

Renal Perfusion and the Renal Hemodynamic Response to Blocking the Renin System in Diabetes

Are the Forces Leading to Vasodilation and Vasoconstriction Linked?

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In three groups of subjects, those with type 2 diabetes and nephropathy, those with type 1 diabetes without nephropathy, and healthy volunteers subjected to short-term hyperglycemia, we observed a counterintuitive relationship. In all three groups, baseline renal plasma flow (RPF) was positively correlated with the RPF response to blocking the renin-angiotensin system (RAS). This seems paradoxical in that an opposite result would have been expected if angiotensin-dependent renal vasoconstriction was responsible for the renal vasodilator response to RAS blockade. This suggests a link between the renal vasodilator response, mediated by nitric oxide (NO), and the activation of the intrarenal RAS. The complex interrelationships between hyperglycemia, insulin, NO, and the RAS may result in phenotypes that indicate varying risk of diabetic nephropathy and underlying genetic polymorphisms. *Diabetes* 51:2025–2028, 2002

Some years ago, we reported an observation that we now know was misinterpreted (1). Somewhat to our surprise, we found that baseline renal plasma flow (RPF) and glomerular filtration rate were highly and positively correlated with the renal hemodynamic response to blocking the renin-angiotensin system (RAS) with irbesartan in patients with type 2 diabetes and nephropathy (Fig. 1). The relationship was counterintuitive in that an opposite relationship would have been anticipated if angiotensin-dependent renal vasoconstriction was responsible for the renal vasodilator response to the angiotensin antagonist. According to this simple model, increased angiotensin-dependent renal vasoconstriction would both lower baseline RPF and enhance the

response to the blocker. We attributed the opposite relationship, i.e., a larger renal vasodilator response to the angiotensin antagonist in the patient with the highest baseline RPF, to an influence of the stage of the nephropathy process. According to this interpretation, high basal RPF, a widely recognized stage in diabetes thought to have pathogenetic consequences, occurred early in the process; our findings were shown to indicate that angiotensin II played a more substantial early role.

More recently, we identified a similar relationship in patients with type 1 diabetes who received both an angiotensin II antagonist, candesartan, and an ACE inhibitor, captopril, as part of a protocol designed to ascertain whether renal vascular response to ACE inhibition primarily reflected a reduction in angiotensin II formation, which it did (2). Based on the response to captopril, we divided the patients into a normal response group, in which the renal vasodilator response to captopril was equal to that in the normal subjects, and an accentuated response group, in which the renal vasodilator response to captopril and candesartan was enhanced (Fig. 2).

To our surprise, the only feature that distinguished the two groups was baseline RPF. RPF, once again, was elevated in those patients with enhanced renal vasodilator response to the two blockers. Unlike the study in type 2 diabetes that involved only patients with nephropathy, the patients with type 1 diabetes were free of nephropathy. Thus, it was difficult to attribute the influence of baseline renal hemodynamic state to the stage of the process, as we had previously done. With baseline RPF as the independent variable, the relationship between baseline renal perfusion and the response to blockers in the patients with type 1 diabetes who were free of nephropathy was in striking accord to that found in the patients with type 2 diabetes and nephropathy (Fig. 3).

Even more compelling information on the relationships has emerged from studies on the influence of hyperglycemia on renal hemodynamics in normal humans. We reported that short-term hyperglycemia in healthy subjects leads, within a few hours, to both renal vasodilatation and an enhanced renal vasodilator response to captopril (3). Despite a consistent and narrow blood glucose level of 150–170 mg/dl, a wide range of renal vasodilator responses occurred with hypergly-

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Received for publication 2 October 2001 and accepted in revised form 11 February 2002.

N.K.H. has received support from Astra-Zeneca and Bristol-Meyers Squibb. eNOS, endothelial nitric oxide synthase; L-NAME, *N*^G-nitro-L-arginine methyl ester; L-NMMA, *N*^G-monomethyl-L-arginine; NO, nitric oxide; PRA, plasma renin activity; RAS, renin-angiotensin system; RPF, renal plasma flow.

