Insulin induces the correlation between renal blood flow and glomerular filtration rate in diabetes – Implications for mechanisms causing hyperfiltration

Liselotte Pihl, Patrik Persson, Angelica Fasching, Peter Hansell, Gerald F. DiBona and Fredrik Palm

1Division of Integrative Physiology, Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
2Department of Internal Medicine and Physiology and Biophysics, University of Iowa, Iowa City, Iowa, USA.
3Division of Nephrology and Hypertension, Georgetown University, Washington DC, USA.
4Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden

*These authors contributed equally.

Corresponding author:
Fredrik Palm, PhD.
Uppsala University
Department of Medical Cell Biology
Biomedical Centre, Box 571
751 23 Uppsala, Sweden
Phone +46 18 471 4182, Fax +46 18 471 4938
E-mail Fredrik.Palm@mcb.uu.se

Abstract

Glomerular filtration rate (GFR) and renal blood flow (RBF) are normally kept constant via renal autoregulation. However, early diabetes results in increased GFR and the potential mechanisms are debated. Tubuloglomerular feedback (TGF)-inactivation, with concomitantly increased RBF, is proposed but challenged by the finding of glomerular hyperfiltration in diabetic adenosine A1-receptor deficient mice which lack TGF. Furthermore, we consistently find elevated GFR in diabetes with only minor changes in RBF. This may relate to use of a lower streptozotocin dose which produces a degree of hyperglycemia which is manageable without supplemental suboptimal insulin administration, as has been used by other investigators. We therefore examined the relationship between RBF and GFR in diabetic rats with (diabetes+insulin) and without suboptimal insulin administration (untreated diabetes). As insulin can affect NO release, the role of nitric oxide (NO) was also investigated.

GFR, RBF and glomerular filtration pressures were measured. Dynamic RBF autoregulation was examined by transfer function analysis between arterial pressure and RBF.

Both diabetic groups had increased GFR (+60-67%) and RBF (+20-23%) compared to controls. However, only diabetes+insulin displayed a correlation between GFR and RBF ($R^2=0.81$, $P<0.0001$). Net filtration pressure was increased in untreated diabetes compared to both other groups. The difference between untreated and insulin-treated diabetics disappeared after administering L-NAME to inhibit NO synthase and subsequent NO release.

In conclusion, mechanisms causing diabetes-induced glomerular hyperfiltration are animal model-dependent. Supplemental insulin administration results in a RBF-dependent mechanism, whereas elevated GFR in untreated diabetes is mediated primarily by a tubular event. Insulin-induced NO release partially contributes to these differences.
**Introduction**

Normal kidney function depends on stable levels of glomerular filtration rate (GFR) and renal blood flow (RBF). Several mechanisms cooperate to maintain a constant GFR and RBF, including a highly efficient myogenic response, the tubuloglomerular feedback mechanism (TGF) and hormonal influences. The autoregulation of RBF and GFR is managed by intrinsic renal mechanisms which maintain RBF and GFR within a narrow range when arterial pressure fluctuates.

One of the characteristics of the diabetic kidney is glomerular hyperfiltration (38). Although the mechanisms mediating the increased GFR are under debate, proposed mechanisms involve an inactivation of TGF, via reduced tubular sodium chloride load to the macula densa. This is caused by increased proximal tubular reabsorption of sodium via sodium-glucose co-transport and results in vasodilation of the afferent arteriole with concomitant increase in RBF (41, 52). Indeed, in some (4, 25, 51) but not all studies (37, 40), changes in RBF and GFR have been observed to be closely correlated in diabetes. In addition, the development of genetically modified mice lacking a functional TGF mechanism, but still developing diabetes-induced glomerular hyperfiltration has challenged this explanation (15, 43). Increased synthesis of nitric oxide (NO) with concomitant renal vasodilatation and increased RBF has also been suggested to be involved in diabetic glomerular hyperfiltration (20, 24, 33).

