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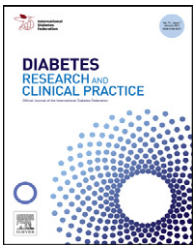

Blockade of the renin–angiotensin system and renal tissue oxygenation as measured with BOLD-MRI in patients with type 2 diabetes☆,☆☆

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ARTICLE INFO

Article history:
Received 18 July 2012
Received in revised form
23 October 2012
Accepted 8 November 2012
Published on line 14 December 2012

Keywords:
BOLD-MRI
Hypertension
Renal
Angiotensin receptor blocker
ACE-inhibitor
Furosemide

ABSTRACT

Aim: To assess whether blockade of the renin–angiotensin system (RAS), a recognized strategy to prevent the progression of diabetic nephropathy, affects renal tissue oxygenation in type 2 diabetes mellitus (T2DM) patients.

Methods: Prospective randomized 2-way cross over study; T2DM patients with (micro)albuminuria and/or hypertension underwent blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI) at baseline, after one month of enalapril (20 mg qd), and after one month of candesartan (16 mg qd). Each BOLD-MRI was performed before and after the administration of furosemide. The mean R_2^* (=$1/T_2^*$) values in the medulla and cortex were calculated, a low R_2^* indicating high tissue oxygenation.

Results: Twelve patients (mean age: 60 ± 11 years, eGFR: 62 ± 22 ml/min/1.73 m^2) completed the study. Neither chronic enalapril nor candesartan intake modified renal cortical or medullary R_2^* levels. Furosemide significantly decreased cortical and medullary R_2^* levels suggesting a transient increase in renal oxygenation. Medullary R_2^* levels correlated positively with urinary sodium excretion and systemic blood pressure, suggesting lower renal oxygenation at higher dietary sodium intake and blood pressure; cortical R_2^* levels correlated positively with glycemia and HbA1c.

Conclusion: RAS blockade does not seem to increase renal tissue oxygenation in T2DM hypertensive patients. The response to furosemide and the association with 24 h urinary sodium excretion emphasize the crucial role of renal sodium handling as one of the main determinants of renal tissue oxygenation.

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☆ The results presented in this paper have not been published previously, except, in part, as an oral presentation at the 5th International Meeting of the French Society of Hypertension, December 15–16, 2011.

☆☆ Sources of support: This study was in part supported by a research grant from the Swiss Society of Hypertension (Astra Zeneca grant), the Swiss National Science Foundation (FN 32003B-132913), by the Centre d'Imagerie BioMédicale (CIBM) of the University of Lausanne (UNIL), the Swiss Federal Institute of Technology Lausanne (EPFL), the University of Geneva (UniGe), the Centre Hospitalier Universitaire Vaudois (CHUV), the Hôpitaux Universitaires de Genève (HUG) and the Leenaards and the Jeantet Foundations. Menno Pruijm is supported by a SPUM-grant from the Swiss National Science Foundation (33CM30-124087).

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http://dx.doi.org/10.1016/j.diabres.2012.11.004

1. Introduction

The incidence of type 2 diabetes mellitus (T2DM) is rising worldwide, and T2DM has become one of the major causes of chronic kidney disease [1]. The pathogenesis of diabetic nephropathy – a condition that affects 30% of all T2DM patients – is incompletely understood, and probably the result of metabolic, hemodynamic and inflammatory mechanisms among which the well recognized activation of the renin–angiotensin system [2].

Adequate renal tissue oxygenation is critical for the maintenance of a normal renal function [3]. Renal tissue hypoxia is the consequence of a difference between oxygen delivery and consumption. In theory, several factors can cause renal hypoxia, including oxidative stress, altered renal hemodynamics, increased glomerular filtration rate, tubular hypertrophy and increased active transport of electrolytes [4]. All these factors have been described in animal models and/or patients with diabetic nephropathy. Renal tissue hypoxia has been reported in the kidneys of diabetic mice, suggesting that renal hypoxia might be a key player in the development of diabetic nephropathy [5]. Until recently, the measurement of renal tissue oxygenation in humans was not possible. A relatively new and validated technique called Blood Oxygenation-Level Dependent MRI (BOLD-MRI) now enables non-invasive assessment of renal tissue oxygenation in humans [5–8]. BOLD-MRI uses the paramagnetic properties of deoxyhemoglobin to assess cortical and medullary oxygenation. BOLD-MRI measurements do not require the administration of contrast products and can be repeated several times in the same person without any side effects, making it an interesting tool to study renal structural and functional properties in T2DM patients.

The use of blockers of the renin–angiotensin system (RAS-blockers) as antihypertensive and antiproteinuric medication has been particularly effective in slowing the progression of renal disease in T2DM [9], and all existing guidelines advise to introduce an angiotensin converting enzyme inhibitor (ACEI) or an angiotensin II type 1 receptor blocker (ARB) in patients with type 2 diabetes, as soon as microalbuminuria is detected or in case of hypertension [10].

Their effectiveness has historically been based on their ability to lower systemic blood pressure, to increase renal blood flow, to lower intraglomerular pressure and to reduce proteinuria. RAS blockers have several other properties that may also lead to renal protection such as the inhibition of growth factors, solute transport, and macrophage proliferation as well as anti-inflammatory effects [11]. Among them, animal studies have suggested that administration of RAS blockers leads to an increase in renal tissue oxygenation [12,13].

