

Reduced Nitric Oxide Concentration in the Renal Cortex of Streptozotocin-Induced Diabetic Rats

Effects on Renal Oxygenation and Microcirculation

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Nitric oxide (NO) regulates vascular tone and mitochondrial respiration. We investigated the hypothesis that there is reduced NO concentration in the renal cortex of diabetic rats that mediates reduced renal cortical blood perfusion and oxygen tension (Po₂). Streptozotocin-induced diabetic and control rats were injected with L-arginine followed by N ω -nitro-L-arginine-methyl-ester (L-NAME). NO and Po₂ were measured using microsensors, and local blood flow was recorded by laser-Doppler flowmetry. Plasma arginine and asymmetric dimethylarginine (ADMA) were analyzed by high-performance liquid chromatography. L-Arginine increased cortical NO concentrations more in diabetic animals, whereas changes in blood flow were similar. Cortical Po₂ was unaffected by L-arginine in both groups. L-NAME decreased NO in control animals by 87 \pm 15 nmol/l compared with 45 \pm 7 nmol/l in diabetic animals. L-NAME decreased blood perfusion more in diabetic animals, but it only affected Po₂ in control animals. Plasma arginine was significantly lower in diabetic animals (79.7 \pm 6.7 vs. 127.9 \pm 3.9 mmol/l), whereas ADMA was unchanged. A larger increase in renal cortical NO concentration after L-arginine injection, a smaller decrease in NO after L-NAME, and reduced plasma arginine suggest substrate limitation for NO formation in the renal cortex of diabetic animals. This demonstrates a new mechanism for diabetes-induced alteration in renal oxygen metabolism and local blood flow regulation. *Diabetes* 54:3282–3287, 2005

Nitric oxide (NO) regulates vascular tone in resistance vessels and thereby blood perfusion in most capillary beds. Systemic inhibition of NO synthase (NOS) decreases renal blood perfusion, both in the cortex and in the medulla (1–3), and increases mean arterial blood pressure (4). Previous investigations *in vitro* have also shown that NO is a potent

competitive inhibitor of oxygen consumption (5) at the level of cytochrome oxidase, the terminal electron acceptor in mitochondria (6). Therefore, the magnitude of the inhibition of oxygen consumption by NO will increase at low Po₂ (7).

Long-term hyperglycemia is associated with increased oxidative stress, i.e., increased production of reactive oxygen species (ROS) (8–11). ROS can react with NO, forming peroxynitrite, and thus decrease the bioavailability of NO (11). The bioavailability of NO and formation of peroxynitrite are also highly dependent on superoxide dismutase, as modeled by Buerk et al. (12). Decreased influence of NO has therefore been suggested to be involved in the increased renal cortical cellular oxygen consumption closely associated with manifest diabetes (10,13,14). Furthermore, involvement of endogenous competitive NOS inhibitor asymmetric dimethylarginine (ADMA) in the development of vascular complications has gained increasing support over the last few decades (15).

Because NO regulates the delivery of oxygen to tissue both by setting the level of vascular tone and blood pressure and by inhibiting cellular oxygen consumption, alterations in NO activity might contribute to the development of diabetes-induced renal hypoxia. The current study aimed to investigate whether there is a diabetes-induced alteration in the NO concentration in the renal cortex and, if so, study the importance of this for renal cortical blood perfusion and oxygenation.

RESEARCH DESIGN AND METHODS

Inbred male Wistar-Furth rats, which weighed 250–330 g, were purchased from B&K Universal (Sollentuna, Sweden). Animals had free access to water and standard rat chow (R3; Ewos, Södertälje, Sweden) throughout the study. The local animal ethics committee at the University of Uppsala approved all experiments.

Diabetes induction. Diabetes was induced by an injection of streptozotocin (STZ; 45 mg/kg body wt; Sigma-Aldrich, St. Louis, MO) in the tail vein. Animals were considered diabetic if blood glucose concentrations increased to \geq 15 mmol/l within 24 h after STZ injection and remained elevated. Blood glucose concentrations were determined with test reagent strips (MediSense, Bedford, MA) from blood samples obtained from the cut tip of the tail in all animals.