Studies from this laboratory consistently showing elevated GFR but only minor changes in RBF in diabetic rats suggest that other mechanisms are mediating the glomerular hyperfiltration in diabetes (37, 40). However, these rats did not receive suboptimal supplemental insulin treatment, which is in contrast to other laboratories that commonly administer daily low-dose insulin. One common observation from the studies using insulin supplementation is increased RBF (25, 46), suggesting differences in GFR regulation in these two rat models of insulinopenic diabetes depending on whether or not supplemental insulin administration was employed. It was therefore of interest to investigate the relationship between RBF and GFR in diabetic rats with and without suboptimal insulin supplementation throughout the course of diabetes to further clarify the
mechanisms behind the diabetes-induced hyperfiltration. Furthermore, as insulin is known to induce NO production, especially in diabetes, measurements were performed before and after acute NO synthase (NOS) inhibition. We tested the hypothesis that low-dose insulin administration affects RBF in diabetes and thereby the determinants of glomerular hyperfiltration.
Materials and Methods

All chemicals were from Sigma Aldrich (St. Louis, MO, USA) and of the highest grade available if not otherwise stated.

Animals and induction of diabetes. Adult male Sprague Dawley rats (300-420 g) were purchased from Charles River (Sulzfeld, Germany). The rats were kept in groups of 2-3 with free access to standard rat chow and tap water. All experiments were performed in accordance with the National Institutes of Health guidelines for use and care of laboratory animals and approved by the Institutional Animal Care and Use Committee of Uppsala University. Rats were allocated to one of three groups, controls (n=17), untreated diabetes (n=16) or diabetes+insulin (n=18) for investigation of all parameters except the micropuncture experiments. Additional rats were allocated to micropuncture experiments, controls (n=4), untreated diabetes (n=4) and diabetes+insulin (n=4). Diabetes was induced by intravenous injection of streptozotocin (55 mg/kg) into the tail vein. Animals were considered diabetic if blood glucose levels increased to >16 mmol/l within 48h after streptozotocin administration. Animals in the group diabetes+insulin were treated with a single subcutaneous injection of insulin once a day for ten days prior to the acute experiment (9 IU/kg/day; Lantus, Sanofi Aventis, Frankfurt am Main, Germany) (diabetes+insulin), whereas the remaining diabetic animals received no treatment (untreated diabetes). Normoglycemic rats served as control (control). Acute experiments investigating kidney function were performed 14±2 days after diabetes induction in both diabetic groups and compared to age-match controls.

Surgical procedure and measurements of GFR and RBF. The animals were anesthetized by an intraperitoneal injection of thiobutabarbital (Inactin, 80-120 mg/kg), tracheostomized and placed on a heating pad to maintain core body temperature at 37.5°C. The left femoral vein was catheterized for continuous infusion of Ringer solution, at a rate of 10 ml/kg/h (diabetics) or 5 ml/kg/h (controls). The carotid artery was catheterized for continuous recording of arterial pressure (AP) and withdrawal of blood. The left kidney was exposed through a subcostal flank incision, immobilized in a plastic cup, embedded in pieces of saline-soaked cotton wool and covered with
paraffin oil (Apoteksbolaget, Gothenburg, Sweden). An ultrasound probe (Transonic Systems, Ithaca, NY, USA) was placed around the left renal artery for continuous recording of RBF. The left ureter was catheterized close to the renal pelvis for collection of urine for subsequent analysis and the urinary bladder was catheterized to allow urinary drainage. GFR was measured by clearance of $^3$H-inulin (American Radiolabeled Company, St. Louis, MO, USA). $^3$H-inulin was given as a continuous infusion (185 kBq/kg/h) in Ringers solution. The radioactivity of $^3$H-inulin in plasma (10 µl) and urine (1 µl) was measured using standard liquid scintillation technique. Urine volumes were measured gravimetrically. Approximately 45 minutes were allowed for recovery and for the plasma concentration of $^3$H-inulin to reach steady-state. Baseline parameters were measured (Baseline) for 40 minutes and followed by i.v. administration of a single bolus dose of $\text{N}^\omega$-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) followed by a continuous i.v. infusion of L-NAME (3 mg/kg/h). Ten minutes were allowed for L-NAME to achieve its steady state effect, thereafter all parameters were followed for another 40-minute period (After L-NAME). All parameters were continuously recorded using MacLab or PowerLab instruments (AD Instruments, Hastings, UK).