To our knowledge, the impact of RAS blockade on renal tissue oxygenation has never been investigated in T2DM patients. The aim of this prospective randomized cross-over study was therefore to assess the chronic effect of RAS blockade on renal tissue oxygenation using BOLD-MRI and to compare, on this behalf, an angiotensin converting enzyme inhibitor with an angiotensin II type 1 receptor blocker.

2. Methods

2.1. Subjects

T2DM patients with chronic kidney disease stage 1–4 (estimated creatinine clearance (4D-MDRD) >15 ml/min/1.73 m^2) were eligible for this study. Patients were either already on treatment with an ACEI or ARB or had a formal indication to start one (hypertension, (micro)albuminuria or both). Other inclusion criteria were: age ≥18 years, type 2 diabetes according to the definition of the World Health Organization [14], no illicit drug intake or substance abuse, and the ability to understand the study protocol. Exclusion criteria were: intolerance to study drugs, known renal artery stenosis, a serum potassium >5.0 mmol/l, and a contra-indication to MR-imaging such as claustrophobia or the presence of a pacemaker or other implanted metallic device.

2.2. Study protocol

Patients were recruited at the outpatient clinic of the nephrology and hypertension department of the university hospital in Lausanne and at a diabetes outpatient clinic. After explaining the nature and purpose of the study, written informed consent was obtained from each patient. The protocol was approved by the local institutional review committee (Ethical Committee of the Canton de Vaud, Switzerland). Baseline physical examination and office blood pressure measurement were performed at screening and at each of the three study visits. Blood pressure (BP) was measured five times by an experienced physician using an automated Omron 705IT oscillometric device according to the recommendations of the European Society of Hypertension [15]; each reported BP was the mean of the last four (out of five) BP measurements.

In total, three BOLD-MRI measurements were performed per participant: the first at baseline, the second after one month of treatment with the ACEI enalapril (20 mg qd, taken in the morning), and the third after one month of treatment with the ARB candesartan (16 mg qd, taken in the morning). Patients treated with a RAS blocker underwent a wash-out period of two weeks before the baseline BOLD-MRI. After the baseline visit, patients were randomized to start either with enalapril or with candesartan with a switch to the other treatment after one month. There was no washout period between the two treatment phases. The choice of these two drugs as representatives of the ACEI- and ARB-drug classes was based on their similar pharmacokinetics (t_{max} 3–4 h, $t_{½}$ 9–11 h) [16,17]. The treatment duration of one month was the estimated time necessary to obtain the maximum antihypertensive effect of each drug. On the day of MRI-measurements – as on all the other days throughout the study – patients took the study drug at 9 o'clock in the morning, and MRI measurements were performed at the peak effect of the drugs. All concomitant (including antihypertensive) medication was continued throughout the study, yet changes in dose or the introduction of new drugs were not allowed.

Participants were maintained on their regular diet. Dietary sodium intake was kept as stable as possible during the study, since salt intake has been shown to influence the R_2^* signal [18].

Salt intake was verified each time before BOLD-MRI by a 24 h urine collection (dosing volume, creatinine- and sodium-concentrations); 24 h urinary sodium excretion is considered as the best way to estimate dietary sodium intake [19,20]. On the day of each BOLD-MRI measurement, the patients took a light breakfast before 8 am. An identical oral hydration protocol was followed by each participant at home (loading dose of 5 ml/kg of water at 9 am, followed by 3 ml/kg/h), in order to avoid as much as possible differences in renal perfusion induced by differences in volume status. The patients presented at 12 pm at the study center. Upon arrival, an intravenous catheter was inserted into an antecubital vein. Thirty minutes later blood was drawn to dose plasma renin activity (PRA), aldosterone, sodium, potassium and glucose, serum creatinine, blood urea nitrogen and hemoglobin as described previously [21]. BOLD-MRI was performed at 13 pm in the radiology department, before and 15 min after the injection of 20 mg of furosemide.

2.3. BOLD-MRI

BOLD-MRI is a non-invasive method to assess tissue oxygen bioavailability in humans, using deoxyhemoglobin as an endogenous contrast agent. Deoxyhemoglobin is a paramagnetic molecule that induces magnetic field perturbations, in contrast to oxyhemoglobin, a diamagnetic substance. Field perturbations lead to a faster signal attenuation in gradient echo T_2^*-weighted sequences. Therefore, increased concentration of deoxyhemoglobin present in hypoxic tissue leads to an increase of the MRI signal attenuation. Acquisition of MR images with increasing echo times allows computation of an exponentially decreasing function of the signal. This decay constant is an estimate of the relaxivity R_2^*, defined as $1/T_2^*$, related to the concentration of deoxyhemoglobin, according to the equation:

$$R_{2*} = R_{2*_{2,0}} + \alpha \times p_{Hb} + \beta \times p_{Hb}$$

In this equation, $R_2^*{}_{2,0}$ is the relaxivity of fully oxygenated hemoglobin and p_{Hb} is the part of deoxygenated hemoglobin. $p_{Hb} = 1 - Y$, with Y as the fraction of oxygenated hemoglobin [22]. Since the concentration of blood (de)oxyhemoglobin is proportional to the partial pressure of oxygen (pO_2) of blood, and blood pO_2 is in balance with tissue pO_2, R_2^* as measured by BOLD-MRI can be considered as a marker of tissue pO_2 [6,7]. The relationship between R_2^* levels and tissue pO_2 is considered to be linear, as described previously by Pedersen et all in pigs exposed to different levels of respirator supplied oxygen fractions undergoing simultaneous measurement of renal R_2^* levels using BOLD-MRI and direct pO_2 measurement using oxygen-sensitive microelectrodes [23].

