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Urinary adiponectin excretion rises with increasing albuminuria in type 1 diabetes ☆

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ABSTRACT

Aim: Urinary adiponectin (u-adiponectin) excretion has been suggested to reflect early glomerular damage. Inspired by this, we studied the levels of u-adiponectin in type 1 diabetic patients with different levels of urinary albumin excretion (UAE).
Methods: U-adiponectin was analysed by ELISA in type 1 diabetic patients: Fifty-eight with normoalbuminuria (<30 mg albumin/24 h), 43 with persistent microalbuminuria (30–300 mg/24 h) and 44 with persistent macroalbuminuria (>300 mg/24 h). For comparison, a control group of 55 healthy individuals was included.
Results: U-adiponectin increased with increasing levels of UAE ($p < 0.01$). U-adiponectin median (interquartile range): Normoalbuminuria 0.38 (0.14–1.31), microalbuminuria 1.12 (0.20–2.68), macroalbuminuria 9.20 (1.10–23.35) and controls 0.09 (0.06–0.24) μg/g creatinine. Levels were unrelated to sex, age, cholesterol, diastolic BP and BMI. U-adiponectin was weakly associated with increasing systolic BP and HbA_{1c} ($r^2 < 0.1$, $p < 0.05$), but strongly related to increasing UAE ($r^2 = 0.57$, $p < 0.001$) and decreasing eGFR ($r^2 = 0.26$, $p < 0.001$). The relationship between UAE and u-adiponectin was significant in all groups and independent of eGFR, BMI, BP and HbA_{1c}. Furthermore, u-adiponectin was associated with markers of tubular damage ($p < 0.01$).
Conclusion: U-adiponectin rises with increasing levels of UAE in patients with type 1 diabetes. This is in accordance with the hypothesis that loss of adiponectin may reflect glomerular and/or tubular damage.

1. Introduction

Thirty to forty percent of all patients with diabetes develop nephropathy, which is the most common cause of end-stage renal disease (ESRD) in Western countries (Parving, Mauer, & Ritz, 2004). Furthermore, proteinuric patients have an increased mortality compared to the general population and likewise when compared to diabetic patients without kidney disease (Borch-Johnsen, Andersen, & Deckert, 1985; Rossing, Hougaard, Borch-Johnsen, & Parving, 1996). This is mainly explained by the increased risk of ESRD and cardiovascular disease (CVD) (Borch-Johnsen et al., 1985). Therefore, prevention of diabetic kidney disease plays a pivotal role in the treatment of diabetic patients.

Adiponectin is an adipocyte derived peptide closely linked to components of the metabolic syndrome and atherosclerosis (Kadowaki & Yamauchi, 2005). Adiponectin appears to decrease vascular inflammation, foam cell formation and cellular adhesion (Gable, Hurel, & Humphries, 2006), events involved in the initiation and progression of vascular lesions. Although adiponectin has some beneficial effects on the vasculature, high concentrations of adiponectin have been associated with microangiopathy in type 1 diabetes (Looker, Krakoff, Funahashi, et al., 2004; Saraheimo, Forsblom, Fagerudd, et al., 2005; Costacou, Zgibor, Evans, et al., 2005; Frystyk, Tarnow, Hansen, Parving, & Flyvbjerg, 2005; Hadjadj, Aubert, Fumeron, et al., 2005). In addition, increased adiponectin levels are predictive of the development of ESRD in type 1 diabetic patients with diabetic nephropathy (Jorsal, Tarnow, Frystyk, et al., 2008; Saraheimo, Forsblom, Thorn, et al., 2008). However, the link between serum adiponectin and albuminuria is so far inconclusive. Three

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experimental studies have reported that adiponectin may have a distinct role in glomerular homeostasis by regulating albuminuria and podocyte function (Sharma, Ramachandrarao, Qiu, et al., 2008; Ohashi, Iwatani, Kihara, et al., 2007; Rutkowski, Wang, Park, et al., 2013). Thus, it is conceivable that the paradoxically elevated levels of adiponectin are a counter-regulatory response to metabolic derangements in type I diabetes and renal failure (Zoccali, Mallamaci, Panuccio, et al., 2003), serving to prevent progressing podocyte damage and decreasing kidney function (Sharma et al., 2008). Finally, a recent study suggested that urinary adiponectin (u-adiponectin) reflects early glomerular damage in type 2 diabetes (von EM et al., 2009). This prompted us to investigate the level of u-adiponectin in type 1 diabetic patients with different levels of UAE. We also investigated the association between u-adiponectin and two markers of tubular injury. Adiponectin exists in different isoforms, however, we have decided to measure the high molecular weight (HMW) isoform which is the biological most active isoform (Pajvani, Hawkins, Combs, et al., 2004).

2. Subjects and methods

2.1. Research design and patients

This cross-sectional study with a prospective follow-up consisted of 145 type 1 diabetic patients and 55 non-diabetic healthy controls (Leth, Andersen, Frystyk, et al., 2008). Type 1 diabetes was defined as age at onset of diabetes younger than 35 years and time to definite insulin therapy less than 1 year. The type 1 diabetic patients were divided into normo-, micro-, and macroalbuminuric groups. Established diabetic nephropathy was defined by persistently increased UAE (>300 mg/24 h) in two out of three consecutive measurements in sterile urines, presence of retinopathy and absence of other kidney or urinary tract diseases (Parving et al., 2004). Microalbuminuria was defined as a UAE of 30–300 mg/24 h, while the absence of diabetic nephropathy (normoalbuminuria) was defined as a persistent UAE less than 30 mg/24 h after at least 15 years of type 1 diabetes in patients not treated with renin–angiotensin system inhibitors such as angiotensin converting enzyme inhibitors (ACEis) or angiotensin II receptor blockers (ARBs).