**Measurements of proximal tubular pressures.** The net glomerular filtration pressure ($P_{\text{net}}$) was estimated by stop-flow technique at baseline in four separate animals from each group. Early proximal tubular segments, 3-4 in each rat, were randomly chosen on the kidney surface. A micropipette, filled with 1 M NaCl colored with Lissamine green and connected to a servo-null pressure system (World Precision Instruments, New Haven, CT, USA) was used to determine proximal tubular free-flow ($P_{\text{ff}}$) and stop-flow pressure ($P_{\text{sf}}$). The latter was determined after tubular flow was blocked with a column of stained mineral oil distal to the pressure micropipette. $P_{\text{net}}$ was calculated according to $P_{\text{sf}} - P_{\text{ff}}$.

**Calculations.** Filtration fraction (FF) was estimated as follows: $\text{FF} = \text{GFR}/\text{RBF}*(1-\text{Hct})$. GFR was calculated as follows: $\text{GFR} = U*V/P$, where $U$ and $P$ denote the concentration of $^3$H in urine and plasma respectively, and $V$ denotes the urine flow (ml/min).

**Transfer function analysis.** The ten last animals from each group included in the linear regression
analysis described above were also used for transfer function analysis. Data used to examine the
dynamic relationship between AP and RBF, i.e., dynamic autoregulation of RBF, were sampled at
40 Hz yielding 96000 data points each for the 40 minute baseline and L-NAME periods. Processing
of AP and RBF data was performed off-line by using previously developed software routines
written for Matlab 7.14 (The MathWorks, Natick, MA, USA). After subtracting the mean value
from the data files, they were digitally low-pass filtered (10 Hz cut-off frequency, finite-impulse
response, order 50) and then resampled to a rate of 20 Hz. These 20 Hz data files were split into
blocks of 8192 data points, yielding a frequency discrimination of 0.002 Hz. Power spectral density
(PSD) of AP and RBF was calculated, as described (12, 13). The TF spectra were calculated from
AP (input) and RBF (output). The TF gain was taken as the quotient of the cross spectrum of input
and output divided by the power spectrum of the input (12, 13). Coherence is a frequency domain
estimate of a linear correlation (i.e., squared coherence, akin to coefficient of determination)
between two signals indicating the degree to which the variance in one signal can be explained by a
linear operation on the other signal (12, 13). The coherence spectra were calculated from AP (input)
and RBF (output). The coherence function was taken as the quotient of the square of the cross
spectrum of input and output divided by the product of the power spectral densities of AP and RBF
(12, 13). These algorithms (applied to the transfer function spectra as well as coherence) involved a
Hanning window and 50% overlap of the blocks. To permit comparison among rats, the TF gain
(magnitude) values over the frequency range have been normalized to the mean value of the renal
vascular conductance for the entire data set. After conversion of the normalized TF gain values into
decibels [20 log (gain)], a mean spectrum was calculated from the consecutive spectra in each rat,
and these were subsequently averaged for all rats. The TF gain corresponds to the ratio of the
amplitude of normalized fluctuations in RBF divided by those of AP. In the presence of normal
renal blood flow autoregulation, fluctuations of RBF are attenuated vs. those of AP causing the TF
gain to be negative. Thus, positive TF gain values indicate impaired renal blood flow autoregulation
(9, 26). Phase and coherence spectra were similarly calculated and averaged. Data over the range of
frequencies for the myogenic response (MR, 0.08–0.18 Hz) and the tubuloglomerular mechanism (TGF, 0.03–0.06) were analyzed (9, 26). The slope of gain reduction in the frequency range of the MR was determined by least squares fitting of the linear region of gain reduction, and the phase peak was estimated as the average phase value within the same frequency interval. In addition, to assess the contribution of the MR, mean gain values in the frequency range of 0.06-0.09 Hz were used to minimize corruption by TGF (<0.06 Hz) and myogenic transients (>0.09 Hz).

To determine the threshold for coherence above which it exceeds zero with a certain significance level, we used the method described by Koopmans (30), which depends on the total number of samples, the total number of blocks, and the nature of the tapering window. In this study with large sample numbers, coherence values >0.1 are significantly different from zero at P < 0.001.