MR measurements were carried out on a 3T whole-body MR system (Trio Tim, Siemens Medical Systems, Erlangen, Germany). In order to acquire as much as possible the same anatomic slices, automatic table position was used throughout the study. Based on single shot fast spin echo localizers, the coronal slice position was oriented in parallel with the long axis of the kidneys. Four coronal slices with good cortico-medullary differentiation were selected from morphological images for functional evaluation with BOLD-MRI (usually in the inner region of the kidneys). All four slices were chosen in the presence of the same nephrologist with experience in radiological imaging (M.P.). All images were obtained in inspiration, in order to reduce diaphragm-dependent variance in kidney position. Twelve T_2^*-weighted images were recorded within a single breath-hold of 17.4 s with a modified Multi Echo Data Image Combination sequence (MEDIC) with the following parameters: repetition time (TR) 68 ms, echo time (TE) 6–52.2 ms (equidistant echo time spacing 4.2 ms), flip angle 20°, field of view (FOV) 400 mm × 400 mm, voxel size 1.6 mm × 1.6 mm × 5 mm, bandwidth 700 Hz/pixel, matrix 256 × 256. The range of TE was limited to 52.2 ms in order to avoid, also for voxels with a lower signal-to–noise ratio, to get into the area of the Rician distribution of noise. All images were exported to a standard personal computer for analysis with a home-built IDL program (Interactive Data Language, Boulder, CO, USA). R_2^* maps were calculated voxel by voxel using a Levenberg–Marquardt least-squares algorithm to fit an exponential function to the signal intensities measured for each echo time. For each coronal slice of 12 T_2^* images, the one with the best cortico-medullary differentiation was selected (usually the one with the lowest TE = 6 ms). On this image, regions of interest (ROIs) were drawn manually by the same experienced investigator, blinded for the study phase (L.H), as shown in Fig. 1. ROIs were traced in the form of circles of equal size (containing approximately 10 voxels each) in the medulla and the cortex (two in the cortex and two in the medulla in each kidney). The reported R_2^* value was the mean value of 16 ROIs for the medulla and the cortex (4 slices, each slice 4 ROI in the cortex and 4 in the medulla). The procedure was repeated for all four coronal series obtained after the administration of furosemide. This technique has been shown to have a good reproducibility (mean coefficient of variance of 3% in the

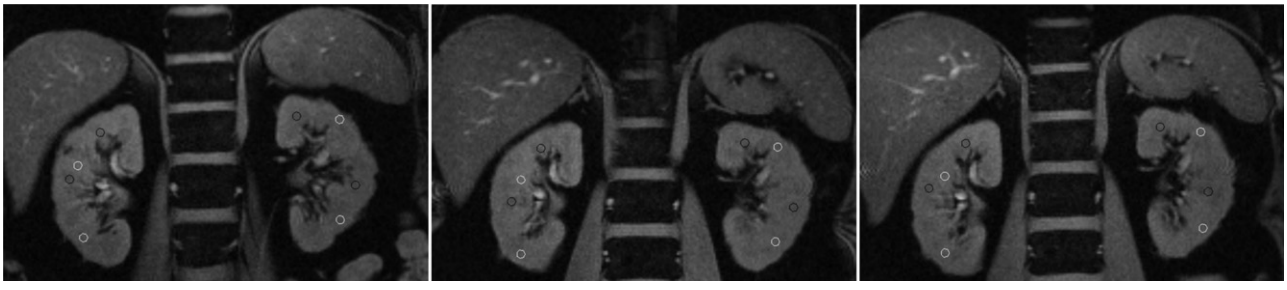

Fig. 1 – Coronal section through both kidneys of one participant, showing the T_2^* image at baseline (left), after one month of candesartan (middle), and after one month of enalapril (right). The selected regions of interest (ROI) are represented as black (medulla) and white (cortex) circles. The reported cortical and medullary R_2^* values of each participant are the mean values of 16 ROIs (4 slices through both kidneys, each slice 2 ROIs in the cortex and 2 in the medulla per kidney).

medulla and 4% in the cortex), for different slice directions (axial, coronal) [8,24]. Medullary/cortical R_2^* ratio (MCR2*) was calculated for each participant by dividing mean medullary R_2^* by cortical R_2^* levels [25].

2.4. Laboratory parameters

Serum creatinine was measured using the Jaffe kinetic compensated method (Roche Diagnostics, Switzerland, intra-assay variability 0.7–2.9%). Estimated glomerular filtration rate (eGFR) was calculated with the 4D-MDRD-formula [26]. PRA, aldosterone, urea nitrogen, hemoglobin, potassium and sodium were measured at each study visit as described previously [21]. Glycemia was measured with the Glucose dehydrogenase technique (Roche diagnostics), and HbA1c by chromatography (pack 220-0101, Bio-Rad, Dreieich, Germany).

