Effect of Diabetes on Renal Medullary Oxygenation During Water Diuresis

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OBJECTIVE — To study the effect of water diuresis on renal medullary and cortical oxygenation in patients with diabetes using blood oxygenation level–dependent magnetic resonance imaging (BOLD MRI).

RESEARCH DESIGN AND METHODS — Nine mild diabetic subjects (48 ± 2.7 years of age, six women) and nine nondiabetic subjects of similar age and sex, all without known vascular or renal disease, were studied noninvasively by MRI before and during water diuresis.

RESULTS — Water diuresis induced an increase in medullary oxygenation in control subjects, producing a decrease in R_2* (apparent spin-spin relaxation time) of 1.89 ± 0.27 ($P <$ 0.01), but no significant change in the group of diabetic subjects.

CONCLUSIONS — These findings in middle-aged diabetic subjects, which resembled those previously described in elderly subjects >65 years of age, suggest early impairment of adaptive vasodilatation within the renal medulla in diabetes.

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The renal medulla of mammalian kidneys operates in a state of relative hypoxia compared with other organs and with the renal cortex because of the counter-current flow of blood through its looped capillaries, which permits the excretion of a concentrated urine (1). The state of oxygenation of renal cortex and medulla can be assessed noninvasively in human subjects using blood oxygenation level–dependent magnetic resonance imaging (BOLD MRI). Normal young men and women exhibit a marked increase in renal medullary oxygenation during water diuresis that is demonstrable by this technique (2,3). This adaptive change, which probably reflects an increase in medullary capillary blood flow, is attenuated with aging and suppressed in young subjects by inhibition of cyclooxygenase (3).

Medullary hypoxic injury characterizes the phenomenon of acute renal failure (4), and it was therefore of interest to examine the renal medullary response to water diuresis in patients with diabetes—a condition thought to predispose to acute renal failure (5,6). In order to avoid the nonspecific effects of renal scarring resulting from obvious diabetic nephropathy to the greatest extent, we selected nine patients with adult-onset diabetes without microalbuminuria, hypertension (blood pressure <150/90 mmHg), or renal insufficiency and compared them with nine healthy control subjects. The results suggest a defect in adaptive change in renal medullary oxygenation in diabetic subjects.

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Abbreviations: BOLD, blood oxygenation level dependent; MRI, magnetic resonance imaging; NO, nitric oxide; PGE_2, prostaglandin E_2.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

Study plan

To compare responses to water diuresis in subjects with and without diabetes, we recruited nine individuals (six women and three men) 36–63 years of age (mean age 48 ± 2.7 years),with mild type 2 diabetes controlled with diet and oral sulfonylurea drugs, and nine healthy individuals (six women and three men) without diabetes, 39–59 years of age (mean age 51 ± 2.2 years). The mean duration of diabetes in the diabetic subjects was 6.5 years (median 7 ± 4.75 years, range 0.1–15). The following exclusion criteria were applied to subjects in all groups: smoking any amount of cigarettes during the previous 6 months, cardiovascular disease (coronary artery disease, arrhythmia, or congestive heart failure), history of stroke or transient ischemic attack, peripheral vascular disease (symptoms of claudication and/or absence of peripheral pulses), chronic renal disease, severe dyslipidemia (triglycerides >600 mg/dl or cholesterol >300 mg/dl), or any other serious chronic disease requiring active treatment. Subjects were also excluded if they were on any of the following medications: any type of antihypertensive, lipid-lowering agent, glucocorticoid, antineoplastic agent, psychoactive agent, or bronchodilator. In addition, diabetic patients with proliferative retinopathy, peripheral somatic neuropathy, and/or insulin or troglitazone therapy were excluded from the study. In all subjects, the medical history (aside from diabetes) was essentially negative, arterial blood pressure was <150/90 mmHg, a complete blood count and urinalysis was normal, and the serum creatinine concentration was <1.2 mg per 100 ml. The excretion of microalbumin was <200/μg per gram of creatinine in all subjects, and the groups were not significantly different from each other in height or weight. The study was approved by the Committee on

Clinical Investigation of the Beth Israel Deaconess Medical Center, and all volunteers gave written informed consent before participating in the study.

