Glucose-induced Increases in Renal Hemodynamic Function

Possible Modulation by Renal Prostaglandins

BERTRAM L. KASISKE, MICHAEL P. O'DONNELL, AND WILLIAM F. KEANE

SUMMARY

Increased glomerular filtration rate and kidney size early in the course of experimental and human diabetes may be important in the pathogenesis of diabetic nephropathy. Factors causing these renal functional changes are unknown. The isolated, perfused rat kidney (IPRK) was used to study the effects of elevated glucose levels on kidneys from normal and diabetic rats in the absence of complex systemic effects of in vivo hyperglycemia. It was found that acute increases in perfusate glucose levels caused sustained dose-dependent vasodilatation in both normal and diabetic isolated kidneys. Furthermore, in normal kidneys, raising perfusate glucose to levels seen in moderately severe diabetes caused increased inulin clearance (C_{in}). In contrast, equal osmolar concentrations of mannitol caused sustained vasoconstriction and a slight decrease in C_{in}. Prostaglandin synthetase inhibitors reduced glucose-induced vasodilatation by 50% and prevented the increase in C_{in} that followed the addition of glucose to normal kidneys. Thus, these studies demonstrated that elevated glucose levels caused significant vasodilatation and increased C_{in} in the IPRK, and these glucose-induced hemodynamic changes were attenuated by prostaglandin synthetase inhibitors. It is possible that these glucose-induced effects may be important determinants of increased glomerular function in early diabetes. DIABETES 1985; 34:360–64.

Increased glomerular filtration rate and kidney size have been described in humans soon after the onset of insulin-dependent diabetes mellitus (IDDM).[1,2] In experimental models of diabetes, similar changes in renal function and kidney size have also been observed.[3,4] The mechanisms responsible for these renal hemodynamic changes are unknown. Diabetes is associated with alterations in levels of glucose, insulin, glucagon, and growth hormone, as well as changes in production and responsiveness of the kidney to several vasoactive agents.[5–10] These factors may influence renal hemodynamics independently or in concert with each other. Thus, it is difficult to assess the individual contribution of any of these factors to diabetic renal hemodynamic changes seen in vivo. The isolated, perfused rat kidney (IPRK) is a useful technique to evaluate the influence of plasma factors on renal function without eliciting systemic effects. The purpose of the present study was to investigate the role of elevated glucose on the function of the isolated kidney. In addition, kidneys from diabetic rats were perfused to determine whether endogenous changes contribute to altered in vivo renal function.

From the Nephrology Division, Department of Medicine, Hennepin County Medical Center, University of Minnesota, Minneapolis, Minnesota.
Address reprint requests to Bertram L. Kasiske, M.D., Regional Kidney Disease Program, 701 Park Avenue, Minneapolis, Minnesota 55415.
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MATERIALS AND METHODS

Isolated, perfused kidney technique. Kidneys were obtained using the surgical technique described by Nishiitsutsuji-Uwo.[11] Briefly, the mesenteric artery was cannulated and flow of oxygenated, albumin-free perfusate was initiated. The right renal artery was then cannulated, the kidney was rapidly removed, and perfusion with oxygenated, albumin-containing perfusate was begun using an apparatus similar to that described by Maack.[12] One-hundred milliliters of perfusate was continuously recirculated at a perfusion pressure maintained at 100 mm Hg distal to the cannula tip by varying the rate of flow. Renal artery pressure was determined from pressure measured proximal to the cannulated kidney by subtracting the pressure caused by the cannula alone at the same perfusate flow.[13] Perfusate was equilibrated with 95% O_2 and 5% CO_2, and filtered with two in-line 5-μm filters. Pressure, flow rate, temperature, and pH were continuously monitored during each experiment.

