Kidney Oxygenation During Water Diuresis and Endothelial Function in Patients With Type 2 Diabetes and Subjects at Risk to Develop Diabetes

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The aim of the present study was to examine the relationship among water diuresis-induced changes in renal oxygenation, endothelial function, and various metabolic parameters in type 2 diabetic patients and healthy subjects at risk of type 2 diabetes. Thirty-eight subjects with type 2 diabetes (D: age, 54 ± 10 years, mean ± SD, 24 men) and 7 healthy subjects with parental history of type 2 diabetes or with impaired glucose tolerance (IGT) (relatives [R]: age 46 ± 11 years, 4 men) were included. Laser Doppler imaging scanning was used to measure vasodilatation in the forearm skin in response to iontophoresis of 1% acetylcholine (Ach) and 1% sodium nitroprusside (SNP), and ultrasound was used to measure the flow-mediated dilation (FMD) and nitroglycerin-induced dilation (NID) in the brachial artery. Renal oxygenation was assessed by magnetic resonance imaging (MRI) before and during water diuresis. A decrease in the magnetic parameter R2* implies an increase in oxygenation. Renal medullary oxygenation did not improve with diuresis in either group (D: −0.5 ± 1.9, R: −0.4 ± 2.1, *P* = not significant [NS]). The renal cortical oxygenation showed a small, but statistically significant, improvement after diuresis in the 2 groups (D: −0.6 ± 1.1, R: −0.5 ± 0.5, *P* < .05). There were no correlations between the change in cortical R2* (R2* post-minus R2* prewater diuresis) and the micro- and macrovascular reactivity. The postdiuresis renal cortical R2* was negatively correlated with both the Ach- and SNP-induced skin vasodilation (% change over baseline)(*r* = −.40, *P* < .01 and *r* = − .39, *P* < .05, respectively), while no correlation existed with the FMD and NID. The baseline renal cortical oxygenation was also negatively correlated with the SNP-induced skin vasodilation (*r* = −.36, *P* < .05) and positively correlated with the fasting plasma glucose, total cholesterol, and vascular cell adhesion molecule (VCAM) concentrations (*r* = .34, *P* < .05, *r* = .31, *P* < .05 and *r* = .37, *P* < .05, respectively). These preliminary findings suggest an association between the kidney cortical oxygenation and the skin microvascular reactivity, glycemia, and lipidemia. Water diuresis failed to produce an improvement in renal medullary oxygenation in both patients with diabetes and subjects at risk for diabetes.

DIABETES IS ONE of the leading causes of vascular disease. Impairment of endothelial function has been proposed as the main common pathophysiologic pathway that is responsible for the development of vascular disease in both the micro- and macrocirculation.[1-3] Coronary and peripheral arterial diseases are the 2 main consequences of the involvement of the large vessels.[4] In the microcirculation, the most prominent changes occur at the retina, kidney, and peripheral nerve and are associated with the development of the diabetic complications in these organs.[5] Studies from our unit have shown that endothelial function is impaired in subjects with impaired glucose tolerance (IGT) and healthy subjects at risk of developing diabetes, as well as in diabetic patients.[6]

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Recent technologic advances in the field of magnetic resonance imaging (MRI) have allowed the evaluation of the state of oxygenation of the renal cortex and medulla noninvasively using blood oxygenation level dependent MRI (BOLD MRI).[7,8] This technique is based on hemoglobin's magnetic properties where oxyhemoglobin is diamagnetic and deoxyhemoglobin is paramagnetic[9] and can be used to detect changes in oxygenation that reflects tissue oxygen consumption, delivery, and/or blood flow.

Previous studies in our unit have shown that water diuresis increases the oxygenation of the renal medulla in young healthy subjects, and that this is related to local vasodilation and increased blood flow due to increased production of prostaglandin E2.[10] In contrast, water diuresis-induced vasodilation within the renal medulla is impaired in elderly healthy subjects and in middle-aged diabetic patients.[11]

We hypothesized that in diabetes these water-diuresis induced changes in oxygenation are associated with the changes in the skin microvascular reactivity and brachial artery flow-mediated dilation (FMD). In the present study, we have examined this relationship in patients with type 2 diabetes and healthy subjects with parental history of type 2 diabetes or IGT.