Statistical analysis. Any correlation between the dependent (Y axis) and independent variable (X axis) in Figs 4A-B, 7A-B and 8A-B, was analyzed using least squares linear regression analysis using Prism software to test if the regression slopes were significantly different from zero (version 5; GraphPad Software, La Jolla, CA, USA). The value Sy.x is the standard deviation of the vertical distances of the points from the regression line. All other statistical calculations analyzing differences between groups or the effect of L-NAME within the same group were performed using SAS for Mixed Models in order to account for the study design consisting of both unpaired data (between groups) and paired data (within groups) (SAS Institute Inc, Cary, NC, USA) (35) assigning individual rats as a random effect. Mixed Model analysis was also used to analyze if the regression slopes presented in Figs 4A-B, 7A-B and 8A-B differed between the groups. For the transfer function analysis data, one-way ANOVA was employed. Unpaired t-test was used where appropriate and Bonferroni correction was made for multiple comparisons. For all comparisons, P<0.05 (two-tailed) was considered statistical significant and all data are presented as mean±SEM.
Results

All STZ-treated animals were hyperglycemic compared to untreated controls. Hematocrit, body weight, baseline MAP and the response of MAP to NOS inhibition were not statistically different between any of the groups (Table 1).

In the baseline period, both diabetic groups developed glomerular hyperfiltration compared to controls (Fig. 1). The increase in GFR was accompanied by elevated RBF in the diabetes+insulin group (Fig. 2) and by increased filtration fraction (FF) in the untreated diabetes group (Fig. 3). Accordingly, a correlation between GFR and RBF was observed only in the diabetes+insulin group, and the slope of the regression line in diabetes+insulin was statistically different from both control (P=0.0020) and untreated diabetes (P=0.0118; Fig. 4A). The increased GFR in the untreated diabetes was accompanied by a decreased Pff resulting in increased Pnet (Fig. 5).

NOS inhibition resulted in significantly decreased GFR only in diabetes+insulin (Fig. 1), but decreased RBF (Fig. 2) and increased FF (Fig. 3) in all groups. After NOS inhibition, diabetes+insulin still displayed a significant correlation between GFR and RBF, but the slope of the regression line in the diabetes+insulin did not statistically differ from that of the untreated diabetes (P=0.1703; Fig. 4B).

In the baseline period, controls exhibited the expected normal pattern of renal blood flow autoregulation (Fig. 6 and Table 2). The MR was characterized by a corner frequency of 0.21 Hz, below which gain decreased sharply from positive to negative values. The TGF was characterized by a local gain maximum between 0.03 and 0.06 Hz which was accompanied by a positive phase value. Neither untreated diabetes nor diabetes+insulin affected the MR response. Diabetes+insulin exhibited a significantly more negative gain at 0.03-0.06 Hz compared to control but otherwise there were no statistical differences among the groups in the TGF response.

After L-NAME, there were increases in gain at the corner frequency of circa 0.2 Hz in all groups but these did not reach statistical significance. The situation was similar with respect to
the slope of gain between 0.08 and 0.18 Hz. The local gain maximum in the TGF frequency range tended to become more negative in control whereas the opposite response was seen in untreated diabetes and diabetes+insulin. While the gain values in the high frequency range above the corner frequency are unusual, they are not critical to the overall analysis as they are outside the frequency range of interest.

Positive correlations between GFR and blood glucose were observed in the two diabetic groups, both before (Fig. 7A) and after NOS inhibition (Fig. 7B). However, only diabetes+insulin displayed a statistically significant correlation between RBF and blood glucose during baseline (Fig. 8A), which was not observed after NOS inhibition (Fig. 8B).
Discussion

The main new finding from the present study is that a correlation between RBF and diabetes-induced glomerular hyperfiltration develops only if concomitant suboptimal insulin treatment is administered. The reason for this difference between diabetic rats receiving insulin and untreated diabetic rats is likely to involve NO since NOS inhibition results in a significantly decreased GFR in the diabetes+insulin rats, as well as a correlation between RBF and GFR in the untreated diabetes rats. The conclusion that different mechanisms are mediating the glomerular hyperfiltration in the two diabetic groups is supported by the micropuncture data. Untreated diabetes rats displayed decreased $P_{ff}$ resulting in increased driving force for filtration, $P_{net}$, whereas diabetes+insulin rats displayed a RBF-related increase in GFR.