All subjects were asked to take nothing by mouth after 8:00 P.M. on the night before the study. At 8:00 A.M the next morning, they reported to the Clinical Research Center, where they were weighed; their vital signs were measured; a sample of blood was drawn for a complete blood count, blood urea nitrogen, serum creatinine, blood glucose, and HbA_{1c}; and a timed sample of urine was obtained by spontaneous voiding. They were then taken to the magnetic resonance suite, where baseline MRI images were obtained in the supine position. The subjects were then taken out of the magnet and asked to void again for a second urine collection. They then drank 20 ml flavored water/ kg body wt within 15 min to induce a water diuresis. Urinary output was measured every 15 min. After it exceeded 5 ml/min, the subject returned to the magnet, where a second set of BOLD MRI measurements was obtained. Immediately after this, a final sample of urine was obtained. In no case was glycosuria detected during water diuresis.

Laboratory methods

Urinary osmolality was determined using freezing point depression with an Osmette osmometer (Precision Instruments, Sudbury, MA). Urinary creatinine concentration was determined using a rate-dependent modification of the Jaffe reaction (SMAC II; Technicon Instruments, Tarrytown, NY).

Urinary samples for determination of prostaglandin E_2 (PGE_2) were immediately aliquoted into containers acidified with 2N HCl. Samples were then adjusted to pH 3–4. PGE_2 was assayed in duplicate on diluted urine samples using a standard double-antibody radioimmunoassay with reagents obtained from New England Nuclear (Dupont, Boston, MA). Inter- and intraassay coefficients of variation were 20 and 5.1%, respectively.

The endogenous formation of nitric oxide (NO) is thought to be reflected in the urinary excretion of NO_3^- plus NO_2^- (7). Urinary concentrations of (NO_3^- + NO_2^-) were determined by reducing nitrate to nitrite and then measuring the concentration of the latter after its reaction with the Griess reagent to form a colored dye (8). Reagents for this procedure were obtained from Roche Diagnostics.

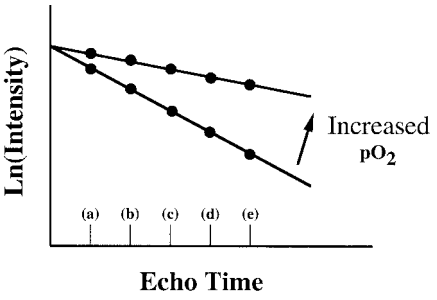

Figure 1—*Correlation of BOLD MRI with pO_2. The deoxygenation of hemoglobin changes its magnetic resonance, termed R_2* (apparent spin-spin relaxation rate). R_2* can be estimated from signal intensity measurements made at several different echo times (a–e). The slope of log_e (intensity) versus echo time determines R_2* and is directly related to the amount of deoxygenated blood (R_2* = slope ~ conc[deoxyHB] ~ blood pO_2 ~ tissue pO_2). A decrease in the slope implies an increase in the pO_2 of blood. Because blood pO_2 is thought to be in rapid equilibrium with tissue pO_2, changes in BOLD signal intensity or R_2* should reflect changes in the pO_2 of the tissue.*

MRI methods

MRI takes advantage of the energy emitted as radio waves by water molecules when their alignment in a strong magnetic field is changed. The BOLD MRI technique depends on the principle that hemoglobin (because of its iron content) changes its magnetic qualities depending on whether the hemoglobin is in the oxygenated or deoxygenated form. This change in the magnetic property of hemoglobin in turn influences the MRI signal from the neighboring water molecules in a predictable way. Because the ratio of oxyhemoglobin to deoxyhemoglobin is related to the pO_2 of blood, and the pO_2 of capillary blood is thought to be in equilibrium with the surrounding tissue, changes estimated by BOLD MRI can be interpreted as changes in tissue pO_2 (Fig. 1). All measurements were performed on a 1.5T whole-body scanner (Vision; Siemens Medical Systems, Erlangen, Germany). We used R_2^* ($=1/T_2^*$) as the parameter to reflect relative oxygenation status (2). We utilized a multiple gradient echo with selective water excitation pulses (mGRE) sequence (TR/TE/Flip angle = 60 ms/6–51 ms/40°) to acquire 16 T_2^* weighted images within a single breath-hold of <15 s, which permits calculation of R_2^* maps (9). We used a modified sequence involving selective water excitation pulses, which increased the range of echo times slightly, 6–51 ms. Both of these sequences give equivalent results as far as R_2^* calculations are concerned (9). The advantage of the selective water excitation is better delineation between renal medulla and intrarenal fat on R_2 maps. A decrease in R_2^* can be interpreted as an increase in tissue pO_2 (Fig. 1).