Surgical procedures. At 4 weeks after allocation to the study, all animals were anesthetized with an intraperitoneal injection of thiobutabarbital (120 mg/kg body wt nondiabetic, 80 mg/kg body wt diabetic animals, Inactin; Sigma-Aldrich), placed on an operating table maintained at 37°C, and tracheostomized. Polyethylene catheters were placed in the right femoral artery and in the right femoral vein. The arterial catheter was used to monitor blood pressure (Statham P23dB; Statham Laboratories, Los Angeles, CA). The catheter in the vein was used for infusion of Ringer solution to compensate for loss of body fluid and for infusion of substances. The urinary bladder was catheterized to allow urinary drainage. The left kidney was exposed by a left subcostal flank incision, immobilized in a plastic cup, and embedded in pieces

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ADMA, asymmetric dimethylarginine; GFR, glomerular filtration rate; L-NAME, N ω -nitro-L-arginine-methyl-ester; NOS, NO synthase; ROS, reactive oxygen species; SDMA, symmetric dimethylarginine; STZ, streptozotocin.

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of cotton wool soaked in saline. The surface of the kidney was covered with paraffin oil (Apoteksbolaget, Gothenburg, Sweden) to avoid evaporation. The left ureter was catheterized for collection of urine for subsequent analysis.

Simultaneous measurements of renal NO activity, blood perfusion, and oxygen tension. Animals were allowed a 60-min recovery period followed by 3×20 min of measurements. After 20 min of control measurements, a single bolus dose of the NO substrate L-arginine (50 mg/kg body wt i.v.; Sigma-Aldrich). After another 20 min, the unspecific NOS inhibitor N ω -nitro-L-arginine-methyl-ester (L-NAME; Sigma-Aldrich; 10 mg/kg body wt i.v.). Glomerular filtration rate (GFR) was estimated by measurements of inulin clearance. For this purpose, [3 H]inulin (185 kBq/ml; American Radiolabeled Company, St. Louis, MO) dissolved in saline was initially given as a bolus dose of 185 kBq and then infused ($5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v.). Urine and arterial blood samples were taken for subsequent analyses. Renal cortical NO concentration was measured by recessed Whalen-type gold microsensors (tip dimensions $\leq 5 \mu\text{m}$), which were fabricated from glass micropipettes (16). Microsensors were polarized at +700 mV (amperometric method) relative to an Ag/AgCl reference electrode. NO microsensors were calibrated in deoxygenated buffer, bubbled with either 100% nitrogen or a mixture of NO and nitrogen (17). Because of the characteristics of these microsensors and the fact that calibration only can be achieved in vitro, merely relative changes of NO activity can be recorded during measurements in vivo. Cortical blood perfusion was measured with laser-Doppler flowmetry (probe 411, 0.45 mm optical density, PF 4001-2; Perimed, Stockholm, Sweden) (10). Oxygen tension (Po_2) was measured with a polarographic technique, using modified Clark-type microelectrodes (4–6 μm outer diameter; Unisense, Aarhus, Denmark) (18,19). Electrodes were two-point calibrated in water saturated with $\text{Na}_2\text{S}_2\text{O}_5$ or saturated with air at 37°C, respectively. All electrodes were inserted into the renal cortex (approximate distance from the renal surface 1.0 mm) with micromanipulators under a stereomicroscope. All measured parameters were continuously recorded with a MacLab instrument (AD Instruments, Hastings, U.K.) connected to a Macintosh Power-PC 6100.

At the end of the experiments, a blood sample was withdrawn from the arterial catheter to estimate the hematocrit. The left kidney was dissected to verify the sites of measurements. If any site of measurement was found not to be correctly located, this recording was excluded. Renal weights were also determined.

Measurements of blood perfusion-dependent renal oxygenation. A clamp was placed on the left renal artery. Renal cortical blood perfusion and Po_2 were measured, as described above, after renal artery occlusion in nondiabetic ($n = 7$) and diabetic animals ($n = 7$). The clamp was tightened stepwise 5–6 times in each animal. Readings of blood perfusion and Po_2 were performed after each time the clamp was tightened and stable values had been observed for at least 1 min.

