**Association of serum uromodulin with adipokines in dependence of type 2 diabetes**

Cornelia Then1,2, Christian Herder3,4,5, Barbara Thorand6,7, Chaterina Sujana7,8, Margit Heier7,9, Christa Meisinger10,11, Annette Peters6,7,12, Wolfgang Koenig12,13,14, Wolfgang Rathmann15, Michael Roden3,4,5, Michael Stumvoll16, Haifa Maalmi3,4, Holger Then17, Uta Ferrari1, Jürgen Scherberich18, Jochen Seissler1,2,6, KORA-Study Group

1 Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, LMU München, Germany;

2 Clinical Cooperation Group Diabetes, Ludwig-Maximilians-Universität München and Helmholtz Zentrum München, Munich, Germany;

3 German Center for Diabetes Research (DZD), Partner Düsseldorf, Germany;

4 Institute of Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine-University Düsseldorf, Germany;

5 Department of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf;

6 German Center for Diabetes Research (DZD), Partner München-Neuherberg, Germany;

7 Institute of Epidemiology, Helmholtz Zentrum München – German Research Center for Environmental Health (GmbH), Neuherberg, Germany;

8 Institute for Medical Information Processing, Biometry, and Epidemiology, Pettenkofer School of Public Health, LMU Munich, Munich, Germany;

9 KORA StudyCentre, University Hospital Augsburg, Augsburg, Germany;

10 Independent Research Group Clinical Epidemiology, Helmholtz Zentrum München – German Research Center for Environmental Health (GmbH), Neuherberg, Germany;

11 Chair of Epidemiology, University of Augsburg, University Hospital Augsburg, Augsburg, Germany;

12 DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany;

13 Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany;

14 Deutsches Herzzentrum München, Technische Universität München, Munich, Germany;

15 Institute of Biometrics and Epidemiology, German Diabetes Center, Leibniz Institute at Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany;

16 Department of Medicine, University of Leipzig, Leipzig, Germany;

17 Freie Waldorfschule Augsburg, Germany;

18 Klinikum München-Harlaching, Teaching Hospital of the Ludwig-Maximilians-Universität, Munich, Germany.

**Corresponding author:** Cornelia Then, Medizinische Klinik und Poliklinik IV - Klinikum der Ludwig-Maximilians-Universität, Ziemssenstraße 1, 80336 München, Germany. Fax: 004989440054956; Phone: 004989440052111; E-mail: [cornelia.then@med.uni-muenchen.de](mailto:cornelia.then@med.uni-muenchen.de)

**Short title** Serum uromodulin and adipokines

**Abstract**

**Background** The renal tubular glycoprotein uromodulin is associated with obesity and type 2 diabetes, but the underlying mechanisms are elusive. We investigated the association of serum uromodulin with adipokines and tested the effect modification by diabetes status.

**Methods** The associations of serum uromodulin with eight adipokines were assessed in 795-1080 participants of the KORA F4 study aged 62-81 years using linear regression models adjusted for sex, age, BMI, estimated glomerular filtration rate and diabetes. Significant associations were assessed for effect modification by diabetes status. We further tested using logistic regression whether adjustment for the significant adipokines affected the association of uromodulin with type 2 diabetes.

**Results** Serum uromodulin was inversely associated with chemerin and retinol-binding protein-4 after multivariable adjustment (p < 0.001) and Bonferroni correction for multiple testing. No significant association was observed between uromodulin and the other adipokines (leptin, adiponectin, secreted frizzled-related protein 5, progranulin, omentin-1 and vaspin) after correcting for multiple testing. The association of uromodulin with chemerin and retinol-binding protein-4 was stronger in participants with type 2 diabetes than in participants without diabetes (p for interaction < 0.05). However, inclusion of chemerin and retinol-binding protein-4 in logistic regression models did not attenuate the association of serum uromodulin with diabetes.

**Conclusions** Serum uromodulin was inversely associated with the predominantly pro-inflammatory adipokines chemerin and retinol-binding protein-4. The associations were stronger in participants with type 2 diabetes compared to participants without diabetes. However, the association of serum uromodulin with type 2 diabetes was independent of chemerin and retinol-binding protein-4.