MATERIALS AND METHODS

Subjects

Thirty-eight type 2 diabetic patients and 7 subjects at risk for diabetes were included in the study. Diabetes was defined according to the recommendations of the American Diabetes Association (ADA) Expert Committee on the Classification and Diagnosis of Diabetes.[12] At risk for diabetes was defined as either having a first-degree relative with type 2 diabetes or IGT defined as a 2-hour blood glucose value between 140 to199 mg/dL during a 75-g oral glucose tolerance test (OGTT). This group included 6 subjects with normal glucose tolerance (NGT)

and 1 subject with IGT. To avoid confounding factors known to affect endothelial function, the following exclusion criteria were applied: smoking any amount of cigarettes during the previous 6 weeks, subjects with unstable cardiovascular disease or cardiac arrhythmia, congestive heart failure, stroke within the last 6 months, uncontrolled hypertension, chronic renal disease (creatinine $\geq$ 1.5 mg/dL), macroalbuminuria (expressed as albumin/creatinine ratio $>$300 μg/mg), severe dyslipidemia (triglycerides $>$600 mg/dL or cholesterol $>$300 mg/dL), or any other serious chronic disease requiring active treatment. Subjects on angiotensin-converting enzyme inhibitors were also excluded from the study.

Volunteers for the study were recruited through local and newspaper advertisement at the Joslin Diabetes Center and Beth Israel Deaconess Medical Center in Boston. The protocol was approved by the institutional review board at each center, and all participants gave written informed consent.

Procedures

Volunteers attended the Joslin Diabetes Center Clinical Research Center to perform the clinical and laboratory evaluations. Subjects were studied after an overnight fast. Plasma glucose, total serum cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, liver function tests, electrolytes, blood urea nitrogen (BUN), and creatinine were measured using the Synchron CX analyzer (Beckman/Coulter, Fullerton, CA). Low-density lipoprotein (LDL) cholesterol was calculated from these results. Routine urinalysis was also performed. The glycosylated hemoglobin (HbA_{1c}) (normal range, 4% to 6%) was determined in whole blood using ion-exchange high-performance liquid chromatography (HPLC). Plasma insulin was measured using a radioimmunoassay (RIA) method. Soluble vascular cell adhesion molecule (sVCAM) and endothelin-1 (ET-1) were measured in plasma by an enzyme-linked immunosorben assay (ELISA) method (R&D Systems, Minneapolis, MN). Tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) were measured by chemiluminescent immunoassay. von Willebrand factor (vWF), plasma activator inhibitor (PAI) antigen, and tissue plasminogen activator (tPA) antigen were also measured by an ELISA method (Diagnostica Stago, Parsippany, NJ).

Vascular Reactivity Tests

All eligible participants returned for a second visit to the Microcirculation Laboratory at the Beth Israel Deaconess Medical Center to perform the vascular reactivity tests. All measurements were performed in the morning while the subjects were in a fasting state. The investigators who performed the vascular reactivity measurements were blinded to the medical history of the subjects. These studies were performed in a temperature-controlled room (24°C to 26°C) and after a 30-minute acclimatization period. The vascular reactivity of the forearm skin microcirculation was evaluated by Laser Doppler perfusion imaging measurements before and after the iontophoresis of acetylcholine chloride (Ach, endothelium-dependent vasodilation) and sodium nitroprusside (SNP, endothelium-independent vasodilation) as previously described.[13] The reproducibility of the technique has been previously reported by our group.[14] The coefficient of variation of the baseline measurement was 14.1% and during maximal hyperemic response after the iontophoresis, it was 13.7%.

To assess the endothelium-dependent reactivity in the macrocirculation, the FMD was measured by high-resolution ultrasound with a 10.0 MHz linear array transducer and an HDI Ultramark 9 system (Advanced Technology Laboratories, Bothel, WA). Reactive hyperemia is produced by inflating a pneumatic tourniquet distally to the brachial artery to 50 mm Hg above the systolic pressure for 5 minutes and then deflating it. Measurements of brachial artery diameter were made 1 minute after cuff deflation and results are expressed as percent increase over baseline. This protocol is described in detail elsewhere.[15] Endothelium-independent vasodilation in the macrocirculation was assessed by studying brachial artery diameter changes 5 minutes after the administration of 400 μg sublingual nitroglycerine (nitroglycerine-induced dilation, NID). This test was performed 15 minutes after the reactive hyperemia test and after obtaining a new baseline reading.

