Title: Serum uromodulin predicts cardiovascular complications and mortality in dependence on glucose tolerance status: Results from the KORA F4/FF4 study

Running title: Uromodulin and cardiovascular outcome

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Abstract

Objective: Serum uromodulin is a novel biomarker for kidney function and has further been associated with adverse cardiovascular outcome in coronary patients. Here, we analyzed the association of serum uromodulin with cardiovascular outcome in the population-based KORA F4/FF4 study stratified for glucose tolerance status.

Research Design and Methods: In 1119 participants of the KORA F4 study aged 62-81 years, serum uromodulin was measured and the associations with cardiovascular events, cardiovascular mortality and overall mortality were assessed using logistic regression models stratified for glucose tolerance status. After a mean follow-up time of 6.5 years, 605 participants where reevaluated and renal outcome was assessed depending on glucose tolerance status.

Results: Low serum uromodulin was significantly associated with past and incident cardiovascular events, cardiovascular mortality (OR 0.55; 95% CI 0.36 - 0.85) and overall mortality (OR 0.65; 95% CI 0.49 - 0.86). This effect was stronger in glucose tolerant subjects and disrupted in participants with prediabetes or type 2 diabetes. Serum uromodulin correlated with the estimated glomerular filtration rate in all participants at baseline (p < 0.001), but was significantly associated with kidney function decline only in glucose tolerant subjects (p < 0.01).

Conclusions: Serum uromodulin is an independent novel predictor of cardiovascular events and mortality in the general population. The predictive value of serum uromodulin depends on the glucose tolerance status.

Key words: Serum uromodulin, sUmod, diabetes type 2, prediabetes, myocardial infarction, apoplexy, cardiovascular event, mortality, outcome, kidney function

Type 2 diabetes mellitus is related to a doubled risk of cardiovascular events and mortality. However, the specific causes of cardiovascular complications in diabetes still remain largely unknown. One possible link between diabetes and cardiovascular disease is uromodulin, a glycosylphosphatidylinositol-anchored protein synthesized in tubular cells of the ascending limb of Henle´s loop (1). Uromodulin is primarily a urinary protein, released into the urinary tract by proteolytic cleavage. Uromodulin exerts anti-lithogen, anti-infective and immunomodulatory functions (2–6) and is critical for renal integrity. Mutations of the uromodulin-coding gene may cause severe kidney damage, including recurring urinary tract infections, cystic kidney disease, familial juvenile hyperuremic nephropathy and congenital nephrolithiasis (1,7,8). Even small changes in uromodulin concentration or function, caused for instance by uromodulin loci variants, may provoke and accelerate kidney disease (9–15).

Due to active secretion by endocytotic vesicles from the basolateral side of tubular cells, uromodulin is also present in the circulation and there represents a novel biomarker for tubular integrity (16–18). The physiological functions and significance of uromodulin in the circulation remain largely unknown, but uromodulin seems to play a role beyond renal homeostasis. Two recent studies investigating serum uromodulin (sUmod) in coronary patients showed an association with type 2 diabetes, cardiovascular events and mortality (19–21).

Since the predictive value of sUmod in the general population is still unexplored, we here investigated the association of uromodulin with cardiovascular complications and total mortality in the population-based KORAF4/FF4 cohort stratified for glucose tolerance status.