2.5. Statistics

Clinical data were analyzed using STATA 11.0 (StataCorp, College Station, TX, USA). Based on an expected medication-induced difference in renal R_2^* values of 10%, an alpha of 0.05 at two sided significance level, and using the highest standard deviation obtained in former studies, we needed to include 12 patients to have a power of 80% and 15 patients to have a power of 90%. Quantitative variables were expressed as mean ± standard deviation, or as median (25th–75th interquartile range), as appropriate. Qualitative variables were expressed as number of patients and percentage. Comparisons between study phases were analyzed with ANOVA or Cuzick's non-parametric test statistics, as appropriate. Spearman's rank test was used to examine correlations. In case of non-normal distribution, variables were log-transformed. A two-sided p-value <0.05 was considered statistically significant.

3. Results

Thirty-eight patients fulfilled the inclusion criteria and were initially interested to participate. Two patients had contraindications to MR imaging, 24 patients declined after detailed explanations of the study protocol. Twelve patients (9 recruited at the university hospital of Lausanne, three at the outpatient diabetes clinic) agreed to participate and signed the informed consent form; their baseline characteristics are shown in Table 1. All patients were of Caucasian origin and were hypertensive or had microalbuminuria (MAU); two patients had overt proteinuria (of respectively 6.7 and 10.0 g/24 h). The values of clinical parameters during the study are shown in Table 2. Although there was a trend toward lower BP, higher PRA and lower plasma aldosterone levels during RAS blockade, differences between the study phases were not statistically significant.

An example of MR images obtained in one participant is illustrated in Fig. 1. Mean values for cortical and medullary R_2^* and their changes after one month of candesartan and after one month of enalapril for each individual are shown in Fig. 2.

Table 1 – Baseline characteristics of the patients.

N = 12	Variable
Age (years)	60 ± 11 (39–74)
Male Gender (%)	75
Duration of diabetes (years)	11 ± 7 (5–28)
Duration of hypertension (years)	17 ± 7 (8–29)
Body Mass Index (kg/m^2)	35 ± 3 (29–40)
(Micro)albuminuria (%)	100
Treatment of diabetes (%)	
Metformine	66
Thiazolidinedione	16
Sulfonylurea	25
Insulin	66
Treatment of hypertension (%)	
ACEI	33
ARB	66
Beta-blocker	50
Calcium antagonist	58
Systemic vasodilators	16
Thiazide diuretic	66
Number of antihypertensive drugs per patient	3 ± 1 (1–4)

Variables are shown as mean ± SD (min–max), or as percentage of total number of patients, as appropriate.

Table 2 – Clinical characteristics of the patients at baseline and under candesartan or enalapril therapy.

N = 12	Baseline	Candesartan	Enalapril
Weight (kg)	99.3 ± 15	100 ± 16.0	99.8 ± 16
SBP (mmHg)	137 ± 11	134 ± 12	134 ± 11
DBP (mmHg)	75 ± 14	73 ± 9	73 ± 10
Urinary sodium (mmol/24 h)	123 ± 42	116 ± 39	111 ± 36
Urinary potassium (mmol/24 h)	86 ± 30	68 ± 17	69 ± 31
HbA1c (%)	7.8 ± 1	7.7 ± 1	7.8 ± 1
Glycemia (mmol/l)	7.2 ± 3	7.9 ± 3	7.2 ± 2
Hematocrit (%)	38 ± 4	38 ± 3	39 ± 4
Serum sodium (mmol/l)	137 ± 3	138 ± 3	137 ± 2
Serum potassium (mmol/l)	3.9 ± 0.6	4.1 ± 0.8	4.1 ± 0.7
Serum creatinine (μmol/l)	123 ± 63	130 ± 78	130 ± 79
eGFR (ml/min/1.73 m^2)	62 ± 22 (23–85)	61 ± 22 (19–83)	60 ± 23 (19–85)
Aldosterone (pg/ml)	94 ± 80	83 ± 100	73 ± 81
Plasma renin activity (ng/ml/h)	0.7 ± 0.6 (0.1–1.4)	3.0 ± 3.1 (0.1–9.5)	3.2 ± 3.8 (0.2–11.9)

Variables are shown as mean ± SD (min–max). No significant changes were noted in any of the shown parameters (ANOVA statistics). SBP: systolic blood pressure, DBP: diastolic blood pressure, eGFR: estimated glomerular filtration rate, HbA1c: glycated hemoglobin.