2.2. Baseline clinical and laboratory investigations

Blood pressure (BP) was measured with a standard mercury sphygmomanometer using an appropriate cuff size. The measurements were performed twice in the resting state, and the measurements were averaged. From venous samples, plasma lipid levels were determined by standard methods. HbA1c was determined by standard high-performance liquid chromatography (HPLC) techniques with normal values ranging from 4.1% to 6.4%.

Serum creatinine concentration was determined by an enzymatic modified Jaffe's method. At the day of the investigation, UAE was measured in a spot urine sample by an enzyme immunoassay and expressed as urinary albumin/creatinine ratio (Feldt-Rasmussen, Dinesen, & Deckert, 1985). The estimated glomerular filtration rate (eGFR) was estimated by the Modification of Diet in Renal Disease equation (Levey et al., 1999). Diabetic retinopathy was assessed by fundus photography after pupillary dilatation and graded nil, simplex, and proliferative retinopathy, respectively. Based on standardized questionnaires, current smokers of one or more cigarettes/cigars/pipes per day were classified as smokers and all others as non-smokers. Previous non-fatal CVD-events were considered present in patients with a history of admission for stroke or myocardial infarction (MI). Urine Liver Fatty Acid Binding Protein (u-LFABP) and urine Neutrophil Gelatinase-Associated Lipocalin (u-NGAL) were measured in spot urine samples by a two-step sandwich enzyme-linked immunosorbent assay and an ELISA-based method, manufactured by Bioporto and R&D Systems, respectively.

2.2.1. Measurement of u-adiponectin

The morning spot urine samples were stored at −80 °C until analysis. U-adiponectin was analysed by using a microtiter plate "sandwich" format enzyme-linked immunosorbent assay (ELISA) technique (ALPCO Diagnostics, USA) and expressed as u-adiponectin/creatinine ratio. The assay was used for the quantitative, selective determination of HMW adiponectin. The detectable analytical range for u-adiponectin is 0.075 to 4.8 μg/L with intra- and inter-assay variation of <10% and <15%, respectively. Values below the analytical sensitivity of 0.075 μg/l cannot be detected and were assigned zero.

2.2.2. Follow-up

The GFR was measured annually in all macroalbuminuric patients on Steno Diabetes Center. We followed the development in kidney function until 2012, with an average follow-up time of 6 years. Patients were followed until last visit on Steno Diabetes Center or time of death. The change in kidney function was calculated by linear regression in patients with at least 3 measurements of GFR.

The study was performed in accordance with the Helsinki Declaration. The local ethics committee approved the study and all patients gave their informed consent.

2.3. Statistics

Normally distributed variables are given as means ± SD, whereas non-normally distributed variables were log transformed before analysis and given as medians (range). Comparisons between groups were performed by an unpaired Student's *t*-test, ANOVA or linear regression as appropriate. A χ^2-test was used to compare non-continuous variables. *P*-values ≤0.05 were considered statistically significant. All calculations were performed using a commercially available program (SPSS for Windows, version 14.1, Chicago, IL, USA).

3. Results

The clinical characteristics of the participants are listed in Table 1. The four groups were similar with respect to BMI, sex and systolic BP. The group of patients with macroalbuminuria was younger. The three diabetic groups had a decreased diastolic BP and increased HbA_{1c} compared to the healthy controls. Furthermore, the diabetic groups had an increased number of previous CV events, however, no significant differences were seen between the three diabetic groups. U-adiponectin (log transformed) increased with increasing levels of albuminuria (Table 1). U-adiponectin median (interquartile range): Normoalbuminuria 0.38 (0.14–1.31) μg/g creatinine, microalbuminuria 1.12 (0.20–2.68), macroalbuminuria 9.20 (1.10–23.35) and non-diabetic, normoalbuminuric controls 0.094 (0.055–0.24) ($p < 0.01$ between all groups, Fig. 1). U-adiponectin was unrelated to sex, age, cholesterol, diastolic BP and body mass index (BMI). However, increasing levels of u-adiponectin were weakly associated with increasing systolic BP and HbA_{1c}, ($r^2 < 0.10$, $p < 0.05$ for both) and strongly related to increasing albuminuria ($r^2 = 0.57$, $p < 0.001$) and decreasing eGFR ($r^2 = 0.26$, $p < 0.001$). The relationship between albuminuria and u-adiponectin levels was significant in all albuminuria groups, independent of eGFR, BMI, BP and HbA_{1c}.

Serum adiponectin levels have previously been measured and published elsewhere (Leth et al., 2008) and these data were used to calculate the ratio between urine and serum HMW adiponectin levels. The ratio increased with increasing albuminuria: Normoalbuminuria 1.4 (0.9–2.0) μg/mg, microalbuminuria 3.2 (1.1–5.3), macroalbuminuria 21.8 (9.0–34.5) and controls 1.1 (0.3–1.8) ($p < 0.01$). U-adiponectin was partly explained by the serum adiponectin concentration ($r^2 = 0.21$, $p = 0.001$).