000005798)

28 Fedak D, Kuźniewski M, Fugiel A, Wieczorek-Surdacka E, Przepiórkowska-Hoyer B, Jasik P, Miarka P, Dumnicka P, Kapusta M, Solnica B, *et al.* Serum uromodulin concentrations correlate with glomerular filtration rate in patients with chronic kidney disease. *Polskie Archiwum Medycyny Wewnetrznej* 2016 **126** 995–1004. (https://doi.org/10.20452/pamw.3712)

29 Delgado GE, Kleber ME, Scharnagl H, Krämer BK, März W & Scherberich JE. Serum uromodulin and mortality risk in patients undergoing coronary angiography. *Journal of the American Society of Nephrology* 2017 **28** 2201–2210. (https://doi.org/10.1681/ASN.2016111162)

30 Leiherer A, Muendlein A, Saely CH, Ebner J, Brandtner EM, Fraunberger P & Drexel H. Serum uromodulin is a predictive biomarker for cardiovascular events and overall mortality in coronary patients. *International Journal of Cardiology* 2017 **231** 6–12. (https://doi.org/10.1016/j.ijcard.2016.12.183)

31 Youhanna S, Weber J, Beaujean V, Glaudemans B, Sobek J & Devuyst O. Determination of uromodulin in human urine: influence of storage and processing. *Nephrology, Dialysis, Transplantation* 2014 **29** 136–145. (https://doi.org/10.1093/ndt/gft345)