**Key words** Adipokines; serum uromodulin; sUmod; type 2 diabetes; uromodulin; chemerin; retinol-binding protein-4

1. **Introduction**

The nephroprotective glycoprotein uromodulin is expressed exclusively in the renal tubular system. The larger portion of uromodulin of is released into the urine, exerting anti-lithogenic and anti-infective effects in the urinary tract [1–4]. Regarding anti-infective properties, urinary uromodulin forms meshwork to trap microorganisms and exhibits pro-inflammatory functions [4]. In vitro, uromodulin purified from urine has been shown to activate neutrophils and macrophages [4] and to stimulate myeloid dendritic cells via Toll-like receptor 4 to acquire a fully mature dendritic cell phenotype [5].

A smaller fraction of uromodulin is secreted basolaterally into the systemic circulation (serum uromodulin, sUmod) [4,6,7]. SUmod has recently been established as a novel, tissue-specific marker of kidney mass and function [4,7–10]. However, the physiological role of uromodulin in the circulation is largely unknown [6,7]. In contrast to the mainly pro-inflammatory functions of urinary uromodulin, current data point towards a systemic immunomodulatory effect of sUmod. In mice, uromodulin knockout entailed enhanced granulopoiesis and systemically increased neutrophil numbers [11] as well as an increased inflammatory response in ischemia-reperfusion kidney injury models, suggesting a protective role of uromodulin by decreasing inflammation [12]. In vitro, uromodulin displays binding capacity for neutrophils [13], immunoglobulin G [14] and tumor necrosis factor-α [15]. Uromodulin can furthermore inhibit the activation of the classical complement pathway by binding of complement 1q and collectin-11 [4,16]. Interestingly, it has been shown that the binding capacity of uromodulin for molecules, e. g. collectin-11, depends on uromodulin glycosylation [4,16]. In humans, sUmod showed an inverse association with several makers of subclinical inflammation [17].

In addition, epidemiological studies showed an inverse association of sUmod with cardiovascular and metabolic risk factors, including arterial hypertension [18], diabetes [19,20], obesity [21], and the metabolic syndrome [21]. Thus, uromodulin might play a role at the intersection of subclinical inflammation and obesity/diabetes-related nephropathy.

Adipokines constitute a critical link between obesity, inflammation and diabetes [22]. Some adipokines correlate with kidney function and may be involved in obesity- and diabetes-associated renal disease [23–26]. Data on a possible association of sUmod with adipokines are not available. Given the putative immunomodulatory properties of sUmod, we hypothesized that sUmod was inversely associated with pro-inflammatory adipokines, whereas no or positive associations were expected for anti-inflammatory adipokines. We further asked the questions, whether associations of sUmod with adipokines were influenced by diabetes status, since altered glycosylation of sUmod may have the potential to change its functional properties, and whether adipokines influence the relation of sUmod with type 2 diabetes. Thus, we investigated the association of sUmod with the primarily pro-inflammatory adipokines chemerin, retinol-binding protein-4 (RBP4) and leptin, as well as with the primarily anti-inflammatory adipokines adiponectin, secreted frizzled-related protein 5 (SFRP5), progranulin, omentin-1 and visceral adipose tissue-derived serine protease inhibitor (vaspin) in the population-based KORA F4 study. Furthermore, we examined possible effect modifications in the observed associations by diabetes status.

1. **Methods**
   1. **Study participants**

The KORA (Cooperative Health Research in the Region of Augsburg) F4 study (2006–2008) is a follow-up examination of the population-based KORA S4 study (1999-2001) and involved 3080 participants from the general community. Recruitment and eligibility criteria [27] as well as study design, standardized sampling methods and data collection (medical history, medication, anthropometric measurements) [28] have been described in detail previously. All study participants gave written informed consent. The study was approved by the Ethics Committees of the Bavarian Medical Association and the Bayerische Landesärztekammer in adherence to the declaration of Helsinki (approval number 06068). SUmod was measured in 1119 participants of the KORA F4 study aged 62 - 81 years with available serum samples (from a total of 1161 participants in this age group). All variables necessary for the analyses were available in 1080 participants for the analysis of RBP4, leptin, adiponectin, omentin-1 and SFRP5, in 806 participants for the analysis of chemerin and progranulin, and in 795 participants for the analysis of vaspin.