Perfusate consisted of modified Krebs-Henseleit bicarbonate buffer to which 6.1 g/dl fraction V bovine serum albumin (Armour) was added. Perfusate was dialyzed for 6 h against Krebs-Henseleit buffer equilibrated with 95% O_2 and 5% CO_2 using a large surface area, hollow-fiber dialyzer. Dialyzed perfusate was frozen at −20°C in 100-ml aliquots until use. Glucose 100 mg (5.5 mM), ^{14}C-inulin, and 1 ml of

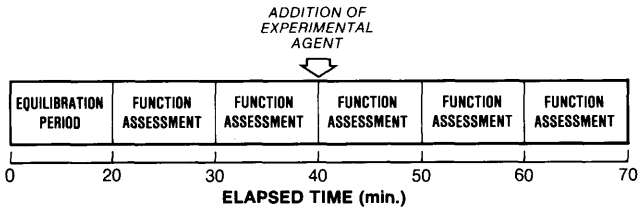

FIGURE 1. A schematic representation of the experimental protocol is depicted. Functional assessment included urine volume, urine sodium excretion ($U_{Na}V$), urine to plasma inulin concentration ratio (U/P_{in}), inulin clearance (C_{in}), and fractional reabsorption of sodium (FRNa).

a solution containing 14 amino acids (Travenol Laboratories, Inc., Deerfield, Illinois) were added to 100 ml of perfusate immediately before use. Perfusate was then filtered with a 0.22-μm filter. Final electrolyte composition (in mM) was: sodium, 138; potassium, 4.5; chloride, 113; bicarbonate, 28; phosphorous, 5.0; calcium, 1.75; and magnesium, 1.2.

Experimental design. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Willmington, Massachusetts) weighing 225–250 g were made diabetic by the intravenous administration of alloxan monohydrate (Sigma Chemical Co., St. Louis, Missouri) 50 mg/kg body wt. Renal vessels were clamped for 5 min at the time of injection to protect kidneys from alloxan damage as previously described.[3] Kidneys from diabetic rats were studied 1 mo after the induction of disease and compared with age-matched controls. Alloxan-treated rats that developed severe diabetes manifested by marked polyuria, glucosuria, and failure to gain weight were not used in this study.

Perfusion of kidneys followed the same general protocol (Figure 1). After a 20-min equilibration period, serial 10-min urine samples were collected during each perfusion and perfusate was sampled at the midpoint of each urine collection to assess the following functional characteristics: urine volume, urine sodium excretion ($U_{Na}V$), urine/plasma inulin concentration ratio (U/P_{in}), C_{in}, and fractional reabsorption of sodium (FRNa). Baseline functional characteristics were determined during the first two successive 10-min collection periods. An experimental agent (see below) was then added, and functional characteristics were again assessed during three subsequent 10-min urine collections.

In the first set of experiments, the effect of an acute elevation of perfusate glucose was evaluated in normal and diabetic kidneys. Two levels of glucose were added to achieve perfusate glucose concentrations seen in moderate and severe experimental diabetes. In one group of 6 normal and 6 diabetic kidneys, perfusion was begun with 100 ml of standard perfusate and, after determination of baseline function, 200 mg/dl of glucose dissolved in 2 ml of perfusate was added and function reassessed. The addition of 200 mg of glucose raised the perfusate glucose concentration from 95 ± 2 mg/dl to 305 ± 10 mg/dl in normals and from 102 ± 2 mg/dl to 318 ± 7 mg/dl in diabetic kidney perfusions. In another group of 6 normal and 6 diabetic kidneys, 500 mg/dl of glucose was added in the same manner as described above. The addition of 500 mg of glucose raised the perfusate glucose concentration from 101 ± 4 mg/dl to 623 ± 20 mg/dl in normals and from 96 ± 3 mg/dl to 619 ± 9 mg/dl in diabetic kidney perfusions. In both experiments, results were compared with 7 normal and 6 diabetic kidneys perfused with 100 mg/dl glucose throughout. To evaluate the functional effects of an increase in perfusate osmolality, 500 mg/dl of mannitol was added to 6 normal kidneys perfused according to the standard protocol.