Methods

Study participants

The population-based KORA (Cooperative Health Research in the Region of Augsburg) S4/F4/FF4 cohort study initially recruited 4,261 participants in the health survey S4 (1999–2001) in Southern Germany. Eligibility criteria and recruitment for the KORA studies have been described elsewhere (22). 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 and Istanbul. Follow-up examinations took place 2006–2008 (KORA F4 with 3,080 participants) and 2013–2014 (KORA FF4 with 2,279 participants). Participants were lost to follow-up from S4 to FF4 due to participants’ death (455), relocation (296), refusal (570), illness/lack of time (504) and no contact possible (157). SUmod was measured in randomly selected participants aged 62–81 years in the KORA F4 cohort (n = 1119). Of the 1119 participants with sUmod measurements in KORA F4, 130 died and 355 could not be contacted or declined participation in the FF4 survey. Thus, the study cohort in the FF4 examination comprised 634 participants, of which 29 had to be excluded due to missing covariables. The mean follow-up time was 6.5 ± 0.3 years. Study design, standardized sampling methods and data collection (medical history, medication, anthropometric measurements, blood pressure) have been described in detail previously (23,24). Criteria for a pre-known diagnosis of diabetes mellitus were a validated physician’s diagnosis or current use of glucose-lowering agents. After an overnight fasting period, all non-diabetic participants underwent a standard 75 g oral glucose tolerance test. Newly diagnosed diabetes, impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and normal glucose tolerance (NGT) were defined according to the 1999 World Health Organization diagnostic criteria based on both fasting and post-challenge glucose values (type 2 diabetes: ≥ 7.0 mmol/l fasting and/or ≥ 11.1 mmol/l 2-h glucose; IFG: ≥ 6,1 mmol/l and < 7,0 mmol/l; IGT: ≥ 7.8 - < 11.1 mmol/l 2-h glucose). Prediabetes was defined as IFG and/or IGT (2-h glucose ≥ 7.8 - < 11.1 mmol/l). Arterial hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or known hypertension with use of anti-hypertensive drugs.

Laboratory measurements

Blood was collected after an overnight fast of at least eight hours without stasis, and the samples were kept at 4°C until centrifugation. Except for 2-h glucose, all blood parameters were based on fasting blood samples. Serum samples were stored at -80°C until assayed. Measurements of blood glucose, HbA1c, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, serum creatinine, cystatin C and high sensitive C-reactive protein (hsCRP) were performed as described elsewhere (25). Glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (2009) based on serum creatinine and cystatin C. SUmod was determined using the commercially available uromodulin enzyme-linked immunosorbent assay kit (Euroimmun AG, Lübeck, Germany) with a lower detection limit of 2 ng/ml, an intraassay precision of 1.8 - 3.2 % and an interassay precision of 6.6 %. The measurement procedure was performed as described previously (17).

Statistical Analyses

Characteristics of the study participants were compared between men and women using t-tests in case of normally distributed variables. Mann-Whitney U-tests were performed for skewedly distributed variables. Chi-square tests were used to compare binomial proportions. 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, respectively. SUmod was deployed as independent variable. Adjustment for confounders was performed in multinomial logistic and linear regression analyses and is indicated for each observation. The models were fitted for the whole study group and for participants without and with prediabetes/diabetes, respectively. Participants with a diabetes type other than type 2 diabetes or missing co-variables were excluded from the respective analyses. The final number of participants included is indicated for each analysis. The level of statistical significance was set at 5 %. All analyses were performed using the statistical environment R, version 3.4.4.

Results

Study population characteristics

Baseline characteristics of the study population stratified for glucose tolerance status are shown in table 1. Participants with prediabetes/type 2 diabetes had an overall adverse metabolic and cardiovascular risk profile and displayed significantly lower sUmod levels compared to glucose tolerant subjects.

Association of sUmod with cardiovascular events and mortality

Table 2 shows the associations of sUmod with cardiovascular outcome parameters. After adjustment for age, sex, BMI, LDL and HDL cholesterol, arterial hypertension, prediabetes, diabetes, eGFR, hsCRP, smoking status and physical activity, low sUmod levels were inversely associated with past cardiovascular events (OR: 0.72; 95% CI: 0.53 - 0.97; p < 0.05) and a combined cardiovascular endpoint comprising fatal and non-fatal myocardial infarction or stroke as well as cardiovascular death in the total study cohort (OR: 0.62; 95% CI: 0.46 - 0.82; p < 0.001). Further, sUmod was a converse predictor of cardiovascular mortality (OR: 0.55; 95% CI: 0.36 - 0.85; p < 0.01) and overall mortality (OR: 0.65; 95% CI: 0.49 - 0.86; p < 0.01).

