

# RNA oxidation and albuminuria in type 1 diabetes patients

Kasper Broedbaek<sup>a,b,d</sup>, Jon T. Andersen<sup>b,d</sup>, Espen Jimenez-Solem<sup>b,d</sup>, Morten Petersen<sup>b,d</sup>, Maria Lajer<sup>f</sup>, Trine Henriksen<sup>b,d</sup>, Allan Weimann<sup>b,d</sup>, Hans-Henrik Parving<sup>c,e,g</sup>, Peter Rossing<sup>f</sup> and Henrik E. Poulsen<sup>b,d,e</sup>

We examined the relationship between urinary excretion of the RNA oxidation marker 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and kidney injury comparing type 1 diabetes patients with different levels of albuminuria (normoalbuminuria:  $N=58$ , microalbuminuria:  $N=46$ , macroalbuminuria:  $N=46$ ) and a nondiabetic control group ( $N=57$ ). Urinary 8-oxoGuo was measured in spot urine samples using ultraperformance liquid chromatography and tandem mass spectrometry. Urinary 8-oxoGuo was significantly higher in the patients [2.85 (2.45–3.33) vs. 2.49 (2.12–2.96) nmol/mmol creatinine,  $P=0.01$ ]. However, after adjustment for possible confounders of oxidative stress, the differences reduced slightly and became statistically nonsignificant ( $P=0.07$ ). There were no significant differences in the level of 8-oxoGuo between the three groups of patients ( $P=0.53$ ) and there were no significant associations between 8-oxoGuo and estimated glomerular filtration rate or albumin-creatinine ratio. In conclusion,

urinary excretion of the RNA oxidation marker 8-oxoGuo does not differ between type 1 diabetes patients with different levels of albuminuria. *Cardiovasc Endocrinol* 2:103–105 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

*Cardiovascular Endocrinology* 2013; 2:103–105

**Keywords:** albuminuria, diabetes, oxidative stress, 8-oxo-7,8-dihydroguanosine

<sup>a</sup>Department of Clinical Biochemistry, <sup>b</sup>Laboratory of Clinical Pharmacology, <sup>c</sup>Department of Endocrinology, Rigshospitalet, <sup>d</sup>Department of Clinical Pharmacology, Bispebjerg Hospital, <sup>e</sup>Faculty of Health Sciences, University of Copenhagen, Copenhagen, <sup>f</sup>Steno Diabetes Center, Gentofte and <sup>g</sup>Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark

Correspondence to Kasper Broedbaek, MD, PhD, Department of Clinical Biochemistry, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark  
Tel: +45 2062 2290; fax: +45 3545 2880;  
e-mail: kasper.broedbaek@rh.regionh.dk

Received 11 March 2013 Accepted 17 June 2013

## Introduction

Oxidative stress is believed to play an important role in the pathogenesis of complications in diabetes, where the generation of reactive oxygen species leads to oxidation of many macromolecules including lipids, proteins, carbohydrates, and nucleic acids [1,2]. 8-oxo-7,8-dihydroguanosine (8-oxoGuo) is a widely used biomarker of oxidative stress to RNA. Urinary excretion of 8-oxoGuo has been receiving increasing attention as it was shown to be an independent predictor of mortality in type 2 diabetes [3,4].

Whether urinary 8-oxoGuo excretion is increased in diabetic patients compared with healthy individuals is unknown, and no studies have investigated the association between urinary excretion of 8-oxoGuo and the presence of diabetes complications.

The aim of this study was to examine the relationship between urinary 8-oxoGuo, assessed in spot urine samples, and kidney injury in a cross-sectional study comparing three groups of type 1 diabetes patients with different levels of albuminuria and a nondiabetic control group.

## Methods

This study was based on data from a cohort used to identify biomarkers of diabetic nephropathy [5]. The population included Caucasian patients with type 1

diabetes from the outpatient clinic at the Steno Diabetes Center. On the basis of albumin excretion in 24-h urine samples that were collected as part of the routine care of the patients before the present study, patients were divided into three groups: 58 with normoalbuminuria [urinary albumin excretion rate (UAER) < 30 mg/24 h], 46 with persistent microalbuminuria (UAER between 30 and 300 mg/24 h), and 46 with persistent macroalbuminuria (UAER > 300 mg/24 h). The control group included 57 nondiabetic healthy individuals. The 24-h urine samples were only used for assessment of inclusion criteria. Groups were matched by sex and duration of diabetes. Assessment of participant characteristics has been described in detail elsewhere [6].

Urinary 8-oxoGuo was measured in spot urine samples at the Laboratory of Clinical Pharmacology, Rigshospitalet, Copenhagen. The urine samples, stored at –80 to –20°C until analysis, were assayed in 2009 for 8-oxoGuo using a validated method of ultraperformance liquid chromatography (UPLC) and tandem mass spectrometry (MS) [7]. 8-oxoGuo was normalized against urinary creatinine concentration.