The MRI signal is influenced by two relaxation mechanisms: spin-lattice (T_1) and spin-spin (T_2) relaxation. Two experimental parameters, repetition time (TR) and echo time (TE) determine the magnitude of T_1 and T_2 influence (or weighting), respectively. In the presence of magnetic inhomogeneities (like the one associated with deoxyhemoglobin), the effective or apparent T_2 relaxation role is higher and termed T_2^*.

Statistical methods

Results are expressed as means ± SEM. Results were analyzed for significance using Student's *t* test, and the differences were considered not significant if $P > 0.05$.

RESULTS

Baseline characteristics in the hydropenic state

The group of nine diabetic patients was comparable at baseline with the control group of nine nondiabetic subjects in age, height, weight, blood pressure, creatinine clearance, fasting urinary osmolality and the urinary excretion of PGE_2, and NO_3/NO_2 (Table 1). The average fasting blood glucose of diabetic subjects was 153 ± 17 mg/dl, and their HbA_{1c} was 7.4 ± 0.46%, compared with 81 ± 2 and 5.5 ± 0.11 in control subjects, respectively. Two-thirds of each group were women. No subject in either group had microalbuminuria, and the resting arterial pressure was <150/90 mmHg in all subjects. BOLD MRI analysis (R_2^*) consistently indicated more hypoxia in the renal medulla than in the cortex in diabetic and control subjects, but the two groups did not differ significantly from each other in this respect.

Effect of water diuresis

During water diuresis, there was a significant increase in oxygenation of the renal medulla in normal volunteers (decrease in

Table 1—*Effects of water diuresis on diabetic and control subjects*

	Control subjects		Diabetic subjects	
	Baseline	Water diuresis	Baseline	Water diuresis
n	9		9	
Age (years)	51 ± 2.2		48 ± 2.7	
Blood pressure (mmHg)	125 ± 4.5/68 ± 2.7		134 ± 3.5/76 ± 4	
Height (cm)	165 ± 4		165 ± 3	
Weight (kg)	81.18 ± 5.5		85.8 ± 4.4	
Urine flow (ml/min)	0.64 ± 0.06	9.54 ± 0.85*	0.86 ± 0.2	11.6 ± 1.3*
Urinary osmolarity (mOsm/kg)	787 ± 40	83 ± 6*	765 ± 62	86 ± 6*
NO_2/NO_3 (mcg/min)	44 ± 5	48 ± 8	49 ± 15	70 ± 16
PGE_2 (ml/min)	386 ± 102	332 ± 175	249 ± 76	136 ± 16†
Creatine clearance rate (ml/min)	119 ± 6	96 ± 4*	133 ± 7	117 ± 6‡
R_2* medulla	17.4 ± 0.54	15.5 ± 0.5*	16.5 ± 0.84	15.9 ± 0.83
R_2* cortex	12.9 ± 0.25	12.2 ± 0.3	12.5 ± 0.23	11.9 ± 0.3
Change in R_2* with diuresis				
Medulla		−1.89 ± 0.27§		0.7 ± 0.43
Cortex		−0.68 ± 0.25		−0.6 ± 0.15

Data are means ± SEM. *$P < 0.01$ compared with baseline, by paired Student's *t* test; †$P < 0.05$ compared with baseline, by paired *t* test; ‡$P < 0.02$ compared with normal control subjects; §$P < 0.05$.

R_2* of 1.89 ± 027, $P < 0.01$) (Table 1, Fig. 2). This effect was similar in direction to but smaller than the change reported earlier in a younger group of normal women (age 25–31 years, mean 28), in whom the average decrease in R_2* was 4.7 ± 0.7 (3). In diabetic subjects, however, R_2* did not change appreciably with diuresis (−0.7 ± 0.43, $P > 0.05$). The decrease in medullary R_2* induced by diuresis in control subjects was significantly greater than that seen in diabetic subjects ($P < 0.05$).