The predictive value of sUmod for cardiovascular complications and mortality was attenuated in participants with prediabetes or type 2 diabetes

In subjects with prediabetes/type 2 diabetes, multivariate adjustment (for age, sex, BMI, LDL and HDL cholesterol, arterial hypertension, eGFR, hsCRP, smoking status and physical activity) attenuated the inverse relation of sUmod and past cardiovascular events to non-significance. Whereas incident cardiovascular events (OR: 0.39; 95% CI: 0.18 - 0.86; p < 0.05) and the combined cardiovascular endpoint (OR: 0.24; 95% CI: 0.12 - 0.49; p < 0.001) where significantly associated with sUmod in glucose tolerant participants, this association was attenuated to non-significance in subjects with prediabetes/type 2 diabetes by correction for confounders. In glucose tolerant participants, cardiovascular mortality was strikingly reduced (OR: 0.12; 95% CI: 0.03 - 0.43; p < 0.001) and overall mortality substantially (OR: 0.48; 95% CI: 0.27 - 0.85; p < 0.05) lower per standard deviation increase of baseline sUmod. The inverse association of sUmod with cardiovascular and overall mortality was attenuated to non-significance in participants with prediabetes or type 2 diabetes after multivariate adjustment.

Association of sUmod with eGFR in dependence of glucose tolerance status

SUmod was associated with eGFR based on creatinine and cystatin C (p < 0.001). After multivariate adjustment, the association was significantly stronger in participants with prediabetes/type 2 diabetes compared to glucose tolerant subjects (coefficient 0.33 ± 0.04 vs. 0.19 ± 0.04; p for interaction 0.002; table 3). In contrast, sUmod was significantly associated with eGFR in the follow-up examination only in glucose tolerant subjects (0.12 ± 0.04; p < 0.01; table 3), whereas this association was attenuated to non-significance in participants with prediabetes/type 2 diabetes after multivariate adjustment. In line, the risk reduction for chronic kidney disease per standard deviation of baseline sUmod values was stronger in glucose tolerant subjects compared to participants with prediabetes or type 2 diabetes (OR for an incident eGFR < 60 ml/min/1.73 m² after multivariate adjustment: 0.62; 95% CI: 0.40 - 0.96 vs. 1.03; 95% CI: 0.75 - 1.42; table 4).

Discussion

This is the first study investigating the association of sUmod with cardiovascular outcome in a population-based cohort. We demonstrate an inverse relation of sUmod with past and incident cardiovascular events, cardiovascular mortality and overall mortality. Our results are in line with two previous studies assessing sUmod and cardiovascular outcome in patients admitted for coronary angiography (20,26) and a study with 527 type 1 diabetes patients, in which sUmod predicted less coronary artery calcification after a follow-up time of 12 years (27). Two other studies failed to show a significant association of uromodulin with mortality: The health ABC study investigated the association of uUmod with mortality in elderly participants (28) and a Chinese study assessed the predictive value of sUmod for cardiovascular events and all-cause mortality (29) in 2652 patients with chronic kidney disease. In the ABC study, pre-analytic drawbacks determining uUmod may have caused the discrepant results, since uUmod is subjected to polymer formation (1), whereas sUmod is a stable monomeric antigen measurable more reliably (30). In the study of Lv et al., participants suffered from chronic kidney disease and thus expressed lower uromodulin values, wherefore these results may not be generalizable. Possibly, sUmod has a threshold, below which it does not exert its assumed protective functions.

SUmod is inversely related to several cardiovascular risk factors, among them diabetes (19), hypertension (31) and metabolic syndrome, which might be a reason for the protective cardiovascular role of sUmod, although the causal nature of these associations is not yet clarified. Adjustment for traditional cardiovascular risk factors attenuated the relation of sUmod with cardiovascular events and mortality, which, however, remained significant. Further, immunomodulatory uromodulin properties may play a role, since atherosclerosis is partly a condition of chronic subclinical inflammation. Uromodulin is able to bind immunoglobulin G, complement 1q and tumor necrosis factor-α. SUmod correlates with tumor necrosis factor-α, interleukin 6 and interleukin 8 serum levels, indicating a regulative role in innate immunity (32-35). The production of tumor necrosis factor-α, interleukin 1-β and interleukin 1 receptor antagonist by isolated peripheral blood mononuclear cells can be stimulated by addition of uromodulin. Interestingly, addition of serum further enhanced the cytokine production, indicating an influence of serum components on this effect (36). Thus, the immunomodulatory uromodulin properties might be even more pronounced in the circulation than in the urine. Higher sUmod is associated with a higher percentage of lymphocytes and lower percentages of neutrophils and eosinophils (21). Uromodulin receptors in the body remain to be determined for the most part, but possibly include Toll-like receptor 4, via which uromodulin may activate myeloid dendritic cells (37). Adjustment for hsCRP in the current study barley affected the association of sUmod with cardiovascular events and mortality, indicating that sUmod may exert its protective effect not only by attenuation of subclinical inflammation.