The study was carried out according to the principles of the declaration of Helsinki. Written informed consent was obtained from all patients and the study was approved by the local ethics committee.

**Table 1 Clinical characteristics of the control group and type 1 diabetes patients**

	Controls	Diabetes	P value	Diabetes			P value
				Normoalbuminuria	Microalbuminuria	Macroalbuminuria	
N (male/female)	57 (37/20)	150 (81/69)	0.16	58 (30/28)	46 (24/22)	46 (27/19)	0.74
Age (years)	51±11.0	53±10.9	0.12	56±10.8	54±11.2	49±9.6	0.006
Smoker (%)	17.5	29.7	0.08	21.1	28.3	42.2	0.07
Diabetes duration (years)	—	36±11	—	37±11	35±11	34±11	0.39
Systolic blood pressure (mmHg)	131±17	141±21	0.001	138±22	141±23	145±18	0.31
Diastolic blood pressure (mmHg)	83±11	77±11	0.0007	76±11	75±12	79±10	0.17
HbA1c (%)	5.5±0.3	8.6±1.2	<0.0001	8.2±1.1	8.8±1.2	8.8±1.1	0.009
BMI (kg/m <sup>2</sup> )	25.8±3.5	24.9±3.6	0.14	24.7±2.9	25.1±3.7	25.1±4.4	0.80
UACR (mg/g)	5 (3–8)	20 (6–203)	<0.0001	5 (4–8)	26 (11–63)	499 (209–1115)	<0.0001
Creatinine (μmol/l)	95 (87–104)	93 (86–109)	0.56	92 (83–97)	89 (81–101)	125 (102–173)	<0.0001
eGFR (ml/min/1.73 m <sup>2</sup> )	70.5±9.6	64.0±17.4	0.0008	70.3±10.3	70.7±13.4	49.2±19.2	<0.0001
U-8-oxoGuo (nmol/mmol creatinine)	2.49 (2.12–2.96)	2.85 (2.45–3.33)	0.01	2.85 (2.46–3.32)	2.88 (2.58–3.45)	2.78 (2.22–3.20)	0.53

Data are mean±SD or median (interquartile range) unless otherwise indicated

eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; U-8-oxoGuo, urinary 8-oxo-7,8-dihydroguanosine; UACR, urinary albumin excretion rate.

P value refers to the overall difference between groups (analysis of variance).

Data are presented as mean±SD for normally distributed variables or median (interquartile range) for non-normally distributed variables. Comparisons between groups were performed using the  $\chi^2$ -test for categorical variables and analysis of variance for continuous variables. To account for the effects of potential confounders of oxidative stress, analysis of covariance was carried out.

Linear regression analysis was used to assess the association between urinary 8-oxoGuo and markers of renal function: estimated glomerular filtration rate (eGFR) and albumin-creatinine ratio (UACR).

Because of deviation from normal distribution, 8-oxoGuo was log-transformed before calculation.

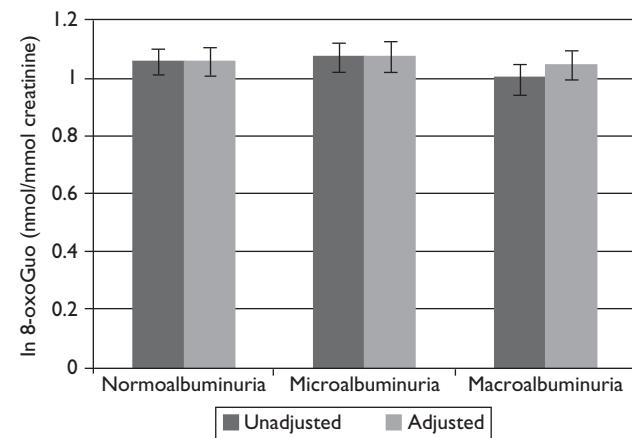
All statistical analyses were carried out using the SAS software, version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Statistical significance was defined as P value less than 0.05. All statistical tests were two sided.

## Results

The characteristics of the control group and patients are shown in Table 1.

Compared with the control group, the patients had significantly higher HbA1c, and UACR, and had significantly lower eGFR. Patients had significantly higher systolic blood pressure, whereas diastolic blood pressure was significantly higher in the control participants. Although not statistically significant, a greater proportion of the patients were smokers.

The three groups of type 1 diabetes patients were well matched in terms of the duration of diabetes, BMI, blood pressure, and sex. Patients with macroalbuminuria were younger than patients with normoalbuminuria and microalbuminuria. Serum creatinine was significantly higher and eGFR was significantly lower in the macroalbuminuric group than in the other two groups. In addition, although not statistically significant, the proportion of participants

**Fig. 1**

Urinary 8-oxoGuo in the three groups of type 1 diabetes patients. Data are least square means±SE of ln 8-oxoGuo. Data were analyzed by analysis of variance and by analysis of covariance after adjustment for potential confounders of oxidative stress: age, sex, smoking status, and HbA1c.

who were smokers differed between the groups, where the proportion increased with the degree of albuminuria. Patients with normoalbuminuria had a significantly lower level of HbA1c.