Criteria for a clinically diagnosed diabetes mellitus were a validated medical diagnosis or current self-reported use of glucose-lowering agents. After an overnight fasting period, all participants without clinically diagnosed diabetes underwent a standard 75 g oral glucose tolerance test. Newly diagnosed diabetes was defined according to the 1999 World Health Organization diagnostic criteria based on both fasting and post-challenge glucose values (diabetes: ≥ 7.0 mmol/l fasting and/or ≥ 11.1 mmol/l 2-h glucose). Participants with a diabetes other than type 2 diabetes (n = 3) or unknown glucose tolerance status (n = 22) were excluded from the analyses.

* 1. **Laboratory measurements**

Blood samples were collected after an overnight fast of at least eight hours and were kept at room temperature until centrifugation. Plasma was separated immediately, serum after 30 min. Samples were assayed immediately or stored at -80°C. Blood glucose levels were assessed using the hexokinase method (GLU Flex; Dade Behring, Marburg, Germany). Serum creatinine was determined with a modified Jaffe test (Krea Flex; Dade Behring). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (2009) based on serum creatinine [29]. Urinary creatinine concentration (Jaffé-method) was determined on a Cobas Mira (Greiner, Bahlingen, Germany). Urinary albumin was measured with an immunoturbidimetric test (Tina-quant\_Albumin in urine, Boehringer Mannheim, Germany) from a single spot urine sample. SUmod 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 described by Steubl et al. [7]. Chemerin serum concentrations were determined using a commercially available ELISA kit (Human Chemerin ELISA, Biovendor, Heidelberg, Germany) with a sensitivity of 0.1 ng/ml and intra- and inter-assay coefficients of variance of 6.0% and 7.6% [30]. Plasma concentrations of RBP4 were measured by immunonephelometry using a BN II analyzer. The intra- and inter-assay coefficients of variation were <10% [31]. Leptin was measured by ELISA (Mercodia, Uppsala, Sweden); intra‐ and inter‐assay coefficients of variation were <10%. Serum levels of omentin-1 and adiponectin were measured using the Human Omentin-1 ELISA (BioVendor, Brno, Czech Republic) and the Human Total Adiponectin/Acrp30 Quantikine ELISA Kit (R&D Systems) with intra-assay CVs of 2.0% and 4.0% respectively, and inter-assay CVs of 3.8 and 8.0% respectively [32]. Progranulin serum concentrations were determined using the Progranulin human ELISA Kit AdipoGen, AdipoGen Inc., Korea [30]. SFRP5 levels were measured using the Enzyme-linked Immunosorbent Assay Kit for Secreted Frizzled Related protein 5 from Cloud-Clone (Houston, Texas) [33]. Serum vaspin concentrations were quantified using a commercial enzyme-linked immunosorbent assay kit (AdipoGen, Seoul, Korea) with a sensitivity of 12 pg/ml. The intra- and inter-assay coefficients of variance were 1.3–3.8% and 3.3–9.1%, respectively [30].