To our knowledge, a dependence of the predictive value of sUmod for cardiovascular outcome on glucose tolerance status has not yet been described. The current data show a predictive value of sUmod for cardiovascular events and mortality only in glucose tolerant subjects after multivariate adjustments, but not in participants with prediabetes or type 2 diabetes. There are several possible reasons for the lacking association of sUmod with cardiovascular events and mortality in participants with prediabetes/type 2 diabetes. Firstly, sUmod properties may be influenced by the hyperglycemic state, possibly due to an altered glycation pattern, which has been demonstrated for uUmod in diabetic individuals (38). Whether such altered glycation patterns and/or altered uromodulin functionality are also present in sUmod, remains to be determined. Secondly, in prediabetic or diabetic individuals, unknown factors or alterations weakening the sUmod effect at the target structures may be present. Thirdly, the hyperglycemic state or other diabetes-accompanying factors may decrease uromodulin secretion, explaining the reduced sUmod levels in diabetic subjects (19). Hypothetically, the reduced sUmod levels in diabetic individuals are below a threshold of effective sUmod functionality. Fourthly, sUmod may be a sensitive marker for early diabetic kidney injury, which is not readily detectable by GFR estimates, since in early diabetic kidney disease, eGFR is often distorted by hyperglycemia-induced hyperfiltration. Therefore, sUmod may be capable to unmask early stages of diabetic kidney disease escaping GFR estimates, and possibly, this early renal injury has a greater impact on cardiovascular health than previously assumed.

Whereas the correlation of sUmod and eGFR was stronger in participants with prediabetes or type 2 diabetes at baseline, the predictive value of sUmod for renal outcome in follow-up was stronger in individuals with normal glucose tolerance. Thus, higher sUmod values in subjects with normal glucose tolerance may reflect nephroprotective properties of uromodulin. A possible explanation for the lacking predictive power of sUmod for eGFR in participants with prediabetes/type 2 diabetes are their lower initial sUmod values that may have been fallen below a hypothetical critical value, beneath which protective uromodulin properties are less effective. Another reason may be that the lower sUmod values in prediabetic/diabetic subjects reveal an otherwise masked early kidney injury that then progresses during the follow-up time. A possible explanation for the stronger association of sUmod with eGFR at baseline may be the attenuated relation of sUmod and eGFR in the higher ranges of both parameters (significance threshold for eGFR in the total cohort: 74 ml/min/1.73 m²; data not shown). Since prediabetic/diabetic participants have lower sUmod levels on average, the relation of sUmod and eGFR might be stronger. In contrast to our data, Leiherer et al. did not find an impact of diabetes on the power of sUmod to predict renal function (39), which might be due to differences in the study population, since in the cohort of Leiherer et al., more than 50% of the 529 participants suffered from significant coronary disease.

Study strengths and limitations

The major strengths of our study are the large, well characterized community-based cohort and the longitudinal design with a follow-up time of 6.5 years. All participants who were not known to be diabetic underwent an oral glucose tolerance test on both examinations. However, only participants aged over 60 years were included. SUmod declines with age (data not shown), so that the association of uromodulin with cardiovascular and renal outcomes in a younger population remains to be confirmed.

Conclusions

This is the first population-based study assessing the association of sUmod with cardiovascular outcome. We here show that sUmod is a novel independent biomarker for cardiovascular events and cardiovascular and overall mortality in the general community and in study participants without prediabetes or diabetes, but not in subjects with (pre-)diabetes. These data suggest a protective cardiovascular effect of sUmod, which is disrupted in the presence of glucose intolerance. Further studies are needed to understand the mechanistic basis of the protective sUmod effect and its modulation in diabetic individuals.