Statistical analysis. All data are presented as group means ± SEM. Differences between two group means were tested for significance at $P < 0.05$ using the unpaired *t*-test. When multiple group means were compared, significance was determined by one-way analysis of variance.

TABLE 1
Characteristics of normal and diabetic kidneys

	Normal (N = 19)	Diabetic (N = 18)	Significance
C_{in}*	0.64 ± 0.05	0.51 ± 0.05	NS
Resistance†	2.86 ± 0.10	2.83 ± 0.13	NS
$U_{Na}V$ (μeq/min)	5.9 ± 0.9	21.7 ± 3.3	$P < 0.001$
FRNa (%)	94.0 ± 0.6	82.9 ± 1.9	$P < 0.001$
Body wt (g)	420 ± 15	325 ± 12	$P < 0.001$
Kidney wt (g)	1.65 ± 0.08	1.86 ± 0.07	$P < 0.05$
Kidney wt/100 g body wt	0.39 ± 0.01	0.58 ± 0.03	$P < 0.001$

*Units: ml/(min × g kidney wt).
†Units: (mm Hg × g kidney wt × min)/ml.

RESULTS

Rats made diabetic with alloxan all had glycosuria. Serum glucose at the time of surgery was 497 ± 59 mg/dl. Diabetic rats gained weight, albeit less than controls. Kidneys from diabetic rats weighed significantly more than control kidneys (Table 1). Baseline resistance and C_{in} were not significantly different between diabetic and normal kidneys. In contrast, $U_{Na}V$ from diabetic kidneys was threefold greater and FRNa was significantly less than in controls (Table 1).

To assess the role of endogenous insulin in the observed differences in $U_{Na}V$, 20 mU/ml of insulin (Eli Lilly and Company, Indianapolis, Indiana) was added to normal and diabetic kidneys after 70 min of perfusion. After the addition of insulin to normal isolated kidneys, $U_{Na}V$ decreased 28.5% (Figure 2). Diabetic kidneys not only had higher baseline

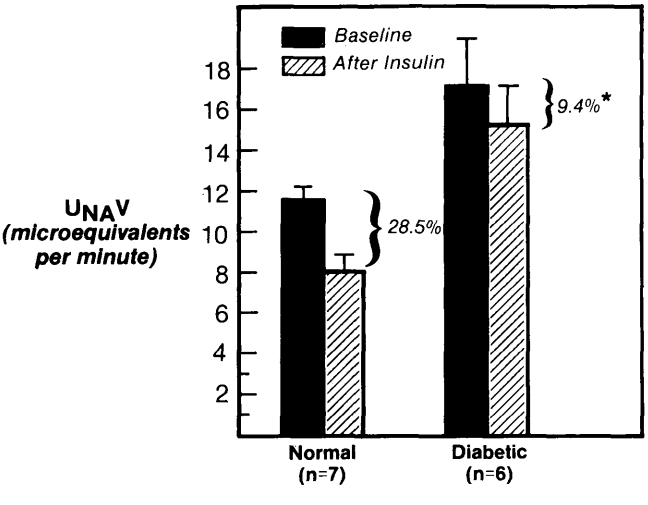

FIGURE 2. Changes in urine sodium excretion ($U_{Na}V$) after the addition of insulin to normal and diabetic isolated kidneys are shown. The decrease in $U_{Na}V$ after insulin was significantly less in diabetic kidneys (9.4%) than in normal kidneys (28.5%). *$P < 0.02$.