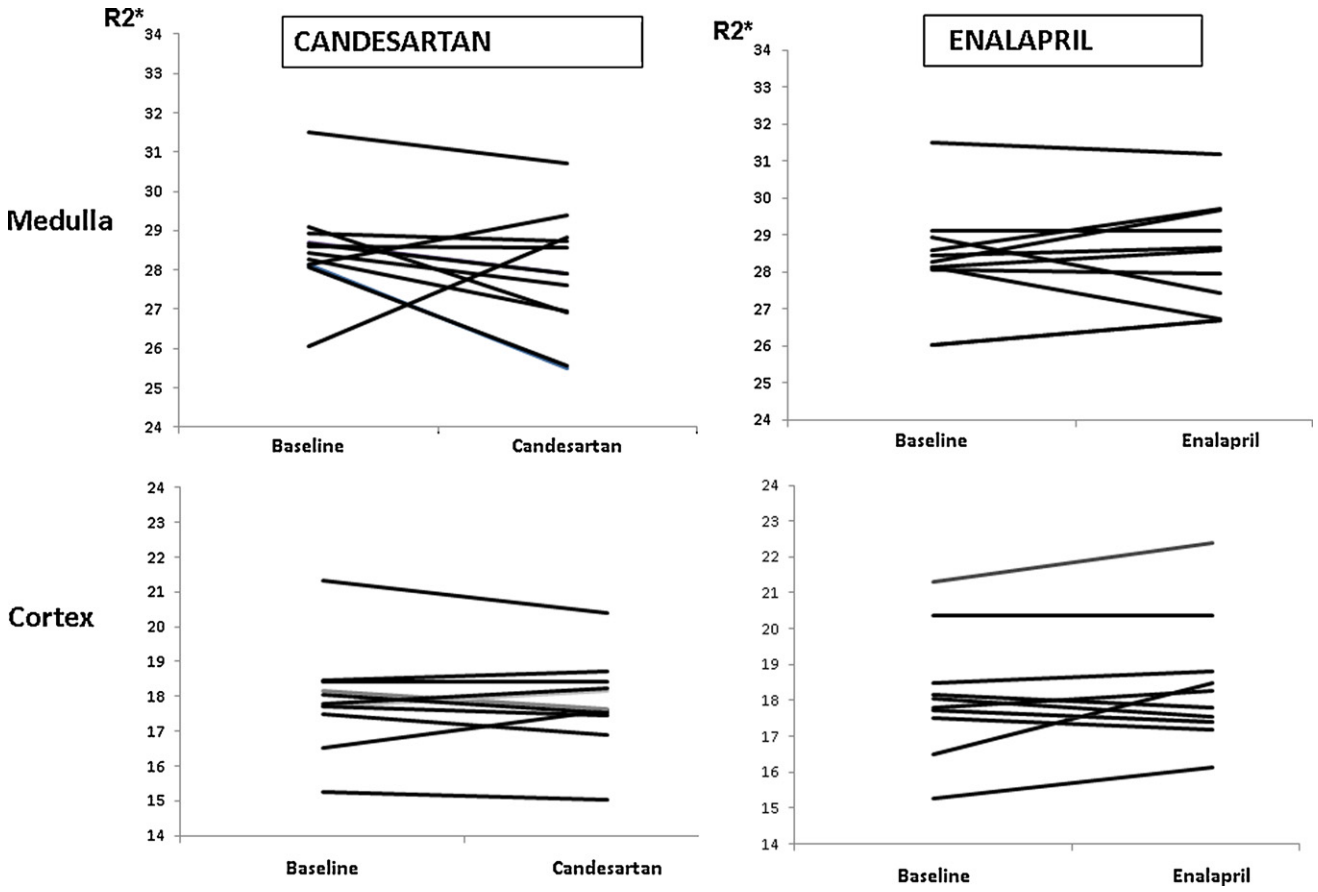

Fig. 2 – Individual R_2^* values (in s^{-1}) in the medulla and the cortex before and after one month of candesartan- and enalapril-intake.

There were no significant changes in cortical R_2^* levels after the introduction of the RAS blockers (cortical baseline R_2^* vs candesartan vs enalapril: respectively 17.9 ± 1.5 vs 17.6 ± 1.5 vs $18.1 \pm 1.9\ s^{-1}$; $p = 0.80$), nor were there any significant changes in medullary R_2^* levels (baseline medullary R_2^* levels vs candesartan vs enalapril respectively $28.7 \pm 1.3\ s^{-1}$ vs $27.9 \pm 1.5\ s^{-1}$ vs $28.7 \pm 1.5\ s^{-1}$, $p = 0.29$).

The situation was different when analyzing the changes induced by the administration of furosemide, with medullary and cortical R_2^* levels significantly lower after the administration of furosemide (medullary R_2^* before furosemide $28.4 \pm 0.2\ s^{-1}$ vs $21.5 \pm 0.4\ s^{-1}$ after furosemide, $p < 0.001$; cortical R_2^* $17.8 \pm 0.3\ s^{-1}$ vs $16.3 \pm 0.4\ s^{-1}$, $p = 0.002$), corresponding to higher local pO_2 levels (Fig. 3A and B). In comparison with baseline, furosemide-induced changes were not significantly lower during the candesartan- and enalapril-phase: furosemide-induced changes in medullary R_2^* levels were respectively $-7.8 \pm 2.6\ s^{-1}$ at baseline, $-6.8 \pm 2.9\ s^{-1}$ under candesartan, and $-7.0 \pm 1.9\ s^{-1}$ under enalapril intake ($p = 0.43$); changes in cortical R_2^* levels were $-1.7 \pm 1.2\ s^{-1}$ at baseline, $-1.1 \pm 1.6\ s^{-1}$ under candesartan and $-1.5 \pm 0.5\ s^{-1}$ under enalapril intake (p trend = 0.66). No changes were observed in MCR2* between the phases before (baseline vs candesartan vs enalapril MCR2*, respectively 1.62 ± 0.18, 1.60 ± 0.17, 1.60 ± 0.17, $p = 0.95$) and after furosemide (respectively 1.33 ± 0.20, 1.33 ± 0.28, 1.35 ± 0.27, $p = 0.98$).