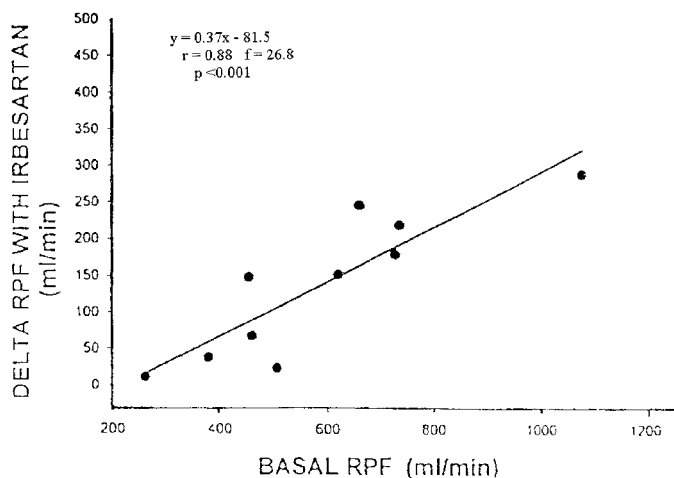


FIG. 1. Relation between basal RPF and change in RPF to 37.5 mg irbesartan in type 2 diabetic patients. (Reprinted with permission. Price DA et al. The paradox of the low-renin state in diabetic neuropathy. *J Am Soc Nephrol* 10:2382-2392, 1999)

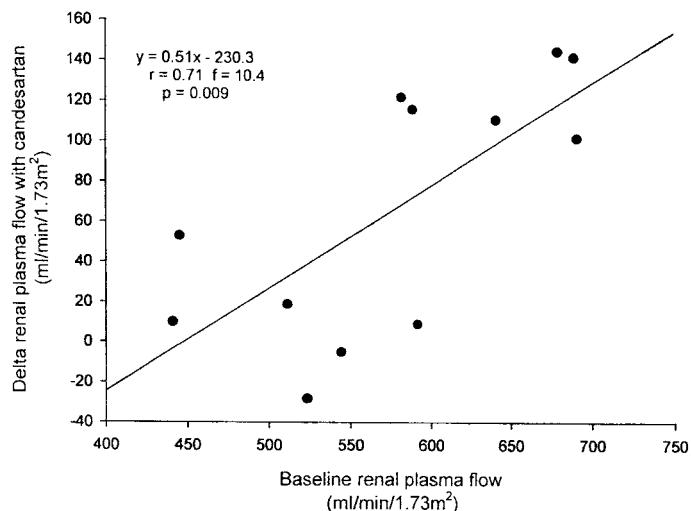


FIG. 3. Relation between basal RPF and response to 16 mg candesartan in type 1 diabetic patients without nephropathy.

emia. Prompted by observations in type 1 diabetic patients, we returned to the studies on the renal response to glucose infusion in normal volunteers and found that the same relationships existed. Baseline renal perfusion at the end of glucose infusion was a major determinant of the renal vasodilator response to captopril (Fig. 4). In this case, clearly, there is no issue of stage of process.

The identification of an essentially identical, counterintuitive relationship under three circumstances—the patient with type 2 diabetes and nephropathy, the patient with type 1 diabetes free of nephropathy, and healthy volunteers under the influence of short-term hyperglycemia—suggests that the vasodilator response and activation of the intrarenal RAS, which led to the enhanced renal vasodilator response to blockers, are linked. The remainder of this speculative report focuses on the answer to these questions. What is known about mechanisms re-

sponsible for the renal vasodilator response to hyperglycemia? What is known about the state of the RAS when that vasodilator pathway is activated? Are these systems linked? What are the pathogenetic consequences of joint activation of these systems?

There are a few endogenous vasodilators that engage the kidney, including nitric oxide (NO), prostaglandins, and bradykinin. Among these, the NO synthase pathway is especially attractive as a candidate, given growing evidence for an interaction between the NO synthase and the RAS (4-23). These interactions include a role for NO in the activation of the intrarenal RAS (4-12). Moreover, there is unambiguous evidence that plasma insulin concentration is a determinant of vascular NO synthase activation, an obvious relevant factor in studies involving either diabetes or the normal human renal response to glucose infusion (24-26).