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Conflict of interest disclosure: All authors declare that they have no conflict of interest associated with this manuscript.

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Table 1: Baseline characteristics of study participants as means ± standard deviation, median (first quartile; third quartile), or proportion (%)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | All subjects | normal glucose tolerance | prediabetes/type 2 diabetes | p †  |
| n | 1119 | 586 | 508 |  |
| Sex (male (%)) | 569 ( 51) | 278 ( 47) | 282 ( 56) | < 0.01 || |
| Age (years) | 70.35 ± 5.48 | 69.41 ± 5.39  |  71.28 ± 5.39 | < 0.01 ‡ |
| BMI (kg/m2) | 28.69 ± 4.49 | 27.62 ± 4.11 | 30.01 ± 4.53 | < 0.01 ‡ |
| Waist circumference (cm) | 98.2 ± 12.2 | 94.96 ± 11.61 | 102.19 ± 11.68 | < 0.01 ‡ |
| Systolic blood pressure (mmHg) | 128.5 ± 19.6 | 125.87 ± 19.24 | 131.52 ± 19.79 | < 0.01 ‡ |
| Diastolic blood pressure (mmHg) | 74.0 ± 10.0 | 73.97 ± 9.83 | 74.08 ± 10.3 | n.s. ‡ |
| Arterial hypertension (%) | 699 (63) | 305 (52) | 376 (74) | < 0.01 || |
| HDL cholesterol (mg/dl) | 55.7 ± 14.1 | 58.71 ± 14.1 | 52.07 ± 13.02 | < 0.01 ‡ |
| LDL cholesterol (mg/dl) | 139.9 ± 35.9 | 142.26 ± 35.77 | 137.09 ± 35.85 | 0.02 ‡ |
| Triglycerides (mg/dl) | 112 (83; 157) | 104 (75; 142) | 128 (92; 184) | < 0.01 § |
| HbA1c (%) | 5.6 (5.4; 5.9) | 5.5 (5.3; 5.7) | 5.9 (5.6; 6.3) | < 0.01 § |
| HbA1c (mmol/mol) | 38 (36; 41) | 37 (34; 39) | 41 (38; 45) | < 0.01 § |
| hsCRP (mg/l) | 1.54 (0.79; 3.19) | 1.4 (0.71; 2.76) | 1.88 (0.92; 3.85) | < 0.01 § |
| eGFR (ml/min) | 77.5 (67.0; 87.5)  | 79.0 (70.5; 88.8) | 75.6 (63.2; 86.3) | < 0.01 § |
| Never smoker (%) | 570 (51) | 301 (52) | 256 (50) | n.s. || |
| Physically inactive ¶ (%) | 556 (50) | 262 (45) | 284 (56) | < 0.01 || |
| sUmod (ng/ml) | 152.5 (110.4; 207.6) | 166.7 (122.2; 207.6) | 136.6 (99.7; 191.0) | < 0.01 § |

† The p value is related to the null hypothesis of differences between glucose tolerant and prediabetic/diabetic participants;

‡ t-Test; § Mann-Whitney U-test; || Chi-square test; n.s. not significant;

¶ Physically inactive: < 1 hour sports/week in winter and summer;

Table 2: ORs (95% CI) for cardiovascular events and mortality in dependence on sUmod as independent variable (per standard deviation): Results of logistic regression modelsstratified for glucose tolerance