Glomerular hyperfiltration is a common observation in type 1 diabetes, both in animal models and in patients (6, 38, 40, 50). As previously reviewed (41), GFR is highly influenced by proximal tubular Na$^+$ reabsorption. Elevated plasma glucose levels result in increased proximal tubular Na$^+$ reabsorption mediated by the Na$^+$-glucose linked transporter (SGLT), resulting in decreased Na$^+$ delivery to the early distal tubule. Both Na$^+$ delivery to the early distal tubule and GFR were normalized by inhibiting SGLT in diabetic rats (50). It was concluded that the diabetes-induced glomerular hyperfiltration involved a TGF-dependent afferent arteriolar vasodilation increasing both RBF and GFR. However, adenosine A1-receptor deficient mice lack the TGF mechanism and still develop diabetes-induced glomerular hyperfiltration (15, 43), which indicates that other mechanisms are involved in the elevated GFR in diabetes. However, the transfer function analysis showed that the myogenic response was not affected by diabetes, untreated or treated with insulin.

One difference between the diabetic animal model we commonly use compared to other laboratories is that suboptimal insulin treatment (37, 40) is not employed, but rather an optimal dose of streptozotocin is selected so as to produce a high, but manageable, blood glucose level. The present study therefore investigated the relationship between RBF and GFR in control
and diabetic rats with and without daily suboptimal insulin administration throughout the course of diabetes. Linear regression analysis revealed a strong correlation between RBF and GFR in diabetes+insulin, which together with the normal $P_{\text{net}}$ is consistent with a TGF-mediated mechanism. In contrast, no significant correlation between RBF and GFR was found in controls and untreated diabetes, suggesting a non-RBF dependent mechanism controlling GFR in these groups under normal conditions. It should be noted that most of the available data from patients with early diabetes-induced glomerular hyperfiltration demonstrate that GFR is increased without concomitant increased RBF (5, 6, 14, 36). However, increased RBF in young diabetic patients has been observed (32), making RBF less predictable than GFR in early diabetes.

Intriguingly, baseline RBF was not significantly different between the two diabetic groups, which was unexpected since the glomerular hyperfiltration in diabetes+insulin was strongly correlated with RBF. However, although the insulin treatment did not normalize the blood glucose in these animals, it resulted in a significantly lower level compared to untreated diabetes. When analyzing the relationship between the blood glucose level and RBF, we found a correlation in the diabetes+insulin group. Using this relationship to calculate the RBF at the blood glucose level found in untreated diabetes results in a corresponding RBF approximately 52% higher in diabetes+insulin compared to untreated diabetes (10.2 vs. 6.7 ml/min/kidney). Similar calculation after NOS inhibition results in a more than 100% higher RBF in diabetes+insulin compared to untreated diabetes (8.1 vs. 3.9 ml/min/kidney). Performing similar corrections of GFR for the different blood glucose levels in the two diabetic groups also resulted in significantly higher GFR in diabetes+insulin, both during baseline (3.5 vs. 2.5 ml/min/kidney) and after NOS inhibition (3.6 vs. 2.2 ml/min/kidney).

Insulin is known to enhance production of NO and has been shown to cause dose dependent vasodilatation of the afferent and efferent arterioles in the isolated perfused kidney, an effect that was abolished after L-NAME (18, 19, 49). This would fit with the increased RBF in diabetes+insulin compared to untreated diabetes. After NOS-inhibition the myogenic response
tended to be enhanced in controls as well as diabetes, untreated or treated with insulin. The TGF response was differentially affected by NOS-inhibition with gain values in the 0.03-0.06 Hz frequency range being significantly more negative in diabetes+insulin than control suggesting improvement in diabetes+insulin. It should be noted that GFR is more influenced by RBF as RBF decreases (1). The slightly decreased GFR in controls and diabetes+insulin, and the statistically unaltered GFR in untreated diabetes after NOS inhibition is in good agreement with previously published results (2, 29, 40).