* 1. **Statistical Analyses**

Clinical characteristics were compared between participants with and without diabetes using t-tests in case of normally distributed variables, Mann-Whitney U-tests for variables with skewed distribution and Chi-square tests for binomial proportions. The associations of sUmod (independent variable) with the adipokines (dependent variables) were assessed in linear regression models. Continuous variables were transformed to a Gaussian distribution by probability integral transformation followed by inverse transform sampling and were used in calculations per 1-standard deviation increase. The models were adjusted for sex, age (continuous, in years), eGFR (continuous, in ml/min/1.73 m2), BMI (continuous, in kg/m2) and type 2 diabetes (yes/no), which have previously been shown to be associated with sUmod [8,19,21]. Smoking was not related to sUmod [17] and therefore not included in the models. To examine possible differences in the association of sUmod with adipokines by type 2 diabetes status, we formally tested for interactions by including interaction terms between sUmod and type 2 diabetes in the total study population and stratified our analyses by diabetes status. For comparison, we also examined the associations between eGFR and the adipokines in the same adjusted models. Finally, we examined the association between sUmod and type 2 diabetes using adjusted logistic regression models and tested whether further adjustment for the adipokines significantly associated with sUmod affected the association. P-values (two-sided) were considered statistically significant if < 0.05. We used Bonferroni correction to account for multiple testing by setting the significance threshold at a p-value of < 0.00625 (0.05 ÷ 8, for eight adipokines). For the interaction terms a p-value < 0.1 was considered significant (< 0.05 after correction for multiple testing accounting for two tests). All calculations were performed using the statistical environment R, version 3.6.0 (R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2019).

1. **Results**
   1. **Study population characteristics**

Table 1 displays the characteristics of the study population. SUmod, adiponectin and omentin-1 were lower in participants with type 2 diabetes, whereas leptin, RBP4, chemerin and vaspin were higher (p < 0.001 for all). Progranulin and SFRP5 did not differ significantly between participants with and without diabetes. Concentrations of sUmod and adipokines in participants with and without type 2 diabetes are additionally presented in Supplementary Figure 1.

* 1. **Inverse association of sUmod and eGFR with pro-inflammatory adipokines**

SUmod was inversely associated with chemerin and RBP4 after multivariable adjustment and correction for multiple testing (p < 0.001 for both; Table 2). A moderate inverse association of sUmod with leptin was present after multivariable adjustment, (p = 0.016), but did not meet the significance threshold corrected for multiple testing.

For comparison with other measures of kidney function, the association of the eGFR with chemerin, RBP4 and leptin was analyzed. The eGFR was associated with chemerin, RBP4 and leptin in the model adjusted for sex, age, BMI, type 2 diabetes and sUmod (p < 0.001 for all, Supplementary Table 1).

Chemerin and RBP4, the two adipokines significantly associated with sUmod after correction for multiple testing, were further investigated regarding their possible associations with the urinary albumin/creatinine ratio. In contrast to sUmod (β: -0.15 ± 0.03; p < 0.001), chemerin (β: 0.02 ± 0.03; p = 0.661) and RBP4 (β: 0.02 ± 0.03; p = 0.561) were not significantly associated with the urinary albumin/creatinine ratio after adjustment for sex, age, BMI, eGFR and type 2 diabetes.

* 1. **Association of sUmod and eGFR with anti-inflammatory adipokines**

SUmod displayed a positive association with adiponectin in the unadjusted model (p < 0.001), which was no longer significant after multivariable adjustment. SUmod was not significantly associated with progranulin, omentin-1, SFRP5 and vaspin in any model (Table 2).

The eGFR was significantly inversely associated with SFRP5 (p < 0.001). Progranulin (p = 0.01) and omentin-1 (p = 0.03) showed a weaker inverse association. No significant association of the eGFR with adiponectin and vaspin was observed (Supplementary Table 1).

* 1. **Stronger association of sUmod with chemerin and RBP4 in participants with type 2 diabetes**

The association of sUmod with chemerin and RBP4 was significantly stronger in participants with type 2 diabetes than in participants without diabetes (p for interaction 0.026 for chemerin and 0.019 for RBP4; Table 3). In contrast, the association of the eGFR with chemerin and RBP4 did not differ significantly between participants with and without diabetes (p for interaction 0.109 for chemerin and 0.596 for RBP4; Supplementary Table 2).

Having found a stronger association of sUmod with chemerin and RBP4 in type 2 diabetes, we tested whether adjustment for chemerin and RBP4, respectively, attenuated the association of sUmod with type 2 diabetes. This, however, was not the case (Supplementary Table 3). Furthermore, we observed similar results when we examined the ratios of sUmod/chemerin and sUmod/RBP4 with respect to the association with type 2 diabetes (Supplementary Table 3).