To further investigate the determinants of R_2^* levels in patients with diabetic nephropathy, Spearman rank testing was performed. For this analysis, associations were first assessed per study phase, and thereafter for all study phases grouped together. Medullary R_2^* levels correlated positively with 24 h urinary sodium excretion ($r = 0.64$; $p = 0.003$), and also with systolic ($r = 0.35$, $p = 0.048$) and diastolic BP ($r = 0.42$, $p = 0.014$) as shown in Fig. 4A and B No correlations were found between medullary R_2^* levels and eGFR ($r = 0.07$; $p = 0.70$), hemoglobin ($r = 0.19$, $p = 0.27$), glycemia, HbA1c or BMI ($r = 0.14$, $p = 0.44$). There were no correlations between cortical R_2^* levels and renal function, BP or dietary sodium intake. However positive correlations were found between cortical R_2^* levels and glycemia ($r = 0.39$, $p = 0.02$) and HbA1c ($r = 0.45$, $p = 0.006$), indicating lower cortical oxygenation at higher blood glucose levels (Fig. 4C).

The furosemide-induced changes in cortical and medullary R_2^* levels did not correlate with BP, BMI, hemoglobin levels, glycemia, HbA1c or eGFR, but correlated positively with 24 h urinary sodium excretion. Hence, furosemide-induced changes in R_2^* level at the cortex ($r = 0.61$, $p = 0.001$) and medulla ($r = 0.50$, $p = 0.01$) were larger at higher salt intake.

4. Discussion

The main findings of this study were that: (1) neither ACE inhibition nor angiotensin II type 1 receptor blockade induced significant changes in R_2^* levels as a measure of renal tissue

oxygenation in T2DM patients with nephropathy, (2) on the contrary, large decreases in medullary R_2^* levels suggesting increases in medullary oxygenation were observed after the administration of furosemide, whether or not the participants were treated with a RAS blocker, (3) medullary R_2^* levels correlated positively with urinary sodium excretion and systemic blood pressure, whereas (4) cortical R_2^* levels correlated positively with glycemia and HbA1c.

There is increasing evidence from animal studies that tissue hypoxia contributes to the progression of kidney diseases by promoting the development of tissue fibrosis. As mentioned earlier, before the development of BOLD-MRI, determination of renal tissue oxygenation was quasi impossible in humans unless very invasive techniques were used. Recent cross-sectional studies in humans have reported higher cortical and medullary R_2^* values in patients with diabetes as compared with healthy age-matched controls, suggesting lower oxygenation in diabetes [27–29]. In our study, cortical as well as medullary R_2^* values in T2DM patients were comparable to those obtained previously in healthy subjects or hypertensive patients [18]. The difference between our findings and previous ones may be explained by the fact that our patients were in an early stage of diabetic nephropathy. Moreover, we were particularly careful to study patients on a standardized fluid intake since acute changes in fluid balance have been shown to modify R_2^* values [30]. In addition, the first studies did not account for medication intake and could not assess the influence of RAS blockade on renal tissue oxygenation. Thus, our results suggest that renal tissue oxygenation is preserved in the early stages of diabetic nephropathy.

This study is the first investigation of the effects of RAS blockade on R_2^* levels as a measure of renal tissue oxygenation

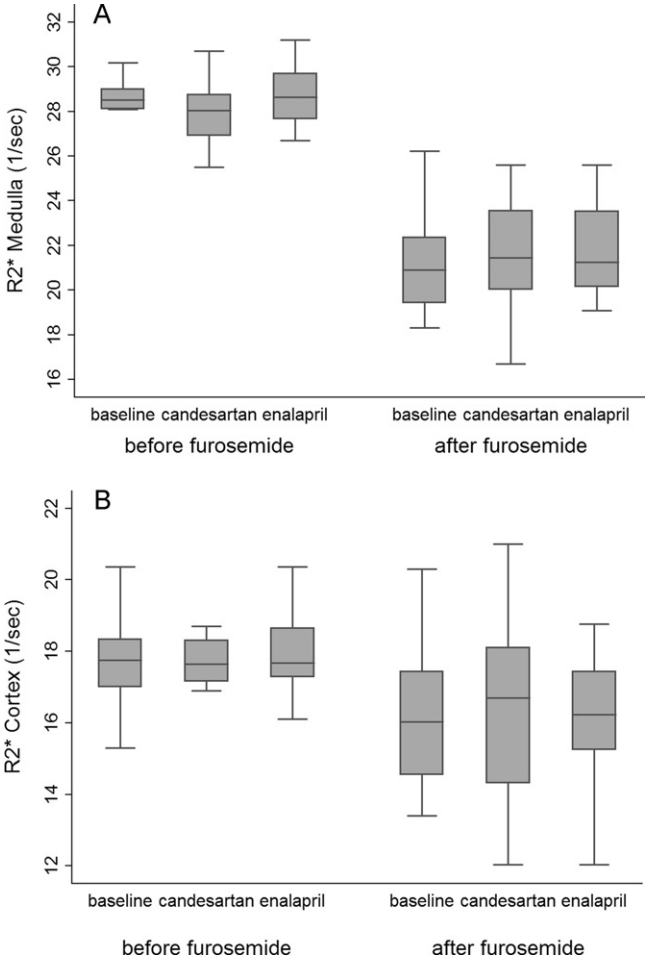

Fig. 3 – R_2^* (in s^{-1}) in the medulla (A) and in the cortex (B) before and after the administration of furosemide.