GFR is determined by the hydrostatic and colloid osmotic forces across the glomerular membrane, as well as the filtration coefficient (Kf). Kf is determined by the permeability of the filtration barrier and its effective filtration area. To further investigate the determinants of GFR in these two models, we performed micropuncture experiments. Pff was reduced in untreated diabetes confirming previous reports (21, 25, 50). Pff in diabetes+insulin was significantly higher compared to untreated diabetes, and was not significantly different from that of controls. Previous studies have shown both reduced (25) and unaltered Pff (48) in diabetic rats receiving insulin. Reduced Pff in diabetes is caused by increased proximal tubular Na+ transport mediated by the SGLT, evident from the finding that inhibition of SGLT by phlorizin increases Pff, with a concomitant reduction in single-nephron GFR, observed exclusively in diabetic rats (50). Also reduced hydraulic resistance in the loop of Henle, due to tubular hypertrophy, has been proposed as a mechanism for the reduced Pff in diabetes (25). The decreased Pff in untreated diabetes together with an unaltered Psf in any of the groups resulted in increased driving force for filtration (Pnet) in untreated diabetes compared to both controls and diabetes+insulin, which is in agreement with previous reports (25, 37, 50). In addition, acute insulin infusion in diabetic rats together with dextrose to maintain hyperglycemia results in normalization of Pnet but with maintained glomerular hyperfiltration (44).

Intrarenal angiotensin II (Ang II) is elevated in the diabetic kidney (22, 39). This can increase efferent arteriolar resistance (53) and might therefore explain the increased FF in diabetes. However, whereas the suboptimal insulin administration normalized Pnet, these animals displayed
increased GFR implying increased $K_f$. This might provide an explanation for RBF-dependent GFR in these animals since increased $K_f$ will drive filtration dynamics closer to filtration equilibrium. It is well-known that GFR in rats is highly RBF-dependent under conditions of filtration equilibrium (1, 8). A lower $K_f$ will increase the importance of $P_{net}$, and the tubular events opposing filtration will have greater influence on the filtration process (1, 27, 28). The unaltered $P_{net}$ in diabetes+insulin could in part be explained by Ang II-mediated efferent arteriolar constriction being offset by NO related vasodilation as shown by DeNicola and colleagues (10). Ang II also contracts isolated glomeruli and a direct association between vasoactive hormones and modulation of mesangial cell tone and GFR has been demonstrated (7, 23). Furthermore, local inhibition of intrarenal NOS reduces $K_f$, suggesting a tonic control of $K_f$ by NO (11). Indeed, NOS-2 (inducible) and NOS-3 (endothelial) are both expressed in endothelial and mesangial cells in the glomerular capillaries (16, 45), and it has been shown that Ang II-mediated reduction in NO activity can reduce active capillary surface area (3). Insulin receptors are expressed in glomeruli (31) and are known to activate NOS (42). The insulin treatment in the present study could therefore have resulted in increased glomerular NO production leading to mesangial cell relaxation and therefore increased $K_f$. This may provide an explanation for the RBF-dependent hyperfiltration in diabetes+insulin even though $P_{net}$ was unaltered.

In conclusion, the results from this study demonstrate that the glomerular hyperfiltration in untreated diabetes is mediated by alterations of tubular origin (i.e. decreased $P_{ff}$), whereas diabetic rats receiving suboptimal insulin treatment develop hyperfiltration caused by hemodynamic alterations (i.e. increased RBF). The available data from patients with early diabetic hyperfiltration demonstrate alterations most similar to the experimental animal model where the glomerular hyperfiltration is of tubular origin (5, 6, 14, 36).

**Perspective and significance**

Numerous physiological functions are either mediated by or modulated by insulin. In
In this study, it was demonstrated that renal hemodynamics and GFR regulation are modulated by insulin although suboptimal insulin administration did not reverse diabetes-induced glomerular hyperfiltration. Previous reports investigating kidney function in early type-1 diabetic patients are in support of diabetes-induced glomerular hyperfiltration being of a tubular origin since these patients displayed normal RBF. Leyssac and co-workers demonstrated that alterations in proximal tubular reabsorption directly affected the driving force for GFR, prior to the activation of TGF (34). Tubular control of GFR in diabetes was later confirmed in adenosine A1-receptor deficient mice lacking a functional TGF mechanism (17).

