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High urinary sulfate concentration is associated with reduced risk of renal disease progression in type 2 diabetes

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ABSTRACT

Diabetes is associated with a high incidence of microvascular disease, including nephropathy. Diabetic nephropathy is the most common cause of chronic kidney disease in the Western world. Sulfate in the urine is the metabolic end product of hydrogen sulfide (H_2S), a recent discovered gaseous signaling molecule. Urinary sulfate has earlier shown beneficial predictive properties in renal transplant recipients. Based on the protective role of exogenous H_2S in experimental models of diabetic nephropathy, we aimed to cross-sectionally investigate the association of sulfate with renal risk markers, and to prospectively investigate its predictive value for renal events in patients with diabetic nephropathy.

Post-hoc analysis on data of the sulodexide macroalbuminuria (Sun-MACRO) trial and the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study was performed. A total of 1004 patients with type 2 diabetes were included. Urinary sulfate concentration was measured and cross-sectionally associated to renal risk markers by linear regression. Multivariable Cox regression analysis was performed to assess the prospective association of sulfate with renal events, which was defined as end stage renal disease or a doubling of baseline serum creatinine.

Mean age was 63 ± 9 years, median sulfate concentration was 8.0 (IQR 5.8–11.4) mmol/L. Urinary sulfate positively associated with male gender, hemoglobin, and negatively associated with albuminuria at baseline. During follow-up for 12 (IQR 6–18) months, 38 renal events occurred. Each doubling of urinary sulfate was associated with a 19% (95%CI 1%–34%) lower risk of renal events, independent of adjustment for potential confounders, including age, estimated glomerular filtration rate (eGFR), and albuminuria.

To conclude, higher urinary sulfate concentration is associated with a more beneficial profile of renal risk markers, and is independently associated with a reduced risk for renal events in type 2 diabetes patients with nephropathy.

1. Introduction

Diabetes is an important risk factor for cardiovascular disease [1]. A high incidence of microvascular complications, including nephropathy has been found in the course of diabetes in patients. Diabetic nephropathy is also the most common cause of chronic renal failure in the Western world [2]. Due to the aging population and Western lifestyle behavior, diabetes and its complications, are significantly increasing [3]. The renal changes are suggested to be caused by the metabolic defect, nonenzymatic glycation of proteins, and hemodynamic changes. The latter leads to glomerular hypertrophy and subsequent glomerular fibrosis. At the renal level the endothelium is important in the initiation and progression of diabetic nephropathy. A family of gaseous molecules including

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nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) is produced at the renal endothelial level and believed to play a beneficial role in oxidative stress, blood pressure regulation and inflammation.

Urinary sulfate is the end product of hydrogen sulfide (H_2S), the most recently discovered gasotransmitter. H_2S is produced enzymatically and non-enzymatically. Non-enzymatic H_2S-production takes place via reduction of bound sulfur [4]. The enzymatic H_2S-production is mediated by three different enzymes. The pyridoxal-5'-phosphate (PLP)-dependent enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) and the PLP-indendent enzyme 3-mercaptopyruvate sulfurtransferase (3MST). The main substrates for endogenous H_2S formation are cysteine and methionine. 3MST produces H_2S from 3-mercaptopyruvate (3-MP), which is produced by the enzymes cysteine aminotransferase (CAT) and D-Amino acid oxidase (DAO) from respectively L-cysteine and D-cysteine H_2S is further oxidized into thiosulfate and finally into sulfate. Small amounts of thiosulfate, the intermediate product of the H_2S pathway, can be found in blood and urine [5,6]. However, urinary sulfate and thiosulfate are not specific markers for endogenous H_2S production, and can also be the products of exogenous H_2S production by sulfate-reducing bacteria in the gut. H_2S is believed to play a regulatory role in different (patho-)physiological processes such as angiogenesis, inflammation and blood pressure regulation [7,8]. H_2S bioavailability is reduced in experimental models of diabetes [9–11]. Previous research has shown that exogenous administration of H_2S is protective in different renal disease models, including diabetic nephropathy [12–14]. In streptozotocin (STZ)-induced diabetic spontaneously hypertensive rats (SHR), treatment with H_2S-donor sodium hydrosulfide (NaHS) inhibits renal impairment and lowers blood pressure [10,15]. Intraperitoneal treatment of NaHS for 12 weeks lowered renal production of reactive oxygen species (ROS) and proteinuria in STZ-induced diabetic Sprague-Dawley rats [16]. Additionally, in STZ-induced diabetic rats, 4 weeks NaHS treatment resulted in improved vascular function. Moreover, *ex vivo* overexpression of CSE reduced ROS production and improved vascular relaxation in hyperglycemic conditions [17].