MRI Methods

All subjects came to the Joslin Diabetic Center and Beth Israel Deaconess Medical Center after an overnight fast. They were then taken to the MRI suite to acquire baseline BOLD MRIs. After the baseline scans, they were taken out of the magnet and asked to drink 20 mL flavored water per kilogram body weight over a period of 15 minutes. Urine collections, obtained by spontaneous voiding, continued every 15 minutes. When the urinary output exceeded 5 mL per minute, they were repositioned in the magnet and BOLD MRI measurements were again obtained.

The MRI measurements were performed on a 1.5T whole body scanner (Vision; Siemens Medical Systems, Erlangen, Germany) using a body coil to transmit and a body phased-array surface coil to receive. Breath-hold technique was used to reduce motion artifacts. A water-selective-excitation multi-TE gradient echo sequence was used to acquire axial view T2* weighted images with TE 6 to 51 ms, step 3 ms, TR 65 ms, a = 30°, slice thickness 5 mm and 2.5 mm gap between slices. The first image has a TE of 6 ms and is designated as the anatomic image of the slice. The R2* map of each slice was generated in gray-scale from the corresponding 16 multi-TE gradient echo images by least squared fit of the slope of $\log_e$ (signal intensity) versus TE plot. Typically, 10 to 15 slices were acquired to cover the kidneys and 16 images corresponding to 1 slice were acquired in 1 breath hold ($\sim$15 seconds).

After the image acquisition, regions of interest (ROIs) were obtained from the medulla and cortex areas in the anatomic images of each slice and then used to obtain R2* values from the corresponding R2* maps. These R2* measurements were put into Microsoft Excel (Redmond, WA) to calculate the mean R2* values of the kidneys pre- and post-water load. If the regional R2* deceased significantly during water diuresis, the region was considered to have an improvement in oxygenation.[16]

Previously obtained MRI data from a group of healthy historic controls without diabetes were used for statistical comparisons. Those subjects were tested at a different time than the diabetes and at risk for diabetes groups.

Statistical Methods

The Minitab statistical package (Minitab, State College, PA) for personal computers was used for the statistical analysis. Analysis of variance (ANOVA) was used for comparisons among groups and Kruskal-Wallis was used when the data were not parametrically distributed. Paired *t* test was used for the difference in the same group before and after diuresis. Pearson's correlation analysis was performed for normally distributed data; otherwise Spearman's correlation analysis was performed. The results are presented as mean $\pm$ SD.

RESULTS

Baseline Characteristics

Table 1 shows the subjects' demographics. Both groups were comparable at baseline in age, systolic, and diastolic blood pressure. The weight was higher in patients with diabetes compared with the at risk group ($P < .05$). The biochemical and endothelial marker measurements are shown in Table 2. Microalbuminuria (urinary albumin-to-creatinine ratio between 30 and 300 μg/mg) was present in 7 subjects in the diabetes

Table 1. Clinical Characteristics of Studied Subjects

	Type 2 Diabetes (D)	At Risk for Diabetes (R)
N	38	7
Age (yr)	54 ± 10	46 ± 11
Gender M/F	24/14	4/3
Diabetes duration (yr)	5.6 ± 5.5	N/A
Weight (kg)*	86.4 ± 13.4	70.8 ± 16.7
Height (m)	1.72 ± 0.09	1.68 ± 0.13
Systolic BP (mm Hg)	126 ± 17	117 ± 14
Diastolic BP (mm Hg)	80 ± 9	78 ± 8

NOTE. Means ± SD.
*D *v* R, $P < .05$.

group and in 1 subject in the at risk for diabetes group. None of the tested diabetic patients had proliferative retinopathy. The diabetes group was comparable at baseline with the at risk group in total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. As expected, HBA_{1c}, fasting glucose and insulin were higher in subjects with diabetes ($P < .05$).

Vascular Reactivity Measurements

The vascular reactivity results are shown in Table 3. No statistically significant differences in the vascular reactivity in the microcirculation were seen between the 2 groups. NID was significantly lower in patients with diabetes ($P < .05$). FMD was also lower in patients with diabetes, but this did not reach statistical significance.