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Past myocardial infarction or stroke | Incident myocardial infarction or stroke | Combined cardiovascular end point † | Cardiovascular mortality | Overall mortality |
| Without adjustment |
| Met criteria (normal glucose tolerance; prediabetes/type 2 diabetes) | 27; 75 | 16; 44 | 31; 86 | 15; 42 | 32; 90 |
| Total study cohort | 0.49 (0.39 - 0.64) \*\*\* | 0.61 (0.45 - 0.83) \*\* | 0.46 (0.35 - 0.59) \*\*\* | 0.39 (0.28 - 0.56) \*\*\* | 0.50 (0.39 - 0.62) \*\*\* |
| Normal glucose tolerance | 0.42 (0.25 - 0.70) \*\*\* | 0.43 (0.23 - 0.83) \* | 0.30 (0.18 - 0.52) \*\*\* | 0.22 (0.10 - 0.50) \*\*\* | 0.42 (0.27 - 0.67) \*\*\* |
| Prediabetes/type 2 diabetes | 0.56 (0.42 - 0.76) \*\*\* | 0.74 (0.52 - 1.06) | 0.46 (0.35 - 0.59) \*\*\* | 0.50 (0.33 - 0.75) \*\*\* | 0.53 (0.40 - 0.71) \*\*\* |
| Adjustment for age, sex, BMI, arterial hypertension, eGFR, HDL, LDL, hsCRP, smoking, physical activity (and prediabetes/type 2 diabetes) |
| Total study cohort | 0.72 (0.53 - 0.97) \* | 0.74 (0.52 - 1.04) | 0.62 (0.46 - 0.82) \*\*\* | 0.55 (0.36 - 0.85) \*\* | 0.65 (0.49 - 0.86) \*\* |
| Normal glucose tolerance | 0.56 (0.32- 0.99) \* | 0.39 (0.18 - 0.86) \* | 0.24 (0.12 - 0.49) \*\*\* | 0.12 (0.03 - 0.43) \*\*\* | 0.48 (0.27 - 0.85) \* |
| Prediabetes/type 2 diabetes | 0.78 (0.54 - 1.12) | 0.90 (0.61 - 1.34) | 0.81 (0.58 - 1.13) | 0.75 (0.46 - 1.22) | 0.74 (0.53 - 1.04) |

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

† non-fatal myocardial infarction or stroke or death due to cardiovascular reasons

Table 3: Association of eGFR (continuous) and sUmod (per standard deviation) stratified for glucose tolerance status: Results of linear regression models

|  |
| --- |
| eGFR (baseline), adjusted for age, sex, BMI, arterial hypertension, hsCRP, LDL, HDL, smoking and physical activity |
| Normal glucose tolerance (n = 429) | 0.19 ± 0.04 \*\*\* |
| Prediabetes/type 2 diabetes (n = 655) | 0.33 ± 0.04 \*\*\* |
| p for interaction | 0.002 |
| eGFR (follow-up), without adjustment |
| Normal glucose tolerance (n = 259) | 0.32 ± 0.05 \*\*\* |
| Prediabetes/type 2 diabetes (n = 346) | 0.28 ± 0.06 \*\*\* |
| eGFR (follow-up), adjusted for age, sex, BMI, arterial hypertension, hsCRP, LDL, HDL, smoking, physical activity and baseline eGFR |
| Normal glucose tolerance (n = 259) | 0.12 ± 0.04 \*\* |
| Prediabetes/type 2 diabetes (n = 346) | 0.01 ± 0.04 |

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

Table 4: ORs (95% CI) for incident chronic kidney disease per sUmod standard deviation: Results of logistic regression models stratified for glucose tolerance status

|  |  |  |  |
| --- | --- | --- | --- |
| eGFR ml/min/1.73 m² | < 60  | < 45  | < 30  |
| Met criteria (normal glucose tolerance; prediabetes/type 2 diabetes) | 49; 77 | 20; 30 | 4; 7 |
| Without adjustment |
| Normal glucose tolerance | 0.58 (0.41 - 0.84) \*\* | 0.31 (0.16 - 0.6) \*\*\* | 0.09 (0.01 - 0.65) \* |
| Prediabetes/type 2 diabetes | 0.89 (0.67 - 1.17) | 0.39 (0.23 - 0.67) \*\*\* | 1.01 (0.46 - 2.2) |
| Adjusted for age, sex, BMI, arterial hypertension, hsCRP, LDL, HDL, smoking, physical activity |
| Normal glucose tolerance | 0.62 (0.40 - 0.96) \*\* | 0.27 (0.12 - 0.60) \*\*\* | 0.06 (0.01 - 0.96) \* |
| Prediabetes/type 2 diabetes | 1.03 (0.75 - 1.42) | 0.50 (0.27 - 0.94) \* | 1.45 (0.59 - 3.56) |

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