Effects of insulin and glucose on NO. Insulin has long been recognized to have vasodilator properties. More recently, studies have provided evidence for insulin-dependent activation of the NO synthase pathway (24-26). Incubation of human vascular smooth muscle cells with

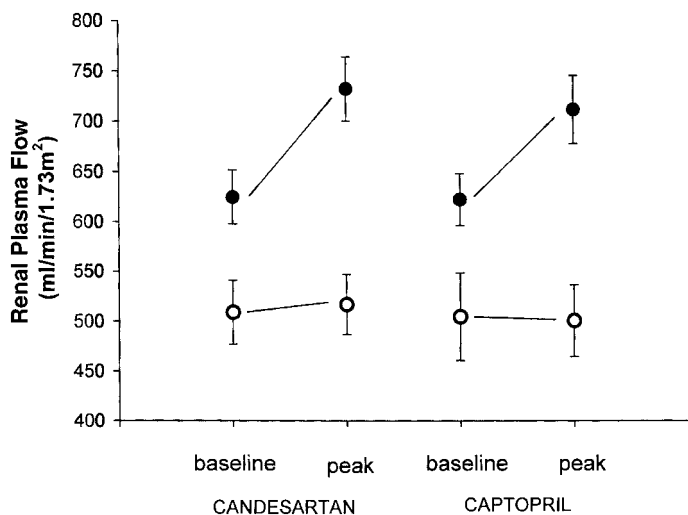


FIG. 2. Baseline and peak RPF response to candesartan and captopril in patients with type 1 diabetes subdivided according to whether the renal response to captopril was in the normal range or accentuated. ○, normal response group; ●, accentuated response group. (Reprinted with permission. Lansang et al. Renal vascular responses to captopril and to candesartan in patients with the 1 diabetes mellitus. *Kidney Int* 59:1432-1438, 2001. Blackwell Science)

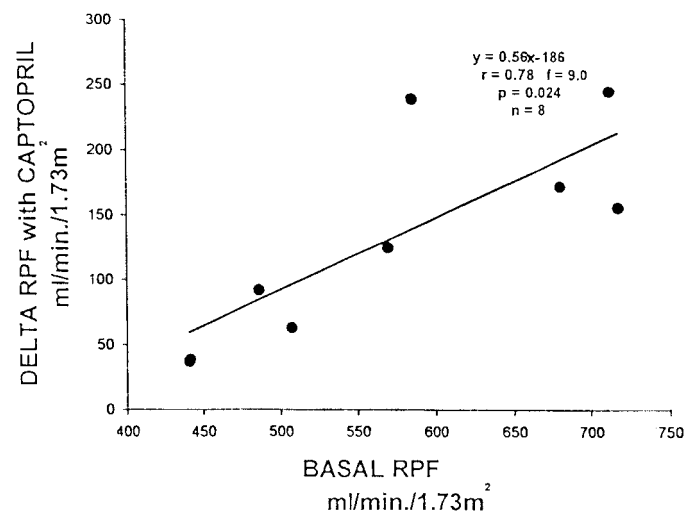


FIG. 4. Relation between basal RPF and the response to 25 mg captopril in healthy volunteers subjected to short-term hyperglycemia.

insulin, for example, caused an increase in cGMP that was blocked by an NO synthase inhibitor. Incubation with insulin also increased NO levels, as measured by L-(3H)-citrulline production from L-(3H)-arginine (24).

The effect of glucose per se on these pathways is even more complex. In studies performed in vitro, free of the complicating effects of a reactive insulin response, the high glucose concentration led to increased endothelial NO synthase (eNOS) messenger RNA and protein expression (27). That effect of an increase in glucose concentration in this system, however, seems insufficient in vivo to counteract the concomitant increased production of superoxide anion, which reacts rapidly with NO to produce toxic products and reduce the effect or concentrations of NO. Thus, despite increased NO synthesis, there is impaired vascular smooth muscle relaxation.

Activation of the RAS and NO system by hyperglycemia. There is ample evidence that hyperglycemia can induce both RAS and NO activation. The former can be manifested in two ways. Hyperglycemia can increase plasma renin activity (PRA) in 8–12 h, as demonstrated in type 1 diabetic patients (28). Another manifestation of hyperglycemia-mediated RAS activation is an enhanced RPF response to ACE inhibition or angiotensin II blockade. In normal individuals, the RPF responses to captopril and eprosartan were significantly greater during hyperglycemia than during euglycemia in spite of similar baseline PRA levels under both conditions, suggesting activation of the intrarenal RAS not reflected in plasma levels (3). In both type 1 and type 2 diabetic patients, the RPF response to either ACE inhibition or angiotensin II blockade was larger than the RPF response in normal subjects, possibly reflecting glucose-induced RAS activation (1,2,29,30).