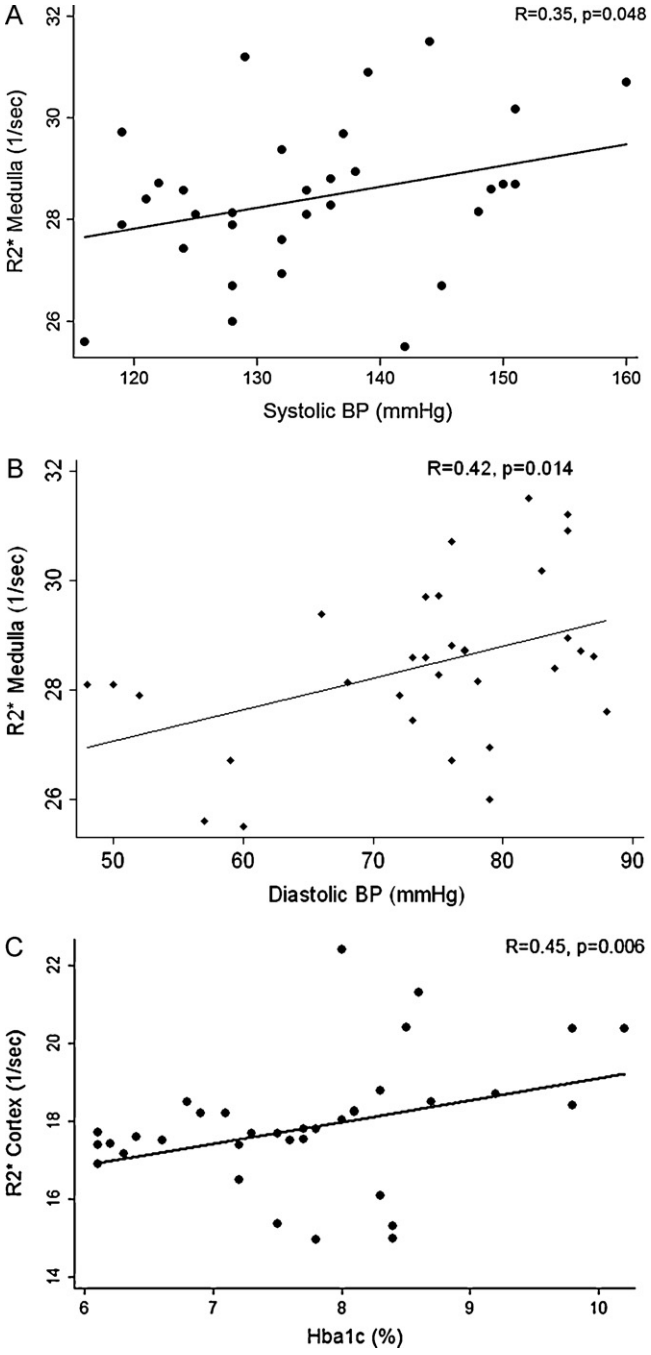

Fig. 4 – Scatterplots showing the associations between medullary R_2^* values and (A) systolic blood pressure and (B) diastolic blood pressure. The association between cortical R_2^* level and HbA1c is shown under C. Each subject is represented thrice in this figure: once under baseline conditions, once under candesartan, and once under enalapril treatment.

in T2DM patients. Because of their ability to increase renal blood flow and urinary sodium excretion in humans [31], we expected that the administration of a RAS blocker would lower R_2^* levels, suggesting increased oxygenation, a mechanism that could have participated in the renal protective effect of these agents. To our surprise, neither enalapril nor candesartan decreased renal cortical or medullary R_2^* levels. These results were obtained after one month of treatment and at peak effect of the two drugs. We did not assess whether these drugs induced acute, transient changes in R_2^* levels. One previous study has evaluated the acute effect of a RAS blocker on R_2^* levels in healthy volunteers and renal transplant patients [25]. In this study, Djamali et al. reported a decrease in cortical but not medullary R_2^* 2 h after the administration of 50 mg of losartan in nine healthy volunteers suggesting an increase in cortical oxygenation with losartan. But, in the same study, no significant change in R_2^* was observed in the ten patients suffering from chronic allograft nephropathy [25]. Although this study did not include patients with diabetes and assessed only the acute effect of one unique dose of losartan, its findings in transplant patients are in line with our study.

The lack of effect of RAS blockade on R_2^* levels as a measure of renal oxygenation in human contrasts with the results obtained in animals. Thus, Norman et al, using the protoporphyrin phosphorescence method in male Sprague-Dawley rats, have reported acute increases in cortical oxygenation upon administration of an ACEI or an ARB [12]. It is possible that RAS blocker-induced changes in pO_2 are smaller in humans than animals, and that the BOLD-MRI technique is not sensitive enough to detect such small changes. To the best of our knowledge, no studies have used BOLD-MRI to assess whether the administration of ACEI or ARBs induces alterations in R_2^* signal in animals, so this remains speculative, and limits quantitative comparisons. Of note, animal studies have generally studied acute rather than chronic medication-induced changes in renal oxygenation, and it might be that in chronic conditions adaptive mechanisms occur that correct eventual acute changes in oxygenation. In line with this hypothesis, Juillard and colleagues have described acute increases in cortical and medullary R_2^* levels the first days after subtotal clipping of the renal artery [32]. Yet four weeks after clipping renal hypoxia could no longer be detected [33].