Urinary levels of 8-oxoGuo in the patient and the control group are shown in Table 1 and in Fig. 1.

Urinary 8-oxoGuo was significantly higher in the patients [2.85 (2.45–3.33) vs. 2.49 (2.12–2.96) nmol/mmol creatinine, P = 0.01]. However, after adjustment for possible confounders of oxidative stress, age, sex, and smoking, the differences reduced slightly and became statistically nonsignificant (P = 0.07).

There were no significant differences in the level of 8-oxoGuo between the three groups of type 1 diabetes patients (P = 0.53). Similarly, after adjustment for possible

confounders of oxidative stress, age, sex, smoking, and HbA1c, no significant differences were observed ( $P = 0.92$ ) (Fig. 1).

There were no significant associations between 8-oxoGuo and eGFR ( $R^2 = 0.009$ ,  $P = 0.26$ ) or UACR ( $R^2 = 0.009$ ,  $P = 0.26$ ). In addition, the patients' use of medication [hypertensive agents (RAAS inhibitors, diuretics,  $\beta$ -blockers, calcium-channel blockers), statins, and acetylsalicylic acid] was not associated significantly with 8-oxoGuo excretion (data not shown).

## Discussion

This is the first study to investigate the association between kidney injury in diabetes and urinary 8-oxoGuo. 8-oxoGuo in spot urine samples did not differ significantly between type 1 diabetes patients with normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively. 8-oxoGuo was not associated with markers of renal function (eGFR and albumin–creatinine ratio).

8-oxoGuo excretion was increased in type 1 diabetes patients compared with the control group, although adjustment for possible confounders of oxidative stress rendered the difference nonsignificant.

Our study has some limitations. Matching was not ideal. Most importantly, the three groups differed significantly in age and in the level of glycated hemoglobin, which are known to be closely associated with nucleic acid oxidation. In addition, although not reaching statistically significance, the proportion of smokers was not the same in all three groups. These differences were accounted for in adjusted analysis (analysis of covariance). Other limitations of this study are that we only measured 8-oxoGuo and no other RNA oxidation markers or metabolites were measured, and that the method used to estimate GFR could be too insensitive to detect small differences in GFR.

We have previously shown that urinary excretion of the RNA oxidation marker 8-oxoGuo assessed using UPLC-MS in freshly voided morning urine samples independently predicted mortality in type 2 diabetes patients both when assessed shortly after diagnosis of diabetes and after 6 years of diabetes duration [3,4]. This association has given rise to

speculations of whether this urinary marker is merely a measure of the kidney function, where patients with renal complications have a greater excretion of 8-oxoGuo than those without. The present study does not support this notion as patients with microalbuminuria or macroalbuminuria did not have greater levels of 8-oxoGuo excretion than patients with normoalbuminuria.

In conclusion, urinary excretion of the RNA oxidation marker 8-oxoGuo assessed in spot urine does not differ between type 1 diabetes patients with different levels of albuminuria.

## Acknowledgements

This study was supported by research funding from the Research Committee at Copenhagen University Hospital – Rigshospitalet (Rigshospitalets Forskningsudvalg), the Danish Medical Research Council, Aase and Ejnar Danielsen Foundation, P. Carl Petersen Foundation, the Augustinus Foundation, and the Lundbeck Foundation.

## Conflicts of interest

There are no conflicts of interest.

## References

- Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**:1615–1625.
- Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 2008; **57**:1446–1454.
- Broedbaek K, Siersma V, Henriksen T, Weimann A, Petersen M, Andersen JT, et al. Urinary markers of nucleic acid oxidation and long-term mortality of newly diagnosed type 2 diabetic patients. *Diabetes Care* 2011; **34**:2594–2596.
- Broedbaek K, Siersma V, Henriksen T, Weimann A, Petersen M, Andersen JT, et al. Association between urinary markers of nucleic acid oxidation and mortality in type 2 diabetes: a population-based cohort study. *Diabetes Care* 2013; **36**:669–676.
- Rossing K, Mischael H, Dakna M, Zurbig P, Novak J, Julian BA, et al. Urinary proteomics in diabetes and CKD. *J Am Soc Nephrol* 2008; **19**:1283–1290.
- Nielsen SE, Sugaya T, Tarnow L, Lajer M, Schjoedt KJ, Astrup AS, et al. Tubular and glomerular injury in diabetes and the impact of ACE inhibition. *Diabetes Care* 2009; **32**:1684–1688.
- Henriksen T, Hillestrom PR, Poulsen HE, Weimann A. Automated method for the direct analysis of 8-oxo-guanosine and 8-oxo-2'-deoxyguanosine in human urine using ultraperformance liquid chromatography and tandem mass spectrometry. *Free Radic Biol Med* 2009; **47**:629–635.