1. **Discussion**

We show an inverse association of sUmod with the pro-inflammatory adipokines chemerin and RBP4. Leptin was also moderately inversely associated with sUmod, although not significantly after correction for multiple testing. In contrast, sUmod was not significantly associated with any of the primarily anti-inflammatory adipokines, although progranulin and omentin-1 showed a moderate, and SFRP5 a strong association with the eGFR. Interestingly, the associations of the adipokines with the eGFR were hardly influenced by adjustment for sUmod. These data indicate a differential association of some adipokines with different measures of kidney function. The association of sUmod with adipokines does not merely seem to reflect the association of kidney function with adipokines, and is in line with previous findings suggesting immunomodulatory effects of sUmod [11,15,17,34].

Chemerin is highly expressed in white adipose tissue and is necessary for adipocyte differentiation [35,36]. Serum chemerin concentrations are positively correlated with BMI and body fat [37]. Further, chemerin is a ligand for the chemokine-like receptor 1 expressed by macrophages, dendritic cells and natural killer cells, exhibiting strong chemotactic properties [38,39]. Thus, chemerin is proposed to constitute an important link between obesity and chronic subclinical inflammation. In this regard, the inverse association of sUmod with chemerin might indicate an immunoregulatory effect of sUmod. At the same time, chemerin is inversely associated with kidney function as measured by eGFR [40,41], which we confirmed in our study. The reason for elevated chemerin levels in impaired kidney function is not completely elucidated yet, but is probably not due to an increased adipose tissue expression [42]. More likely, impaired chemerin excretion may partly explain elevated levels in chronic kidney disease, but interestingly, preclinical studies also showed an increased chemerin expression in kidney tissue itself in response to renal damage: Chemerin was overexpressed and correlated with inflammatory parameters in kidney tissue in rat models of diabetic nephropathy [24,43]. The expression pattern correlated with the site of damage in rat models of glomerulonephritis and hypertensive nephropathy [44]. The exact mechanisms of chemerin expression in the animal models remain unknown, but chemerin is believed to fuel renal inflammation in nephritis [45]. In this context, a contrariwise expression of sUmod and chemerin might constitute a local immunomodulatory setting in kidney disease.

RBP4 is an adipokine derived from adipocytes and hepatocytes, and the specific transport protein for retinol (vitamin A) in the blood stream [46]. Pro-inflammatory functions of RBP4 suggest that RBP4 links obesity to inflammation and insulin resistance [47]. RBP4 levels are associated with BMI, waist circumference and prediabetes [48]. The inverse association of sUmod with RBP4 might mirror a regulatory sUmod effect but may also partly be explained by the fact that both parameters are markers of renal tubular function. Due to its expression pattern, uromodulin is a tissue-specific marker of the ascending limb of Henle´s loop, whereas *urinary* RBP4 is a functional marker for the proximal tubule, since most of the filtered RBP4 is re-absorbed in the proximal tubule [49]. *Serum* RBP4 levels associate inversely with renal function [50], as also shown in the present study. However, the value of serum RBP4 as marker of tubular function is unknown. Since the association of sUmod and RBP4 was largely independent of the eGFR, mechanisms beyond reflecting kidney function may be assumed. A possibility is the binding of pro-inflammatory adipokines in the circulation by sUmod, as shown *in vitro* for markers of inflammation [13–15,34]. Also, kidney tissue-specific expression and/or excretion may explain the eGFR-independent association of sUmod with RBP4 and chemerin.