Inhibition of tubular glucose reabsorption, by blocking the sodium-glucose transporters expressed in the proximal tubule, and the resulting glucosuria has emerged as a novel treatment to reduce plasma glucose levels in diabetic patients resistant to conventional anti-diabetic treatments. However, the results from the present study may imply that inhibition of proximal tubular electrolyte transport reduces GFR in diabetics. Indeed, reduced GFR after inhibition of proximal tubular glucose reabsorption has recently been reported in rats (47). Although a reduction of early glomerular hyperfiltration may be beneficial, caution should be exercised in regard to further reductions in GFR during the development and course of diabetic nephropathy with its progressive decline in GFR.

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Disclosures
None of the authors have anything to disclose.
Figure legends

**Figure 1.** Glomerular filtration rate in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline and after acute L-NAME administration. * denotes P < 0.05 versus corresponding control.

**Figure 2.** Renal blood flow in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline and after acute L-NAME administration. * denotes P < 0.05 versus corresponding control.

**Figure 3.** Filtration fraction in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline and after acute L-NAME administration. * denotes P < 0.05 versus corresponding control.

**Figure 4.** Correlation between glomerular filtration rate and renal blood flow in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline (A) and after acute L-NAME administration (B). The slope of the regression line in Diabetes + Insulin is significant different compared to the slopes of the regression lines in both other groups in (A), whereas the slopes of the regression lines in Untreated Diabetes and Diabetes + Insulin are not statistically different in (B).

**Figure 5.** Free flow (top), Stop flow (middle) and Net filtration pressure (bottom) in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline.
Figure 6. Transfer function analysis in control (left) and diabetic rats with (right) and without (middle) chronic suboptimal insulin treatment during baseline and after acute L-NAME administration.

Figure 7. Correlation between glomerular filtration rate and blood glucose in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline (A) and after acute L-NAME administration (B). None of the slopes in either (A) or (B) are statistically different.

Figure 8. Correlation between renal blood flow and blood glucose in control and diabetic rats with and without chronic suboptimal insulin treatment during baseline (A) and after acute L-NAME administration (B). The slope of the Diabetes + Insulin is significant different compared to the slopes of both other groups in (A), whereas none of the slopes are statistically different in (B).
References


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Table 1. Mean arterial pressure (MAP), hematocrit (Hct), body weight, left kidney weight and blood glucose in Control, Untreated Diabetes and Diabetes + Insulin during baseline and after nonspecific inhibition of nitric oxide synthase (L-NAME).

<table>
<thead>
<tr>
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<th>Control (n=17)</th>
<th>Untreated Diabetes (n=16)</th>
<th>Diabetes + Insulin (n=18)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>After L-NAME</td>
<td>Baseline</td>
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<tr>
<td>Blood glucose (mmol/l)</td>
<td>6.1±0.2</td>
<td>-</td>
<td>24.5±0.9*</td>
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<tr>
<td>Hct (%)</td>
<td>43±1</td>
<td>45±1</td>
<td>43±1</td>
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<td>MAP (mmHg)</td>
<td>95±3</td>
<td>137±2†</td>
<td>98±4</td>
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<tr>
<td>Body weight (g)</td>
<td>369±4</td>
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<td>370±10</td>
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<tr>
<td>Left kidney weight (g)</td>
<td>1.50±0.05</td>
<td>-</td>
<td>2.20±0.09*</td>
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</table>

All values are means±SEM. * denotes P<0.05 compared to Control, # denotes P<0.05 compared to Untreated Diabetes and † denotes P<0.05 versus Baseline within the same group.
Table 2. Characteristics of the transfer function between arterial pressure and renal blood flow.