BOLD MRI Measurements

Table 4 shows the BOLD MRI measurements. The MRI data from the historic controls are also shown in this table. That group included 3 healthy men and 6 women without diabetes (age, 39 to 59; weight, 81.18 ± 16.5 kg; height, 1.65 ± 0.12 m; systolic blood pressure, 125 ± 13.5 mm Hg and diastolic blood pressure, 68 ± 8.1 mm Hg.

Table 2. Results of Biochemical and Endothelial Marker Measurements

Group	Type 2 Diabetes (D)	At Risk for Diabetes (R)
Fasting plasma glucose (mg/dL)*	166 ± 57	86 ± 13
Plasma insulin (μU/mL)* (N = 30, not on insulin therapy)	15 ± 10	6 ± 3
HbA_{1c}*	7.7 ± 1.8	4.9 ± 0.4
Total cholesterol (mg/dL)	197 ± 35	193 ± 44
HDL cholesterol (mg/dL)	53 ± 10	60 ± 17
LDL cholesterol (mg/dL)	118 ± 28	115 ± 37
Triglycerides (mg/dL)	134 ± 67	90 ± 39
VCAM (mg/mL)	730 ± 190	535 ± 97
ET-1 (pg/mL)	1.1 ± 0.3	0.9 ± 0.2
TNF-α (pg/mL)	9.4 ± 18.7	2.2 ± 1.9
CRP (mg/dL)	0.4 ± 0.6	0.3 ± 0.4
VWF (%)	122 ± 49	94 ± 59
PAI-antigen (ng/mL)	41 ± 35	22 ± 15
t-PA-antigen (ng/mL)	8.4 ± 3.3	7.4 ± 6.1

NOTE. Means ± SD.
*D *v* R, $P < .05$.

Table 3. Vascular Reactivity Measurements

Group	Type 2 Diabetes (D)	At Risk for Diabetes (R)
Flow-mediated dilation (% of increase over baseline)	5.4 ± 2.9	7.7 ± 4.7
Nitroglycerin-induced dilation (% of increase over baseline)*	13.4 ± 4.1	20.4 ± 8.4
Acetylcholine-induced skin vasodilation (% change over baseline)	160 ± 80	201 ± 88
Sodium nitroprusside skin-induced vasodilation (% change over baseline)	93 ± 42	85 ± 35

NOTE. Data are means ± SD.
*R *v* D, $P < .05$.

Baseline. There were no differences in the baseline renal cortical or medullary oxygenation among the diabetes group, at risk for diabetes group, and the historic controls (Table 4). As in previous studies, measurements of R2* before water diuresis consistently indicated more hypoxia in the renal medulla than in the cortex in each of these 3 groups.[11]

Response to water diuresis. In subjects at risk for diabetes and in patients with diabetes, the renal medullary oxygenation did not change significantly with diuresis. In contrast, there was a statistically significant increase in oxygenation of the renal medulla in the historic control group (decrease in R2* of -1.9 ± 0.8 $P < .001$). The renal cortical oxygenation showed a small, but statistically significant, improvement after diuresis in all 3 groups (D: -0.6 ± 1.1, R: -0.5 ± 0.5, C: -0.7 ± 0.8, $P < .05$).

Correlations (Diabetes and at Risk for Diabetes Groups)

Cortex. When all subjects with diabetes and at risk for diabetes were considered as 1 group, no correlations were found between the Ach- and SNP-induced vasodilation and the change in R2* (R2* post- minus R2* prewater diuresis) with water diuresis. However, significant correlations existed between the vascular reactivity measurements of the skin microcirculation and the postdiuresis cortical R2*. Thus, both the Ach- and SNP-induced vasodilation (% change over baseline) were associated with the postdiuresis renal cortical R2* ($r = -.40$, $P < .01$ and $r = -.39$, $P < .05$, respectively) (Table 5).

The baseline cortical oxygenation correlated with the fasting plasma glucose, total cholesterol, VCAM, and CRP (Table 6). Both the fasting plasma glucose and CRP also correlated with the postdiuresis R2*. In addition, the total and LDL cholesterol negatively correlated with the change in cortical oxygenation with water diuresis. The correlation between HBA_{1c} and both the pre- and postdiuresis R2* marginally missed statistical significance.

There were no associations between the macrocirculation vascular reactivity and the renal cortical measurements either at baseline or in response to water diuresis. Similar results were found when the subjects with diabetes were analyzed separately from the at risk subjects.