The magnitude of diuresis after water ingestion and the urinary excretion of sodium (data not shown) was similar in diabetic and control subjects, as was the minimum urinary osmolality attained at the height of diuresis. The creatinine clearance measured at the height of water diuresis was slightly greater in diabetic than in control subjects (117 ± 6 ml/min, range 100–137, vs. 96 ± 4.5 ml/min, range 81–113; $P < 0.02$). There were no significant differences in urinary excretion of PGE_2 or NO_3/NO_2 before or during water diuresis between groups. PGE_2 excretion declined during water diuresis ($P < 0.05$) in diabetic subjects, whereas it did not change significantly in control subjects.

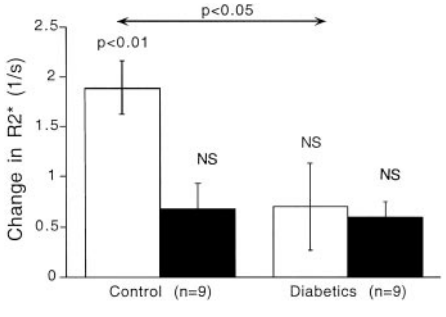

**Figure 2—*Effect of water diuresis on* R_2* *in control and diabetic subjects. The decrease in* R_2* *(improvement in tissue oxygenation) after water diuresis is plotted on the ordinate for renal cortex (■) and medulla (□) in control and diabetic subjects. Values shown are mean ± SE. The* P *values shown reflect the change in* R_2* *produced by water diuresis in each column. NS signifies that the change in* R_2* *from baseline during water diuresis was not significantly different from zero (*P *> 0.05).*

CONCLUSIONS — The main finding of these experiments is that water diuresis failed to produce a significant improvement in renal medullary oxygenation in a group of middle-aged diabetic patients with no other evidence of diabetic nephropathy. This behavior contrasts with that of age- and sex-matched control subjects without diabetes, in whom water diuresis increased oxygenation of the medulla. The diabetic response to water diuresis resembles that seen in much older subjects (>65 years of age) who do not have diabetes or in young nondiabetic subjects who are given ibuprofen, in whom water diuresis similarly failed to improve medullary oxygenation (3). It should be noted that diuresis after water ingestion was not itself inhibited in the diabetic subjects and that their blood pressures and creatinine clearances were normal.

In these middle-aged subjects, urinary excretion of PGE_2 in the control group (average age 51 years) was unchanged by water diuresis on the average, whereas it tended to decline in diabetic subjects (average age 48 years). PGE_2 excretion in the urine rises sharply with water diuresis in young women with a mean age of 28 years and is unchanged or falls in the elderly (average age 69 years) (3). It is likely that in the present study, PGE_2 excretion was strongly influenced by age in both groups.

These results presumably reflect an early deficiency in medullary vasodilatation during water diuresis in diabetic subjects, perhaps owing to a reduction in the local synthesis of prostaglandins, NO, or other endogenous vasodilator substances. The baseline urinary excretions of PGE_2 and nitrate/nitrite were not significantly different in diabetic subjects from the levels measured in control subjects, but these values may not accurately reflect the local concentration of these vasodilating metabolites in the renal medulla. It has been postulated that patients with diabetes (and their relatives) have a generalized defect in endothelial-based vasodilatation, presumably secondary to a deficiency in the production of NO by vascular tissue (10–12). If this deficiency

included the small blood vessels of the renal medulla, it might account for the present findings. It should be emphasized that these diabetic subjects were purposely selected because their diabetes was mild and easily controlled and they had no clinical evidence of microvascular disease. It seems probable (though we have not proven it) that the defect in renal medullary oxygenation represents an early change that is likely to be more pronounced in patients with diabetes that is more severe, of longer duration, or associated with obvious vasculopathy and nephropathy.

The failure of these mildly diabetic subjects to increase the oxygenation of their renal medulla during water diuresis may signal a deficiency in adaptive medullary vasodilatation under other forms of stress as well. It seems possible that this might account, at least in part, for the reported predisposition of patients with diabetes to the clinical occurrence of acute ischemic renal failure (5,6).

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