Measurements of urine parameters. The radioactivity of [3 H]inulin in plasma (10 μl) and urine (1 μl) was measured by liquid scintillation. GFR was then calculated as the clearance of [3 H]inulin. Urine volumes were measured gravimetrically, osmolality by use of a freezing point technique (model 3MO; Advanced Instruments, Norwood, MA), and urinary sodium and potassium concentrations by use of a flame spectrophotometer (IL543; Instrumentation Lab, Milan, Italy).

Measurements of plasma arginine, ADMA, and symmetrical dimethyl-arginine. We withdrew ~ 0.5 ml blood from separate control ($n = 8$) and diabetic animals ($n = 10$). Plasma concentrations of arginine, ADMA, and symmetrical dimethylarginine (SDMA) were determined simultaneously by high-performance liquid chromatography, as described previously (20). In brief, solid-phase extraction on polymeric cation-exchange extraction columns was performed, using monomethylarginine as internal standard. After derivatization with *o*-phthalaldehyde reagent containing 3-mercaptopyrrolic acid, analytes were separated by isocratic reversed-phase high-performance liquid chromatography with fluorescence detection.

Statistical analysis. All values are the means \pm SE. Comparisons between two groups were performed using Student's *t* test for unpaired comparisons. Multiple comparisons between different groups were performed, using ANOVA followed by Fisher's protected least significant difference test. Multiple comparisons within the same group were performed, using repeated-measures ANOVA followed by post hoc test for paired comparisons. Linear regression was used to analyze the relationship between perfusion and oxygenation and multivariate ANOVA when comparing the relationship between blood perfusion and oxygenation between the different groups (Statview; Abacus Concepts, Berkeley, CA). For all comparisons, $P < 0.05$ was considered statistically significant.

RESULTS

Blood glucose concentrations were 5.7 ± 0.2 mmol/l in control animals ($n = 22$) and 23.2 ± 0.5 mmol/l ($P < 0.05$

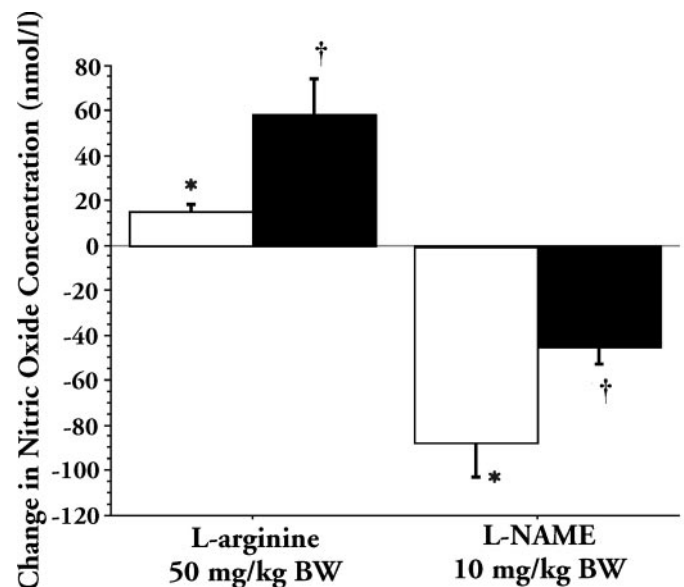


FIG. 1. Maximal changes in bioavailable NO concentration in the renal cortex in control ($n = 7$) and diabetic rats ($n = 8$) in response to intravenous injections of L-arginine and L-NAME. All values are the means \pm SE. * $P < 0.05$ compared with baseline within the same group, † $P < 0.05$ compared with the control group at the corresponding treatment. □, control; ■, diabetes. BW, body weight.

vs. control) in 4-week diabetic animals ($n = 25$). Renal weights were increased in 4-week diabetic animals compared with control animals (1.38 ± 0.05 g, $n = 25$, and 0.97 ± 0.02 g, $n = 22$, respectively; $P < 0.05$).