Table 4. BOLD MRI Measurements

	Type 2 Diabetes (D)		At Risk for Diabetes (R)		Historic Controls (C)	
	Baseline	Water Diuresis	Baseline	Water Diuresis	Baseline	Water Diuresis
R2* medulla	17.2 ± 1.7	16.6 ± 2.0*	16.8 ± 1.3	16.4 ± 1.5*	17.4 ± 1.6	15.6 ± 1.5†
R2* cortex	12.7 ± 1.0	12.1 ± 1.1†	12.6 ± 0.4	12.1 ± 0.4†	12.9 ± 0.7	12.2 ± 0.9†

NOTE. Data are mean ± SD.
*P = NS, compared with baseline.
†$P < .05$ compared with baseline.

Medulla. There was no correlation between the medullary oxygenation either at baseline or after water diuresis with any vascular reactivity measurement. No correlation existed between the baseline medullary R2*, glucose, and total cholesterol (data not shown), while a positive correlation was found with the plasma endothelin levels ($r = .320$, $P < .05$) and a negative correlation with the PAI antigen levels ($r = -.352$, $P < .05$).

There were no correlations between either the cortical or medullary oxygenation and TNF-α, VWF, or t-PA antigen.

DISCUSSION

In this study, we used a novel MRI technique to evaluate tissue oxygenation at the kidney level in subjects at risk of developing diabetes and patients with type 2 diabetes. Although there was no association between the water diuresis-induced change in renal cortical oxygenation and the macro- and microvascular reactivity, measurements of the renal cortical oxygenation after water diuresis were found to be associated with both the endothelium and nonendothelium-dependent vasodilation of the skin microcirculation. In addition, a relationship was found between the fasting plasma glucose, total cholesterol, and the renal cortical oxygenation. Another finding is that the water diuresis failed to improve the renal medullary oxygenation not only in patients with type 2 diabetes, as we had previously shown, but also in healthy subjects who are at risk for type 2 diabetes.

BOLD MRI measures tissue oxygenation, which is a reflection of both oxygen delivery and blood flow, as well as tissue oxygen consumption. In the present study, we showed that measurements of kidney oxygenation pre- and postwater diuresis were associated with the skin endothelial function. However, there was no correlation between the water diuresis-induced change in renal cortical oxygenation and the macro- and microvascular reactivity. As these limited data suggest that vascular changes at the skin level may reflect changes at the renal cortical circulation, more studies are needed to clarify this association.

Further support for the above is provided by the fact that both fasting plasma glucose and total cholesterol were positively correlated with the renal cortical R2*. Both hyperglycemia and hyperlipidemia are known to increase oxidative stress and impair endothelial function.[17,18] It is unclear to us why the total and LDL cholesterol were negatively correlated with the change in cortical oxygenation during water diuresis.

In addition, correlations were found between both medullary and cortical oxygenation and biochemical markers of endothelial activation. Thus, ET-1, a potent vasoconstrictor that is secreted by the endothelium, was associated with decreased medullary oxygenation. This endothelial marker is increased in patients with diabetes and is associated with the development of long-term diabetes complications.[19,20] While PAI-1 correlated with the medullary oxygenation, this was a negative correlation that we could not fully explain.

Another correlation was also observed between the VCAM and the cortical oxygenation. VCAM is secreted by the endothelium in response to glyco-oxidative stress and plays an important role in the development of the initial stages of atherosclerosis. Finally, a negative correlation was found between CRP and both the baseline and postdiuresis cortical R2*, indicating that the higher the CRP the better was the cortical oxygenation. It seems reasonable to suggest that CRP levels reflect inflammation that may be related to increased blood flow in the kidney. We do not have a clear explanation why some endothelial markers correlated with the kidney oxygenation, while others did not.