Hyperglycemia and the associated rise in insulin levels can activate the NO system. In several studies, the increase in limb perfusion caused by glucose was reversed by N^G -monomethyl-L-arginine (L-NMMA), an L-arginine analog that competitively inhibits NO synthesis (31,32). During euglycemic-hyperglycemic clamp, brachial artery blood flow increased in healthy volunteers, but blood flow remained unchanged when L-NMMA was given before the insulin-glucose infusion (31). In another study of healthy volunteers, a greater decline in leg blood flow in response to intrafemoral artery infusion of L-NMMA was seen during a euglycemic-hyperinsulinemic clamp than during infusion of normal saline (32).

Activation of the RAS by NO. Numerous studies support the notion that NO activates the RAS. In the isolated rat kidney, for example, N^G -nitro-L-arginine methyl ester (L-NAME), another NO synthesis inhibitor, inhibited renin release independent of a rise in perfusion pressure (4). In ramipril- or losartan-treated rats, L-NAME abolished the increase in renal renin mRNA, total renin content, renin secretion, or PRA brought about by the ACE inhibitor or the angiotensin II antagonist (5,6). L-NAME was also found to block the rise in renin in rats treated with furosemide or with the adenylyl cyclase activator forskolin (7,8). In rats and dogs and isolated mouse renal juxtaglomerular cells, NO synthesis inhibition by L-NAME or N^G -nitro-L-arginine attenuated the renin release or renin mRNA induced by lowering renal artery pressure (9–12).

Caveats. There are other relationships that may modify

the pathways described above, such as glucose impairing NO-mediated vasodilation. As such, it would seem to have two mechanisms for promoting vasoconstriction, RAS activation, and NO pathway disruption. Hyperglycemia can disrupt NO-mediated relaxation, whereby NO is inactivated by superoxide anion or free radicals in the presence of high glucose levels or by a direct NO-scavenging effect of glucose (27,33–36). Despite this interaction, baseline renal vasodilation is common in diabetes. It is possible that the net balance of these forces is dominated by the attenuating action of NO on the renal hemodynamic effects of angiotensin II. Blockers of NO action enhance the renal vasoconstricting action of angiotensin II (13–15).

To further complicate these relationships, angiotensin II increases NO levels, which can in turn mitigate its vasoconstrictive tone. Both AT₁ and AT₂ receptors seem to be involved (16–19), and the degree of NO response to angiotensin II may depend on the kidney site (20), the NOS isoform involved (18,21), and the duration of angiotensin II stimulation (22,23).

The influence of time would have to be considered. In animal models and in vitro studies, exposure to high glucose levels for a few minutes to 72 h resulted in blunting of NO action (34–36). More prolonged exposure to hyperglycemia (5 days) resulted in increased eNOS gene and protein expression and NO release, but still with a concomitant rise in superoxide anion (27). It is therefore difficult to make conclusions regarding time course. Even with the seeming predominance of NO inhibition during acute exposure to hyperglycemia, this could not be easily translated to human physiology because of the disparate levels of glycemia achieved. Glucose levels attained in these in vitro and animal experiments were from 22 to 44 mmol/l, more than twice the blood glucose levels maintained in the human studies (1–3,27,34–36). This marked difference in glycemia may again affect what force predominates—vasoconstriction in the markedly hyperglycemic animals and tissue cultures or vasodilation in the mildly hyperglycemic humans.

Could it then be the baseline glycemic level that dictates the RBF response? In our aforementioned study on type 1 diabetic patients, neither HbA_{1c} nor fasting blood glucose levels were correlated with the change in RBF (2). On the other hand, in another study of type 1 diabetes, renal hemodynamic response was greater in those with higher HbA_{1c} levels; however, HbA_{1c} was also positively correlated with baseline RBF (37). It is therefore difficult to ascribe the findings to glycemia without exploring this aspect further.

Interaction with gene polymorphisms. It is reasonable to speculate on the genetic interplay that underlie these relationships. The D-allele of the ACE polymorphism and the T-allele of the AGT gene M235T polymorphism confer an increased risk of diabetic nephropathy (38–42). The A14 allele in the NOS2 gene promoter is associated with a low risk of nephropathy (43). It is possible that a combination of the different ACE, AGT, and NOS alleles dictate the baseline renal hemodynamics, the response to RAS blockade in diabetic patients, and the risk of nephropathy.

CONCLUSIONS

In an era where most scientific endeavors gravitate toward the molecular end of the spectrum, it is essential to

recognize the contribution of physiologic observations to the discovery of new mechanistic and genetic pathways. The observation of a paradoxically large renal hemodynamic response to RAS blockade in subjects with high baseline RPF has led to this report. It is possible that baseline RPF and RPF response to RAS blockade is a phenotype not only for risk of nephropathy but also for some underlying gene polymorphisms. These relationships are amenable to direct investigation.