Injection of L-arginine caused a larger increase in renal cortical NO concentration in diabetic animals than in control animals (Fig. 1). Administration of L-NAME resulted in a pronounced decrease in renal cortical NO concentration in both groups, but with the largest decrease in control animals. Basal renal cortical blood perfusion was similar in both diabetic and control animals (318 ± 15 , $n = 8$, vs. 339 ± 17 laser units, $n = 7$, respectively; NS), and it increased after injection of L-arginine and decreased after L-NAME injection in both groups. The decrease after L-NAME administration was largest in diabetic animals (Figs. 2 and 3). Before the injections, basal Po_2 was lower in diabetic compared with control animals (33.6 ± 1.5 mmHg, $n = 8$, vs. 44.1 ± 3.2 mmHg, $n = 7$; $P < 0.05$) (Fig. 4). Injection of L-arginine did not significantly affect renal cortical Po_2 in any of the two groups, whereas L-NAME decreased Po_2 in control animals. There was a trend toward decreased Po_2 after L-NAME administration also in diabetic animals, although this did not reach statistical significance. Mean arterial blood pressure was unaffected by L-arginine injection, but it increased as a result of L-NAME injection in both investigated groups (Table 1).

There was an approximately linear dependence of cortical Po_2 on blood perfusion in both nondiabetic and diabetic animals ($n = 7$ and $P < 0.05$ in both groups, curve fit 0.621 in the nondiabetic group and 0.704 in the diabetic group) (Fig. 5). The relationship between renal cortical blood perfusion and Po_2 was similar in both groups (multivariate ANOVA, $P = 0.23$).

The 4-week diabetic animals had a lower baseline GFR than control animals (Table 1). GFR in control animals was unaffected by L-arginine injection, but it significantly decreased after injection of L-NAME. In diabetic animals,

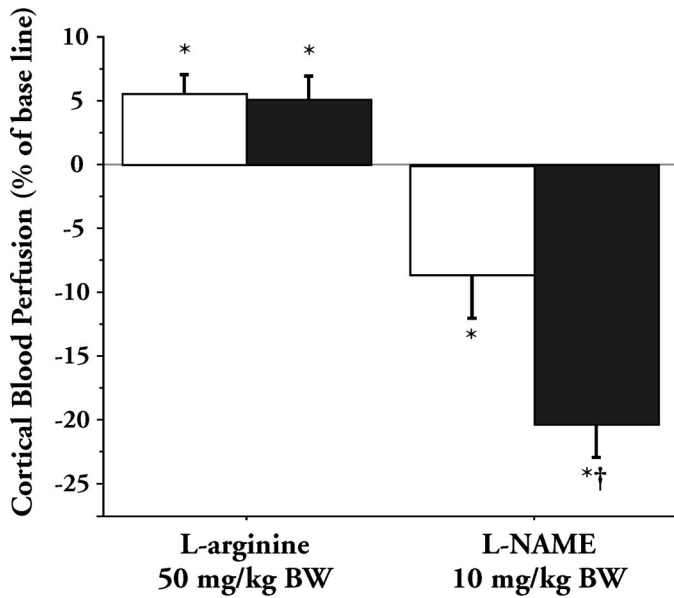


FIG. 2. Average change in blood perfusion in the renal cortex in control ($n = 7$) and diabetic ($n = 8$) rats in response to intravenous injections of L-arginine and L-NAME. All values are the means \pm SE. * $P < 0.05$ compared with baseline within the same group, † $P < 0.05$ compared with the control group at the corresponding treatment. □, control; ■, diabetes. BW, body weight.

L-arginine caused an increase, whereas L-NAME did not alter GFR. Baseline urinary flow rate was 10-fold higher in diabetic animals than in control animals (Table 1). In both groups, the urinary flow rate was unaffected by either of the two injections. Sodium excretion was unaffected by L-arginine injection in control animals, whereas injection of L-NAME resulted in increased sodium excretion (Table 1). None of the injections affected sodium excretion statistically in diabetic animals, although absolute values after L-NAME were more than twice the control level. Neither of the two injections had any effect on potassium excretion in either control or diabetic animals (Table 1). The plasma arginine concentration was significantly re-