The association of sUmod with both chemerin and RBP4 was stronger in participants with diabetes compared to participants without diabetes. This observation may indicate a counter-regulative effect of sUmod in diabetes but may also be explained by a differential expression of these markers in diabetes, namely reduced uromodulin expression in tubular damage and decreased renal clearance/increased expression of chemerin and RBP4 in response to kidney injury. Therefore, we examined, whether the inclusion of chemerin and RBP4, respectively, in the regression models attenuated the known association of sUmod with type 2 diabetes [19]. Additionally, we tested whether the ratio of sUmod and chemerin and RBP4, respectively, displayed a stronger association with type 2 diabetes than sUmod alone and might thus constitute a stronger marker for early tubular damage in diabetes. However, both model variations did not substantially alter the association of sUmod with diabetes, strengthening the value of sUmod as an independent renal marker of diabetes. In line, whereas sUmod was associated with the urinary albumin/creatinine ratio [8], an early measure of diabetic nephropathy, chemerin and RBP4 were not.

**Study strengths and limitations**

To our knowledge, this is the first study to examine the associations of sUmod with adipokines. Strengths of our study include the population-based design with a large, well-characterized study sample with different measures of kidney function. The inclusion of only participants aged 62 - 81 years and of western European origin limits the generalizability of our data. Due to the observational and cross-sectional design of our study, we do not provide data on mechanistic links.

**Conclusions**

SUmod was inversely associated with the pro-inflammatory adipokines chemerin and RBP4, whereas anti-inflammatory adipokines showed no significant association with sUmod. The inverse association of sUmod with chemerin and RBP4 was stronger in participants with type 2 diabetes compared to participants without diabetes and might represent a counterregulative mechanism in diabetes. Possible explanations for a stronger inverse association of sUmod with chemerin and RBP4 in diabetes include an altered binding of these molecules by sUmod in the circulation, possibly related to modified sUmod glycosylation, and/or a contrarywise expression pattern in the kidney due to diabetes-related renal stress. Further preclinical studies should examine the binding potential of uromodulin for chemerin and RBP4 depending on glucose concentrations, the expression patterns of sUmod in context with chemerin and RBP4 in normal and diabetic kidney tissue, and the potential of sUmod to influence adipose cells and their adipokine secretion pattern. The association of sUmod with type 2 diabetes was independent of chemerin and RBP4, respectively, strengthening sUmod as an independent renal marker of diabetes.

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**Conflict of interest disclosure**

CH received a research grant from Sanofi-Aventis. MR reports consulting fees from Eli Lilly, Terra Firma, Sanofi US, Fishawack Group, Target Pharmasolutions, Astra Zeneca, honoraria for lectures from Boehringer-Ingelheim Pharma, Sanofi US, Novo Nordisk, participation in advisory board of Poxel S.A and Servier Laboratories. J. Scherberich has a patent at the University Charite Berlin pending, reports honoraria for lectures, support for attending the German Soc Nephrol. (2019) commission Prevention of kidney diseases, and participated as author of the guidelines for lab.diagn. of AKI and progress, CKD, AWMF national board. The reported disclosures are not directly related to this manuscript. The other authors declare that they have no conflict of interest associated with this manuscript.