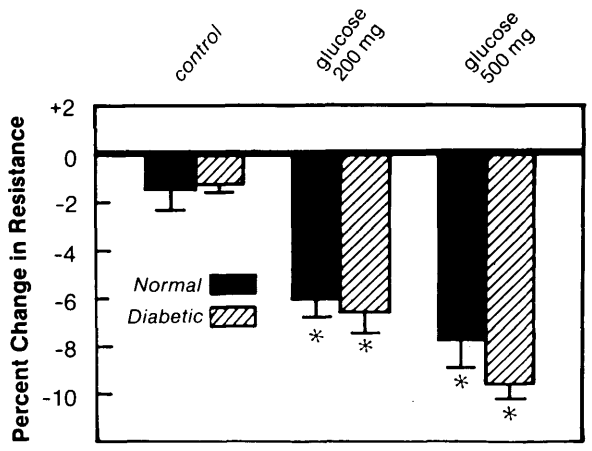

FIGURE 3. Percent change in resistance 5 min after the addition of glucose to normal and diabetic kidneys. *P < 0.001 versus control.

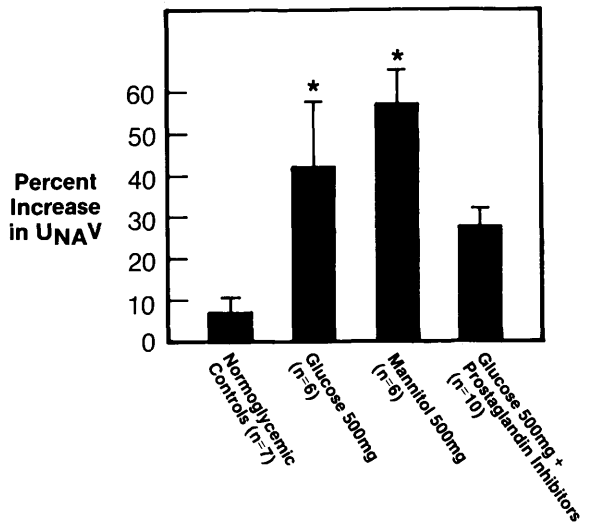

FIGURE 5. Percent change in urine sodium excretion ($U_{Na}V$) after the addition of an experimental agent to normal kidneys. *P < 0.01 versus normoglycemic controls.

$U_{Na}V$ before the addition of insulin, but insulin also failed to decrease $U_{Na}V$ to levels seen in normal kidneys. Indeed, the percent decline (9.4%) in $U_{Na}V$ caused by insulin was significantly less in diabetic kidneys.

Glucose added to both normal and diabetic kidneys caused immediate vasodilatation, which was sustained (Figure 3). These resistance changes were dose-related (Figure 3). Glucose-induced vasodilatation was accompanied by an acute increase in C_{In} in normal and diabetic kidneys (Figure 4). This increase in C_{In}, however, reached statistical significance only when 500 mg of glucose was added to normal kidneys.

The vasodilatation and increased C_{In} after the addition of glucose to isolated kidneys were accompanied by increased $U_{Na}V$ (Figure 5). The 42% (2.5 μeq/min) increase in $U_{Na}V$ after glucose was significantly greater than the 7% (0.7 μeq/min) increment seen in normoglycemic controls (Figure 5). Similar changes in sodium excretion were seen after the addition of glucose to diabetic kidneys.

In contrast to glucose, the addition of 500 mg of mannitol to normal kidneys caused transient vasodilatation of 1–2-min duration followed by sustained vasoconstriction (Figure 4).