In contrast to the absence of changes in R_2^* levels during RAS blockade, the administration of furosemide resulted in an acute drop of medullary and to a lesser degree cortical R_2^* levels, suggesting a diuretic-induced increase in renal oxygenation. A similar drop in R_2^* levels after furosemide has been reported previously in patients with essential hypertension or renal artery stenosis [34,35]. The effect of furosemide on R_2^* levels has been attributed to a reduction of the active oxygen consuming sodium transport in the ascending loop of Henle [35]. There was a trend toward a smaller furosemide-induced change in R_2^* levels after enalapril and candesartan treatment. This could be due to the natriuretic effect of ACEI and ARB's, and the redistribution of the cortico-medullary blood flow occurring after their administration, in favor of the cortex [11,31,36]. Since renal microcirculation was not assessed in this study, we cannot confirm these hypotheses.

Urinary sodium excretion was strongly and positively associated with medullary R_2^* levels, suggesting lower medullary oxygenation in participants with high-urinary sodium excretion. Since urinary sodium excretion is a proxy of dietary sodium intake [19], this finding is in line with our previous work, in which we showed that dietary sodium intake strongly influenced medullary R_2^* levels in normo-and hypertensive volunteers [18]. The influence of both furosemide and urinary sodium excretion on R_2^* levels further emphasizes that renal sodium handling is a key determinant of medullary oxygenation. Of note, at the current stage it is unknown whether low medullary R_2^* levels are renoprotective, and it is preliminary to conclude that the renoprotective properties of low sodium intake are explained by increases in medullary oxygenation. To illustrate this, we found no correlation between kidney function and medullary R_2^* levels, thus confirming a recent study by Michaely et al. [37]. For the same reason, the large furosemide-induced change in medullary R_2^* should not turn furosemide into a drug with renoprotective potential. First, this would not be in accordance with the literature. Second, the diuretic actions of furosemide are short lived (2–4 h), and followed by a long phase of renal sodium retention, during which there is theoretically a decrease in medullary oxygenation.

Finally, both systolic and diastolic BP were positively associated with medullary R_2^* levels in this study. The mechanism whereby a high systemic BP leads to higher medullary R_2^* levels suggesting lower oxygenation cannot be elucidated from the results of this study. It is possible that a chronically high BP in diabetes has a deleterious effect on renal microcirculation. This finding clearly deserves further specific investigations.

Cortical R_2^* levels were relatively independent of covariates, with the exception of positive associations with glycemia and HbA1c. Hyperglycemia increases not only renal blood flow but also GFR in diabetics [38], with in theory increasing effects on tissue oxygenation. The higher cortical R_2^* levels, suggesting lower cortical oxygenation at higher glycemia, might be another example that renal oxygen consumption plays a larger role than renal blood flow in renal tissue oxygenation. On the other hand, it opens hypotheses linking repetitive hyperglycemia with accelerated kidney function decline. All the described associations in this study were unadjusted and should thus be interpreted with caution, and need confirmation in larger studies.

The main limitation of this study was its small sample size. Thus, the study may have lacked power to detect small changes in R_2^* levels. Other authors have shown that renal tissue oxygenation in humans decreases only in case of severely reduced blood flow [39]. Unfortunately we did not assess renal hemodynamics. However, previous studies from our laboratory have demonstrated that renal blood flow increases significantly with the administration of enalapril or candesartan [36,40]. Furthermore, concomitant antihypertensive medication was not interrupted but continued at similar dose throughout the study for safety reasons, which might have caused potential interferences.

Finally, the BOLD-MRI technique itself has been criticized by some [41,42], since it is difficult to acquire the same anatomical slices in each participant when repeating the BOLD-MRI exams. We have previously shown that the intra-observer variability of the cortical and medullary R_2^* values is

low when performed by an experienced investigator [8]. To avoid bias, the same investigator analyzed all BOLD MRI images of our study without knowing the treatment phase or the characteristics of the participants. The fact that similar R_2^* values were found on three different occasions in this study further underlines the reproducibility of BOLD-MRI measurements.

5. Conclusion and perspectives

Taken together, no major change in renal R_2^* levels was detected after repeated administration of RAS-blockers in T2DM patients with diabetic nephropathy. The changes in medullary R_2^* levels observed in response to furosemide or in association with urinary sodium excretion as a proxy of dietary sodium intake suggest that renal sodium handling is one of the main determinants of renal tissue oxygenation. Our data also suggest that renal tissue oxygenation is well preserved in early diabetic nephropathy. Finally, this study provides additional arguments to recommend strict control of blood pressure and glycemia and a low sodium intake in T2DM patients.

Conflict of interest

There are no conflicts of interest.

Acknowledgements

The authors express their gratitude to the participants, the NMR-technicians and to the investigators who have contributed to the data collection, in particular Sylvie Tremblay, Carole Zweiacker, Meryll Cassat and Nicolas Chevrey.

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