<table>
<thead>
<tr>
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<th>Control (n=10)</th>
<th>Untreated Diabetes (n=10)</th>
<th>Diabetes + Insulin (n=10)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>After L-NAME</td>
<td>Baseline</td>
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<tr>
<td>Corner frequency (Hz)</td>
<td>0.206±0.006</td>
<td>0.226±0.003</td>
<td>0.185±0.003</td>
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<td>Gain at corner frequency (dB)</td>
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<td>4.15±1.14</td>
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<td>Phase at corner frequency (degrees)</td>
<td>-4.0±9.2</td>
<td>-19.7±15.1</td>
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<td>Gain, 0.03-0.06 Hz (dB)</td>
<td>-2.36±1.01</td>
<td>-6.78±1.18</td>
<td>-5.46±1.25</td>
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<td>Phase, 0.03-0.06 Hz (degrees)</td>
<td>26.6±5.4</td>
<td>28.4±13.4</td>
<td>38.7±7.5</td>
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<tr>
<td>Gain, 0.06-0.09 (dB)</td>
<td>-4.80±0.65</td>
<td>-6.58±1.12</td>
<td>-5.28±1.09</td>
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<td>Gain, slope 0.08-0.18 Hz, (dB/decade)</td>
<td>18.00±2.05</td>
<td>27.24±2.96</td>
<td>22.12±2.92</td>
</tr>
</tbody>
</table>

Values are means±SEM. See METHODS for calculations. * denotes P<0.01 compared to corresponding Control.
Glomerular Filtration Rate
(ml/min/kidney)

- **Control**
- **Untreated Diabetes**
- **Diabetes + Insulin**

Baseline | After L-NAME
---|---

*P < 0.05*
Renal Blood Flow (ml/min/kidney)

- Control
- Untreated Diabetes
- Diabetes + Insulin

- Baseline
- After L-NAME

P<0.05
Glomerular Filtration Rate (ml/min/kidney)

Renal Blood Flow (ml/min/kidney)

BASELINE

R² = 0.8066
P<0.0001
Sy.x=0.4628

R² = 0.0022
P=0.8643
Sy.x=0.7559

R² = 0.0217
P=0.5728
Sy.x=0.4015

Legend:
- Control
- Untreated Diabetes
- Diabetes + Insulin
Glomerular Filtration Rate (ml/min/kidney)

Blood Glucose (ml/min/kid/min/kidney)

BASELINE

$R^2 = 0.0938$
$P = 0.2319$
$Sy.x = 0.3864$

$R^2 = 0.3636$
$P = 0.0134$
$Sy.x = 0.6037$

$R^2 = 0.4646$
$P = 0.0018$
$Sy.x = 0.7701$

Control
Untreated Diabetes
Diabetes + Insulin
After L-NAME

**Glomerular Filtration Rate (ml/min/kidney)**

**Blood Glucose (mmol/l)**

- Control
- Untreated Diabetes
- Diabetes + Insulin

- $R^2 = 0.5744$  
  $P = 0.0017$  
  $Sy.x = 2599$

- $R^2 = 0.2515$  
  $P = 0.0340$  
  $Sy.x = 0.9511$

- $R^2 = 0.3614$  
  $P = 0.0138$  
  $Sy.x = 0.9197$
Renal Blood Flow (ml/min/kidney)

Blood Glucose (mmol/l)

- Control
- Untreated Diabetes
- Diabetes + Insulin

Baseline

\[ R^2 = 0.2172 \]
\[ P = 0.0499 \]
\[ Sy.x = 2.944 \]

\[ R^2 = 0.1755 \]
\[ P = 0.0942 \]
\[ Sy.x = 2.084 \]

\[ R^2 = 0.0498 \]
\[ P = 0.4060 \]
\[ Sy.x = 2.262 \]
After L-NNAME

**Renal Blood Flow** (ml/min/kidney)

**Blood Glucose** (mmol/l)

- $R^2 = 0.0098$  
  $P = 0.7359$  
  $Sy.x = 1.536$

- $R^2 = 0.0226$  
  $P = 0.5784$  
  $Sy.x = 1.830$

- $R^2 = 0.1581$  
  $P = 0.1023$  
  $Sy.x = 2.785$

Legend:
- **Control**
- **Untreated Diabetes**
- **Diabetes + Insulin**