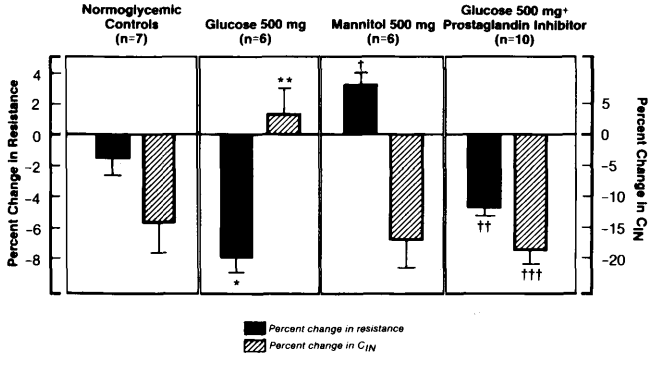

FIGURE 4. Percent change in resistance 5 min after the addition of an experimental agent is shown in solid bars. Percent change in inulin clearance (C_{In}) at 10 min is shown in hatched bars. *P < 0.001 versus normoglycemic controls and mannitol, 500 mg; **P < 0.01 versus normoglycemic controls and mannitol, 500 mg; †P < 0.001 versus normoglycemic controls; ††P < 0.01 versus normoglycemic controls and glucose, 500 mg; and †††P < 0.01 versus glucose, 500 mg.

This increased vascular resistance was accompanied by a slight decline in C_{In} and contrasted with the significant increase in C_{In} seen after the addition of glucose to normal kidneys (Figure 4). The addition of mannitol was also associated with a 57% (5.7 μeq/min) increase in $U_{Na}V$, an increment not significantly different from that seen after glucose (Figure 5).

Glucose has been shown to increase the production of vasodilator prostanoids in vascular tissue.[14] To evaluate whether the observed glucose-induced functional changes were mediated by these vasoactive substances, 500 mg glucose was added to six normal kidneys perfused with indomethacin (6 mM) and four normal kidneys pefused with meclofenamate (6 mM). Since results with meclofenamate and indomethacin were not significantly different, these data were combined for purposes of analysis. Except for $U_{Na}V$, which was significantly increased in kidneys perfused with prostaglandin synthetase inhibitors, baseline functional characteristics were similar (Table 2).

Glucose-induced functional changes were significantly reduced in the presence of prostaglandin synthetase inhibitors (Figure 4). Specifically, glucose-induced vasodilatation was reduced by 50%. Furthermore, the increment in C_{In} after the addition of glucose was not observed in the presence of indomethacin or meclofenamate. With prostaglandin synthetase inhibition, the 27% (2.7 μeq/min) increment in $U_{Na}V$ after glucose addition failed to reach statistical significance com-

TABLE 2
Baseline function of normal kidneys perfused with prostaglandin synthetase inhibitors (N = 10)

C_{In}*	0.63 ± 0.05
Resistance†	2.56 ± 0.08
$U_{Na}V$ (μeq/min)	11.8 ± 2.9‡
FRNa (%)	92.1 ± 1.4

*Units: ml/(min × g kidney wt).
†Units: (mm Hg × g kidney wt × min)/ml.
‡P < 0.05 versus controls (N = 19).

pared with normoglycemic controls ($0.05 < P < 0.1$, Figure 5).

DISCUSSION

Increased glomerular filtration precedes significant glomerular injury in human type I diabetes.[1,2] In experimental models, increased renal blood flow and glomerular filtration rate are followed by glomerular damage that resembles that seen in human diabetes.[3,4] The factors that may be responsible for changes in renal hemodynamic function early in the course of diabetic nephropathy are unknown. Complex interactions between glucose, insulin, glucagon, growth hormone, catecholamines, and other vasoactive agents make it virtually impossible to determine in vivo which factors may cause these renal hemodynamic changes. In this regard, we used the IPRK to study whether high levels of glucose per se influence renal function.

Baseline hemodynamic data in normal and diabetic kidneys perfused with normal levels of glucose demonstrated similar C_{In} and vascular resistances. Elevations of glucose levels comparable to those seen in diabetes caused vasodilatation and increased C_{In} in both normal and diabetic isolated, perfused kidneys. In contrast to these vasodilatory responses, mannitol in equal osmotic concentrations resulted in significant vasoconstriction and an insignificant decline in C_{In}.

It has been shown that prostaglandin metabolism is altered in both glomeruli and other vascular structures in diabetes.[15,16] Furthermore, high levels of glucose may stimulate the production of vasodilatory prostaglandins in vascular tissues.[14] In this regard, our studies demonstrated that prostaglandin synthesis inhibition by meclofenamate and indomethacin significantly reduced glucose-induced vasodilation in normal kidneys. These latter data suggested the possibility that prostaglandins are involved in the glucose-induced hemodynamic changes. However, both meclofenamate and indomethacin have effects that are not limited to prostaglandin synthetase inhibition alone, and it is possible that other mechanisms may be involved.