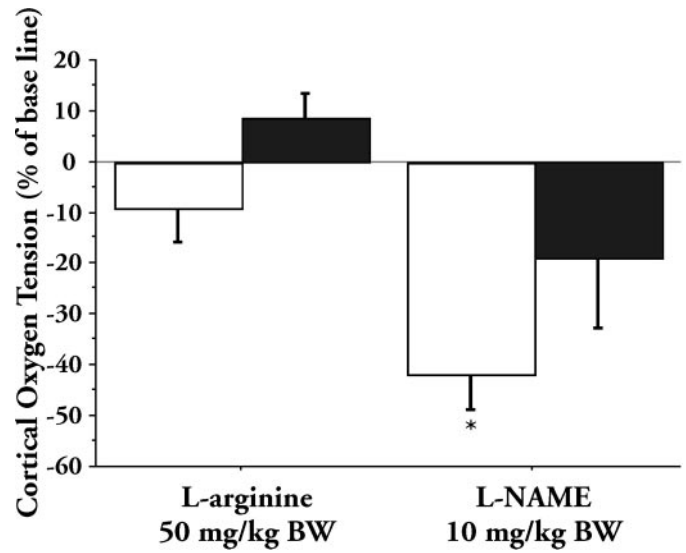


FIG. 4. Average change in oxygen tension in the renal cortex in control ($n = 7$) and diabetic rats ($n = 8$) in response to intravenous injections of L-arginine and L-NAME. All values are the means \pm SE. * $P < 0.05$ compared with baseline within the same group. □, control; ■, diabetes. BW, body weight.

duced in diabetic animals, whereas plasma concentrations of ADMA and SDMA were similar to control animals (Table 2).

DISCUSSION

The current study demonstrates substrate limitation for NO formation with concomitant lower NO concentration in the renal cortex of diabetic animals. These findings demonstrate a new mechanism for diabetes-induced alteration in renal oxygen metabolism and local blood flow regulation, which may have implications for the development of diabetes-induced vascular dysfunction.

So far, the reported effects of hyperglycemia on renal NO concentration and blood perfusion have been highly diverse (rev. in 21). There are several possible explana-

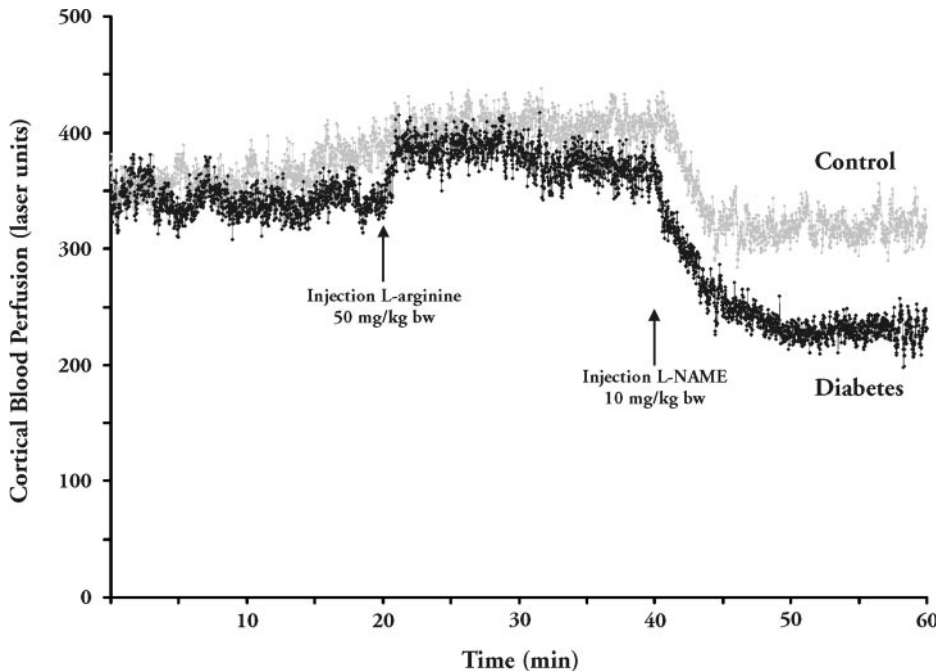


FIG. 3. Representative individual recordings of cortical blood perfusion from one control and one diabetic animal before and after intravenous injection of L-arginine and L-NAME.