Like in experimental models of diabetes, H_2S bioavailability is reduced in patients with type 2 diabetes when compared to healthy subjects [18,19], but the performance of H_2S or its metabolite sulfate as a predictor of renal outcomes in patients with type 2 diabetes and nephropathy is not established.

Based on the above, we hypothesized that urinary levels of H_2S, as measured by its metabolic end product sulfate, could serve as a predictor of renal outcome. We therefore aimed to cross-sectionally investigate the association of sulfate with renal risk markers in type 2 diabetes patients and to prospectively examine its predictive value for renal events in patients with diabetic nephropathy.

2. Materials and methods

2.1. Study population

This study is a post-hoc study of a prospective, randomized, controlled trial (Sun-MACRO), and a prospective population-based cohort-study (the Prevention of Renal and Vascular End-Stage Disease (PREVEND)-study).

2.1.1. Sun-MACRO

The Sun-MACRO (sulodexide macroalbuminuria) trial has been described in detail previously [20]. Briefly, patients aged ≥18 years with a diagnosis of type 2 diabetes mellitus and marked proteinuria (urinary protein excretion ≥0.9 g/24 h or >0.9 mg per gram creatinine) and serum creatinine >1.3 mg/dL were included. Diabetic nephropathy was not biopsy proven, but nephropathy was assumed based on the high urinary protein levels and serum creatinine >1.3 mg/dL (>1.0 mg/dL in females) or eGFR < 60 ml/min/1.73 m^2. Patients with type 1 diabetes mellitus, patients with known additional nondiabetic renal disease and patients with the need for chronic immunosuppressive therapy were excluded. Eligible patients were randomly assigned to treatment with sulodexide 200 mg/day or placebo. After randomization, patients were seen every 3 months and were followed until the occurrence of a renal event which was defined as a confirmed doubling of serum creatinine, serum creatinine >6.0 mg/dL, or end-stage renal disease. The study was conducted according to the Declaration of Helsinki, the institutional review boards of each center approved the trial, and all patients provided written informed consent. This trial was registered with ClinicalTrials.gov (NCT00130312). The trial was stopped at an early stage because of futility of the intervention.

2.1.2. PREVEND

The PREVEND study has been described in detail previously [21]. Briefly, all participants (28–75 years) were inhabitants of Groningen, the Netherlands. The total PREVEND cohort consists of 8592 individuals, of which 6000 with urinary albumin concentration of ≥10 mg/L. Pregnancy and type 1 diabetes were exclusion criteria at the time of inclusion of the subjects. Participants were seen every 3 years to evaluate blood pressure, renal function, albuminuria and general health via a questionnaire. For the purpose of our study we selected all patients with type 2 diabetes at baseline (n = 265). The PREVEND study has been approved by the medical ethics committee of the University Medical Center Groningen and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.2. Renal outcome definition

Primary endpoint of the analyses was a composite renal endpoint, composed of a sustained doubling of baseline serum creatinine or ESRD, defined as a sustained serum creatinine ≥6.0 mg/dl (530 μmol/L), renal transplantation or need for dialysis.

2.3. Urine and plasma parameters

All urine samples in both studies were stored at −80 °C until analysis. Urine samples from the Sun-MACRO cohort and PREVEND study were morning void urine samples en 24-h urine samples respectively. Sulfate concentration at baseline was measured by ion exchange chromatography (type 861; Metrohm, Herisau, Switzerland), using a Metrosep A Supp 4 − 250/4.0 column. Intra-assay and inter-assay variations were 2.0% and 4.3%, respectively.

2.4. Clinical parameters

Serum and urine creatinine were determined using the modified Jaffe, rate-blanked, alkaline picrate method (Roche/Hitachi Modular System) (Sun-MACRO) or Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY) (PREVEND). Urinary albumin was determined by immunoturbidimetry (Roche, Montclair, NJ) (Sun-MACRO) or by nephelometry (BN™II Dade Behring Diagnostic, Marburg, Germany) (PREVEND).

2.5. Statistical analyses

Data analysis was performed using STATA/SE software (Release 13; StataCorp, College Station, TX). Normality was tested with the Shapiro-Wilk and Shapiro-Francia test and visualized with histograms and Q-Q plots. Skewed data were normalized for analyses by