In addition to these glucose-induced hemodynamic changes, increases were also observed in $U_{Na}V$. Diabetic kidneys exhibited a threefold higher baseline $U_{Na}V$ than did normal kidneys. The mechanisms of increased $U_{Na}V$ in isolated, perfused diabetic rat kidneys are unknown. It has been demonstrated that kidneys in normal rats may have significant amounts of receptor-bound insulin at the time of isolated perfusion, even when no exogenous insulin is added to the perfusate.[17] It is possible that the greater $U_{Na}V$ in diabetic compared with normal isolated kidneys may reflect differences in receptor-bound endogenous insulin present at the initiation of perfusion. However, in our experiments, the addition of large amounts of insulin to diabetic isolated, perfused kidneys failed to correct this natriuresis (Figure 2). Moreover, diabetic kidneys were significantly less sensitive than normal kidneys to the antinatriuretic effect of insulin. Thus, a difference in endogenous insulin levels appeared to be an unlikely explanation for the increased sodium excretion seen in isolated, perfused diabetic kidneys. It is also unlikely that the increased sodium excretion is a direct result of tubular damage caused by alloxan.[18] Renal artery clamping has been demonstrated to be effective in protecting kidneys from the renal toxicity of alloxan.[18] It is more probable that the diabetic milieu produced alterations in the kidney itself that ultimately resulted in augmented sodium excretion during normoglycemic, isolated perfusion.

It has been previously demonstrated, in studies with the isolated, perfused dog kidney, that increased $U_{Na}V$ and increased delivery of NaCl to distal tubules is associated with activation of tubuloglomerular feedback mechanisms.[19] We also found that the addition of mannitol to normal isolated rat kidneys in the present experiments led to increased $U_{Na}V$ and sustained vasoconstriction. Thus, it appeared that tubuloglomerular feedback mechanisms are operative during a mannitol diuresis. In contrast, our results demonstrated that the expected increase in vascular resistance associated with increased $U_{Na}V$ did not occur after elevations of perfusate glucose levels in either normal or diabetic kidneys. Since the principal effect of tubuloglomerular feedback is to increase resistance and decrease glomerular filtration in situations of increased sodium chloride delivery to the distal nephron, glucose inhibition of tubuloglomerular feedback could have a permissive role in the observed vasodilation and increased C_{In}. Acute hyperglycemia in vivo has also been shown to increase $U_{Na}V$ without inducing vasoconstriction.[20] The mechanisms responsible for this altered vascular response in hyperglycemia are unknown. It is possible that glucose directly inhibited tubuloglomerular feedback mechanisms or altered the sensitivity of the tubuloglomerular feedback loop to increased $U_{Na}V$.[21]

In conclusion, these studies demonstrated that glucose caused significant vasodilatation in the IPRK. This vasodilatation appeared, in part, modulated by glucose-induced effects on prostaglandin synthesis. Moreover, the results are compatible with glucose inhibition of tubuloglomerular feedback mechanisms that may have a permissive role in glucose-induced vasodilatation. It is possible that these effects of glucose may be important in the increased glomerular function in diabetes mellitus.

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REFERENCES

[1] Mogensen, C. E., and Andersen, M. J. F.: Increased kidney size and glomerular filtration rate in early juvenile diabetes. Diabetes 1973; 22:706–12.

[2] Ditzel, J., and Junker, K.: Abnormal glomerular filtration rate, renal plasma flow, and renal protein excretion in recent and short-term diabetics. Br. Med. J. 1972; 2:13–19.