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1. **Tables**

**Table 1** Characteristics of the study participants a

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **All participants** | **No diabetes** | **Type 2 diabetes** | **p b** |
| **n** | **1080** | **869** | **211** | **-** |
| Women n (%) | 526 (49) | 443 (51) | 83 (39) | < 0.001 c |
| Age (years) | 70.3 ± 5.5 | 69.9 ± 5.4 | 71.7 ± 5.4 | 0.849 d |
| BMI (kg/m2) | 28.7 ± 4.5 | 28.2 ± 4.2 | 31.1 ± 4.8 | 0.026 d |
| Estimated glomerular filtration rate (ml/min/1.73 m2) | 77.9 (67.3; 87.7) | 78.8 (68.8; 88.2) | 72.7 (60.3; 84.5) | 0.274 e |
| Serum uromodulin (ng/ml) | 152.3  (110.6; 207.6) | 160.5 (110.6; 215.0) | 126.3 (86.8; 172.5) | < 0.001 e |
| Leptin (ng/ml) | 14.6 (6.5; 28.5) | 13.7 (6.0; 26.5) | 19.8 (9.71; 42.4) | < 0.001 e |
| Retinol-binding protein-4 (g/l) | 0.043  (0.037; 0.050) | 0.043  (0.037; 0.049) | 0.046  (0.039; 0.055) | < 0.001 e |
| Adiponectin (µg/ml) | 10.1 (6.6; 15.26) | 10.8 (7.3; 16.1) | 7.6 (5.3; 11.3) | < 0.001 e |
| Omentin-1 (ng/ml) | 487 (402; 580) | 490 (406; 580) | 477 (390; 573) | < 0.001 e |
| Secreted frizzled-related protein 5 (ng/ml) | 53.5 (37.8; 67.3) | 52.8 (38.5; 67.4) | 48.2 (35.3; 66.8) | 0.379 e |
| **n** | **806** | **656** | **150** | **-** |
| Chemerin (ng/ml) | 176.5 (140.1; 206.7) | 174.5 (136.5; 204.7) | 183.7 (147.4; 221.5) | < 0.001 e |
| Progranulin (ng/ml) | 138.4 (109.3; 175.6) | 138.2.2 (110.5.; 173.9) | 141.1 (103.4; 189.0) | 0.117 e |
| **n** | **795** | **646** | **149** | **-** |
| Vaspin (ng/ml) | 0.60 (0.35; 1.07) | 0.59 (0.34; 1.06) | 0.68 (0.39; 1.08) | < 0.001 e |

a Data are presentedas mean ± standard deviation, median (first quartile; third quartile), or absolute numbers (%);

b the p value is related to the null hypothesis of no differences between participants with and without diabetes;

c Chi-square test; d t-test; e Mann-Whitney U-test.

**Table 2** Association estimates between serum uromodulin and adipokines: β coefficients ± standard errors from linear regression models are given per standard deviation of serum uromodulin and adipokines.

|  |  |  |
| --- | --- | --- |
|  | Without adjustment | Adjustment for age, sex, BMI, eGFR and type 2 diabetes |
| Chemerin | -0.29 ± 0.03 \*\*\* | **-0.18 ± 0.03 \*\*\*** |
| Retinol-binding protein-4 | -0.32 ± 0.03 \*\*\* | **-0.21 ± 0.03 \*\*\*** |
| Leptin | -0.09 ± 0.03 \* | -0.05 ± 0.02 \* |
| Adiponectin | 0.15 ± 0.03 \*\*\* | 0.05 ± 0.03 |
| Progranulin | -0.02 ± 0.04 | -0.003 ± 0.04 |
| Omentin-1 | 0.01 ± 0.03 | -0.002 ± 0.03 |
| Secreted frizzled-related protein 5 | -0.02 ± 0.03 | -0.02 ± 0.03 |
| Vaspin | -0.01 ± 0.04 | -0.03 ± 0.04 |

\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001;

bold print indicates significance in the fully adjusted model after correcting for multiple testing using the Bonferroni method (p < 0.00625 (0.05 ÷ 8)).

**Table 3** Association estimates between serum uromodulin and chemerin and retinol-binding protein-4, respectively, stratified by diabetes: β coefficients ± standard errors from linear regression models are given per standard deviation of serum uromodulin and chemerin and retinol-binding protein-4, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
| Stratification | Adjustments | Chemerin | Retinol-binding protein-4 |
| Without diabetes | Without adjustment | -0.18 ± 0.04 \*\*\* | -0.27 ± 0.03 \*\*\* |
| Type 2 diabetes | -0.39 ± 0.07 \*\*\* | -0.46 ± 0.07 \*\*\* |
| Without diabetes | Adjustment for age, sex, BMI and eGFR | **-0.14 ± 0.04 \*\*\*** | **-0.17 ± 0.03 \*\*\*** |
| Type 2 diabetes | **-0.30 ± 0.07 \*\*\*** | **-0.39 ± 0.07 \*\*\*** |
| p interaction type 2 diabetes | **0.026** | **0.019** |

\*\*\* p < 0.001;

bold print indicates significance in the fully adjusted model after correcting for multiple testing using the Bonferroni method (p < 0.0125 for the β estimates (0.05 ÷ 4) and < 0.05 (0.1 ÷ 2) for the interaction terms).