TABLE 1

Renal parameters, blood pressure, and hematocrit obtained from control and 4-week diabetic animals during the control period and after administration of L-arginine (50 mg/kg body wt) and L-NAME (10 mg/kg body wt)

	Control animals (<i>n</i> = 7)			Diabetic animals (<i>n</i> = 8)		
	Control	L-arginine	L-NAME	Control	L-arginine	L-NAME
GFR (ml/min)	1.27 ± 0.03	1.26 ± 0.04	1.04 ± 0.02*†	1.09 ± 0.06‡	1.38 ± 0.11*	1.19 ± 0.18
Urinary flow rate (μl/min)	1.7 ± 0.3	1.7 ± 0.3	1.9 ± 0.2	17.5 ± 3.2‡	18.7 ± 3.5‡	14.2 ± 3.0‡
Sodium excretion (pmol/min)	71 ± 19	68 ± 17	94 ± 21*†	156 ± 67	243 ± 98	382 ± 189
Potassium excretion (pmol/min)	354 ± 108	431 ± 93	429 ± 75	436 ± 83	586 ± 99	527 ± 105
Mean blood pressure (mmHg)	111 ± 4	113 ± 5	126 ± 7*	110 ± 2	111 ± 2	120 ± 2*
Hematocrit (%)	45 ± 1	45 ± 1	45 ± 1	46 ± 1	45 ± 1	46 ± 1

Data are the means ± SE. Values are the means of the respective 20-min sampling period. **P* < 0.05 when compared with the corresponding control period; †*P* < 0.05 when compared with L-arginine period in the same group; ‡*P* < 0.05 when compared with the corresponding period for control animals.

tions for these reported differences. The use of different animal models or human populations and different techniques for estimating the renal NO concentration may explain some of the discrepancies. Furthermore, the production and bioavailability of NO are not necessarily identical entities.

In the current study, Whalen-type microsensors were used to record the bioavailable NO concentration in vivo. Direct and online measurements of NO activity are achieved because of the design of the sensors. These highly sensitive sensors have shown their usefulness under various in vivo conditions (17,22,23). By using direct measurements, we observed that NO concentrations in the renal cortex appeared to be markedly changed in diabetic rats. Basal NO concentrations can be calculated with the assumption that L-NAME inhibits all, or close to all, NO synthesis, resulting in 87 nmol/l in nondiabetic animals and only 45 nmol/l in diabetic animals, or a 48% lower basal NO level. Injection of L-arginine resulted in an increased renal cortical NO concentration in both the nondiabetic and diabetic rats within minutes after the injection, suggesting increased production by the intracellularly located NOS, similar to previous observations after systemic administration of L-arginine in nondiabetic animals (24). In the diabetic animals, the increase in NO concentration after L-arginine injection was, however, about fourfold higher compared with control animals. This suggests either a substrate limitation or increased NOS activity, or a combination of both. NO synthesis is highly dependent on cellular transport of arginine and is therefore dependent on extracellular arginine availability. This

availability is regulated by de novo arginine synthesis, cellular arginine transport, and the degradation rate by arginase (25). NO synthesis also depends on O₂ availability and the Michaelis constant (*K_m*) for O₂ for different NOS isoforms (26). The 24% lower average basal tissue P_{O₂} in the renal cortex of diabetic animals compared with nondiabetic animals could be a factor that contributes to their 48% lower basal NO levels. However, our results suggest that reduced L-arginine availability may be a more important factor. Indeed, plasma arginine concentration in the diabetic animals was 38% lower than nondiabetic animals (*P* < 0.05), which is in agreement with the concept of substrate limitation. Furthermore, the current study supports previous findings that tissue NO concentration is tightly dependent of the availability of extracellular arginine (25). The reason for the lower plasma arginine level in the diabetic animals is unclear, but a similar reduction has been observed during dietary salt restriction, and it was shown to be a result of increased hepatic arginine metabolism resulting from excessive cellular transport by the cationic amino acid 2A y⁺ transporter (27).