Previous studies in our unit and elsewhere have shown that endothelial dysfunction is present even before the clinical onset of type 2 diabetes,[6] However, no information is available regarding the effect of having a risk to develop diabetes on renal oxygenation. In the present study, we have shown that the medullary oxygenation during water diuresis is impaired to a similar degree in healthy subjects at risk of developing diabetes and in diabetic patients. This finding suggests that there may be an early defect preceding hyperglycemia. It also indicates that

Table 5. Correlation of the Renal Cortical Oxygenation to Vascular Reactivity

	R2* Cortex (baseline)	R2* Cortex (after diuresis)	Change in R2* With Water Diuresis
Acetylcholine-induced skin vasodilation (% change over baseline)	−0.26 (0.091)	−0.40 (0.009)	−0.226 (0.155)
Sodium nitroprusside skin-induced vasodilation (% change over baseline)	−0.36 (0.017)	−0.39 (0.011)	−0.06 (0.69)
Flow-mediated dilation (% of increase over baseline)	0.02 (0.898)	0.01 (0.958)	0.05 (0.75)
Nitroglycerin-induced dilation (% of increase over baseline)	−0.05 (0.788)	−0.12 (0.508)	−0.01 (0.98)

Table 6. Correlation of the Renal Cortical Oxygenation to Various Metabolic Parameters

	R2* Cortex (baseline)	R2* Cortex (after diuresis)	Change in R2* With Water Diuresis
Fasting glucose	0.34 (0.021)	0.37 (0.017)	0.06 (0.70)
HBA_{1c}	0.27 (0.07)	0.27 (0.08)	0.02 (0.90)
Plasma insulin	−0.05 (0.79)	−0.30 (0.09)	−0.28 (0.11)
Microalb/creatinine ratio	−0.26 (0.09)	−0.16 (0.31)	0.11 (0.49)
Total cholesterol	0.31 (0.038)	−0.08 (0.602)	−0.36 (0.02)
HDL cholesterol	0.01 (0.95)	0.01 (0.94)	−0.02 (0.92)
LDL cholesterol	0.27 (0.07)	−0.09 (0.58)	−0.32 (0.04)
Triglycerides	0.08 (0.59)	−0.06 (0.69)	−0.14 (0.37)
VCAM	0.37 (0.037)	0.28 (0.135)	0.073 (0.70)
ET-1	0.07 (0.68)	−0.06 (0.69)	−0.08 (0.61)
TNF-α	0.03 (0.86)	−0.141 (0.46)	−0.06 (0.74)
CRP	−0.40 (0.013)	−0.37 (0.032)	−0.141 (0.43)
VWF	0.19 (0.22)	−0.07 (0.67)	−0.15 (0.38)
PAI-antigen	−0.10 (0.54)	−0.29 (0.08)	−0.15 (0.39)
t-PA-antigen	−0.01 (0.93)	−0.27 (0.10)	−0.19 (0.24)

NOTE. Data are *r (P)*.

early changes in the vascular reactivity in the prediabetic stage may result in subclinical changes on renal oxygenation.

In contrast to the effects of water diuresis on medullary oxygenation, where no changes were seen in the diabetic patients and the subjects at risk of developing diabetes, water diuresis produced a small, but statistically significant, improvement in cortical oxygenation in the 38 diabetic patients and the 7 subjects at risk for diabetes. More studies will be required to investigate whether this small improvement in cortical oxygenation is impaired in later stages of diabetic nephropathy.

The present study is a preliminary one and, therefore, has its limitations. A major limitation is the lack of a concomitantly studied control group of healthy subjects. Our historic controls, however, were tested in our unit under the same MRI protocol and did not have any major differences in their clinical characteristics as compared with the 2 groups. A second major limitation is that with the current MRI technique, we were able to measure oxygenation by randomly choosing ROIs in both the medulla and cortex. Measurements of selected areas, such as the corticomedullary junction, may have allowed us to better characterize kidney oxygenation. Another limitation may be that hyperglycemia itself may have affected our results by causing intravascular volume depletion and dehydration and thus potentially affecting measurements of renal perfusion. However, none of our subjects was clinically dehydrated according to careful clinical and laboratory evaluation.

In conclusion, in the present study, we have shown that there is no association between the water diuresis-induced change in kidney cortical oxygenation and the skin microvascular reactivity and brachial FMD. However, significant associations existed between the pre- and postdiuresis cortical oxygenation measurements and the vascular reactivity of the skin microcirculation. Furthermore, we found that the cortical oxygenation is associated with glycemia, lipidemia, and selected markers of endothelial activation. An additional finding was that water diuresis failed to improve the renal medullary oxygenation, not only in patients with type 2 diabetes, as previously shown, but also in healthy subjects who are at risk for diabetes. Further studies are needed to confirm and investigate the physiologic significance of these findings.

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