[3] Michels, L. D., Davidman, M., and Keane, W. F.: Determinants of glomerular filtration and plasma flow in experimental diabetic rats. J. Lab. Clin. Med. 1981; 98:869–85.

[4] Hostetter, T. H., Troy, J. L., and Brenner, B. M.: Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int. 1981; 19:410–15.

[5] Viberti, G. C., Strakosch, C. R., Keen, H., Mackintosh, D., Dalton, N., and Home, P. D.: The influence of glucose-induced hyperinsulinaemia on renal glomerular function and circulating catecholamines in normal man. Diabetologia 1981; 21:436–39.

[6] Mogensen, C. E., Christensen, N. J., and Gundersen, H. J. G.: The acute effect of insulin on heart rate, blood pressure, plasma noradrenaline and urinary albumin excretion. The role of changes in blood glucose. Diabetologia 1980; 18:453–57.

[7] Parving, H. H., Christiansen, J. S., Noer, I., Tronier, B., and Mogensen, C. E.: The effect of glucagon infusion on kidney function in short-term insulin-dependent juvenile diabetes. Diabetologia 1980; 19:350–54.

[8] Christlieb, A. R.: Renin, angiotensin, and norepinephrine in alloxan diabetes. Diabetes 1974; 23:962–70.

[9] Ylikorkala, O., Kaila, J., and Vinnikka, L.: Prostacyclin and thromboxane in diabetes. Br. Med. J. 1981; 283:1148–50.

[10] Christensen, N. J.: Plasma norepinephrine and epinephrine in untreated diabetics, during fasting and after insulin administration. Diabetes 1974; 23:1–8.

[11] Nishiitsutsuji-Uwo, J. M., Ross, B. D., and Krebs, H. A.: Metabolic activities of the isolated, perfused rat kidney. Biochem. J. 1967; 103:852–62.

[12] De Mello, G., and Maack, T.: Nephron function of the isolated, perfused rat kidney. Am. J. Physiol. 1976; 231:1699–707.

[13] Loutzenhiser, R., Horton, C., and Epstein, M.: Flow-induced errors in estimating perfusion pressure of the isolated rat kidney. Kidney Int. 1982; 22:693–96.

[14] Jeremy, J. Y., Mikhailidis, D. P., and Dandona, P.: Simulating the diabetic environment modifies in vitro prostacyclin synthesis. Diabetes 1983; 32:217–21.

[15] Rogers, S. P., and Larkins, R. G.: Production of 6-oxo-prostaglandin F_1 and prostaglandin E_2 by isolated glomeruli from normal and diabetic rats. Br. Med. J. 1982; 284:1215–17.

[16] Rosenblum, W. I., and Hirsh, P. D.: Some interrelationships between glucose levels thromboxane production and prostacyclin production in normal and diabetic mice. Prostaglandins 1984; 27:111–18.

[17] Rostand, S. G., Watkins, J. B., and Clements, R. S.: The effect of insulin and of anti-insulin serum on handling of sodium by the isolated perfused kidney of the streptozotocin-diabetic rat. Diabetes 1980; 29:679–85.

[18] Evan, A. P., Mong, S. A., Connors, B. A., Aronoff, G. R., and Luft, F. C.: The effect of alloxan, and alloxan-induced diabetes on the kidney. Anat. Rec. 1984; 208:33–47.

[19] Nizet, A.: Connection between the changes in tubular reabsorption and in glomerular filtration rate induced by replacement of plasma sodium and chloride by isotonic mannitol. Arch. Int. Physiol. Biochim. 1981; 89:35–40.

[20] Blantz, R. C., Tucker, B. J., Gushwa, L., and Peterson, O. W.: Mechanisms of diuresis following acute modest hyperglycemia in the rat. Am. J. Physiol. 1983; 244:F185–94.

[21] Blantz, R. C., Peterson, O. W., Gushwa, L., and Tucker, B. J.: Effect of modest hyperglycemia on tubuloglomerular feedback activity. Kidney Int. 1982; 22:S206–12.