In vitro experiments have shown that the NOS activity in proximal tubules from STZ-induced diabetic rats is elevated in the presence of hyperglycemia (14). However, injection of L-NAME in the diabetic animals did not result in as large a decrease in NO concentration as in control animals, further supporting the theory of substrate limitation for NOS during this condition. Furthermore, an estimation of the actual in vivo capacity of NO bioavailability can be performed when calculating the total NO signal, i.e., adding the NO value in the presence of excessive

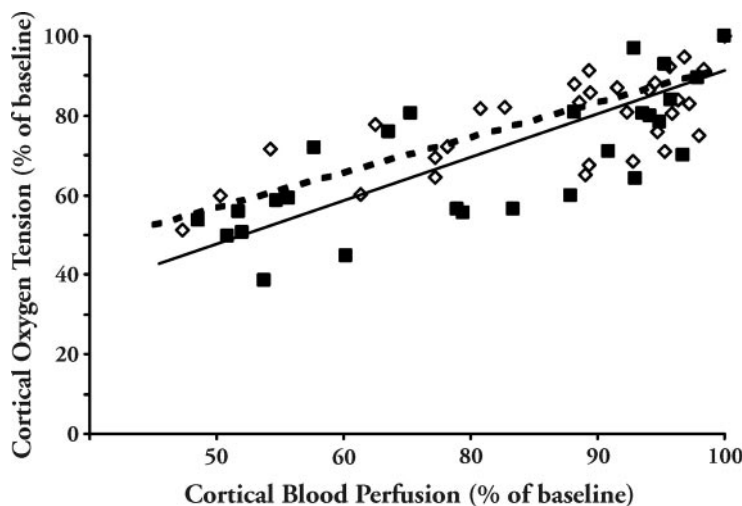


FIG. 5. Relationship between renal cortical blood perfusion and oxygen tension. Each group consist of seven animals with five to six measurements in each animal. ◇ and dotted line, control animals; ■ and solid line, diabetic animals.

TABLE 2
Plasma L-arginine, ADMA, and SDMA in control and 4-week diabetic animals

	<i>n</i>	L-arginine ($\mu\text{mol/l}$)	ADMA ($\mu\text{mol/l}$)	SDMA ($\mu\text{mol/l}$)
Control rats	8	127.9 \pm 3.9	0.599 \pm 0.017	0.328 \pm 0.012
Diabetic rats	10	79.7 \pm 6.7*	0.581 \pm 0.078	0.366 \pm 0.056

Data are the means \pm SE. **P* < 0.05 when compared with control animals.

substrate to the total amount inhibited by L-NAME. This results in 102 nmol/l in nondiabetic and 104 nmol/l in the diabetic animals, displaying similar capacity to produce bioavailable NO when substrate availability is unrestricted.

Other possible contributing factors to the decreased NO concentration in diabetic animals are NOS uncoupling, increased degradation of NO, or inhibition of NOS by endogenous ADMA. Uncoupling of NOS may occur if either the substrate, L-arginine, or the cofactor, tetrahydrobiopterin, is absent (28). Interestingly, uncoupled NOS donates electrons to molecular oxygen instead of L-arginine, resulting in accelerated oxidative stress with a concomitant increase in NO scavenging and oxidation of tetrahydrobiopterin to its inactive forms (21,28,29). In this context, it is noteworthy that the degradation of NO in vivo already during normal conditions is mostly accomplished through scavenging by free radicals and hemoglobin (26), and that both arginine and arginine derivatives per se have been shown to possess ROS scavenging properties (30). Elevated plasma level of the endogenous competitive NOS inhibitor ADMA, as previously reported in other animal models of experimental hyperglycemia (31), could potentially account for the decreased NO observed in the diabetic animals. However, neither ADMA nor SDMA were elevated in the current study, which would dismiss endogenous NOS inhibition as a contributing factor. The discrepancy with the observations made by Cooke and colleagues (31), showing a three-fold ADMA level in STZ- and high-fat diet-induced type 2 diabetes, is likely a result of the different animal model used in that study.

Cortical renal blood perfusion showed a similar increase after L-arginine injection in both nondiabetic and diabetic animals, even though the increase in NO activity was four times larger in diabetic animals. A seemingly similar paradox was observed after L-NAME was administered, with a more pronounced NO decrease in nondiabetic animals even though the decrease in blood perfusion was almost twice as large in diabetic animals. However, these results are in agreement with previous reports by Blantz and colleagues (32) showing that renal blood perfusion in experimental type 1 diabetes is more dependent on NO from the juxtaglomerular neuronal NOS. NO from this source will only contribute to a fraction of the total NO signal measured in the current study, resulting in a paradoxical finding. In this context it should also be noted that excessive ROS formation has been shown to cause antioxidant-preventable endothelium abnormalities and increased reactivity to vasoconstrictors (11,33,34). The pronounced elevation in mean arterial blood pressure after inhibition of NOS was similar in both nondiabetic and diabetic animals and was well within the range for efficient renal autoregulation (35).

It is well documented that the diabetic state induces increased cellular oxygen consumption (10,13,14,36). In the current study, basal renal cortical Po_2 i.e., that recorded before manipulation of NO production, was significantly lower in diabetic compared with control animals. This has also been reported during hypertension, another established state of increased radical production (37). We have previously reported (10) that decreased basal Po_2 in diabetic rats can be prevented by daily treatment with the radical scavenger α -tocopherol throughout the course of diabetes, mainly because of prevention of the diabetes-induced increase in oxygen consumption by renal tubular cells (10). Renal Po_2 is influenced by two factors, namely the delivery of oxygen by the blood and the oxygen consumption within the renal tissue. Alterations in either of these factors are likely to change renal tissue Po_2 if no compensatory mechanism is activated. Because no difference in basal cortical blood perfusion was observed between nondiabetic and diabetic animals in the current study, it is most likely that the increase in renal metabolism, as previously reported (10,13), is the main mechanism responsible for the diabetes-induced decrease in renal cortical Po_2 . Another possible explanation is a reduction in renal cortex capillary density in diabetic animals, but this seems unlikely because inhibition of NOS decreased the renal cortical Po_2 in the nondiabetic animals to a similar level as that recorded in the diabetic animals, despite the more pronounced decrease in blood perfusion in the diabetic animals. Decreased bioavailability of NO will, however, not only cause vasoconstriction, but it will also result in increased oxygen consumption (5). Because the rate at which NO inhibits the mitochondrial respiration is an interplay between the concentrations of both NO and oxygen (7), the renal oxygen consumption is likely to increase more in the nondiabetic than in the diabetic animals after inhibition of NOS because the NO decrease is proportionally larger in the control animals in relation to Po_2 . The dependency of renal blood perfusion for Po_2 was similar in nondiabetic and diabetic animals (Fig. 5). We therefore conclude that the more pronounced decrease in Po_2 in the nondiabetic animals after L-NAME is caused by a higher baseline NO inhibition of the mitochondrial respiration rate. This accounts for the similar absolute Po_2 levels in the two investigated groups after NOS inhibition.

Because of the duration of diabetes, the transient phase of glomerular hyperfiltration, which is present in this animal model during the first weeks after the induction of diabetes, had passed (38). Interestingly, Brown et al. (39) have shown that increased NO activity desensitizes the tubuloglomerular feedback mechanism, which could explain the increased GFR in the diabetic animals observed after L-arginine injection (40). The inverse mechanism could be responsible for the decreased GFR in the control animals observed after inhibition of NO production. There was a 10-fold higher urinary flow rate in the diabetic animals because of the osmotic diuresis. Altering NO production did not significantly affect the urinary flow rate in either of the two groups. The increased sodium excretion in the nondiabetic animals after NO inhibition is most likely an effect of pressure natriuresis. Even though the elevation in arterial pressure in the diabetic animals after L-NAME was similar to that of control animals, the increase in sodium excretion (twofold elevation) did not reach statistical significance due to large variation. It is, however, plausible that the mechanism underlying the higher sodium output after L-NAME in the diabetic animals

is similar to that in the control animals i.e., pressure natriuresis.

In conclusion, diabetic animals have a larger increase in the renal cortical NO concentration after injection of L-arginine compared with control animals which, together with the reduced plasma arginine concentration, demonstrate substrate limitation for NO synthesis by NOS. The decreased total renal NO concentrations in diabetic animals results in reduced tissue P_{O_2} and altered regulation of blood perfusion. These findings demonstrate a new mechanism for diabetes-induced alteration in oxygen metabolism and blood flow regulation, which may have implications for the development of cardiovascular complications during diabetes, but it warrants further investigation.

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