ACKNOWLEDGMENTS

We would like to acknowledge the assistance of Diana Page-Capone in the preparation of this manuscript.

REFERENCES

- Price DA, Porter LE, Gordon M, Fisher ND, De'Oliveira JM, Laffel LM, Passan DR, Williams GH, Hollenberg NK: The paradox of the low-renin state in diabetic nephropathy. *J Am Soc Nephrol* 10:2382-2391, 1999
- Lansang MC, Price DA, Fisher ND, Laffel LMB, Osei SY, Fisher ND, Hollenberg NK: Renal vascular responses to captopril and to candesartan in patients with type 1 diabetes mellitus: angiotensin-mediated renal vascular tone. *Kidney Int* 59:1432-1438, 2001
- Osei SY, Price DA, Fisher ND, Porter LE, Laffel LMB, Hollenberg NK: Hyperglycemia and angiotensin-mediated control of the renal circulation in healthy humans. *Hypertension* 3:559-564, 1999
- Gardes J, Poux JM, Gonzalez MF, Alhenc-Gelas F, Menard J: Decreased renin release and constant kallikrein secretion after injection of L-NAME in isolated perfused rat kidney. *Life Sci* 50:987-993, 1992
- Tharoux PL, Dussaule JC, Pauti MD, Vassitch Y, Ardaillou R, Chatziantoniou C: Activation of renin synthesis is dependent on intact nitric oxide production. *Kidney Int* 51:1780-1787, 1997
- Schricker K, Hegyi I, Hamann M, Kaissling B, Kurtz A: Tonic stimulation of renin gene expression by nitric oxide is counteracted by tonic inhibition through angiotensin II. *Proc Natl Acad Sci U S A* 92:8006-8009, 1995
- Schricker K, Kurtz A: Liberators of NO exert a dual effect on renin secretion from isolated mouse renal juxtaglomerular cells. *Am J Physiol* 265:F180-F186, 1993
- Chatziantoniou C, Pauti MD, Pinet F, Promeneur D, Dussaule JC, Ardaillou R: Renin release is impaired after nitric oxide inhibition. *Kidney Int* 49:626-633, 1996
- Persson PB, Baumann JE, Ehmke H, Hackenthal E, Kirkheim HR, Nafz B: Endothelium-derived NO stimulates pressure-dependent renin release in conscious dogs. *Am J Physiol* 264:F943-F947, 1993
- Scholz H, Kurtz A: Involvement of endothelium-derived relaxing factor in the pressure control of renin secretion from isolated perfused kidney. *J Clin Invest* 91:1088-1094, 1993
- Naess PA, Christensen F, Krikeboenn A, Kill F: Effect on renin release of inhibiting renal nitric oxide synthesis in anesthetized dogs. *Acta Physiol Scand* 148:137-142, 1993
- Schricker K, Della Bruna R, Hamann M, Kurtz A: Endothelium-derived relaxing factor is involved in the pressure control of renin gene expression in the kidney. *Pflugers Arch* 428:261-268, 1994
- Baylis C, Harvey J, Engels K: Acute nitric oxide blockade amplifies the renal vasoconstrictor actions of angiotensin II. *J Am Soc Nephrol* 5:211-214, 1994
- Alberola AM, Salazar FJ, Nakamura T, Granger JP: Interactions between angiotensin II and nitric oxide in control of renal hemodynamics in conscious dogs. *Am J Physiol* 267:R1472-R1478, 1994
- Llinas MT, Gonzalez JD, Salazar FJ: Interactions between angiotensin and nitric oxide in the renal response to volume expansion. *Am J Physiol* 269:R504-R510, 1995
- Thorup C, Kornfeld M, Winaver JM, Goligorsky MS, Moore LC: Angiotensin II stimulates nitric oxide release in isolated perfused renal resistance arteries. *Pflugers Arch* 435:432-434, 1998
- Thorup C, Kornfeld M, Winaver JM, Goligorsky MS, Moore LC: AT₁ receptor inhibition blunts angiotensin II-stimulated nitric oxide release in renal arteries. *J Am Soc Nephrol* 10 (Suppl. 2):220-224, 1999
- Siragy HM, Carey RM: The subtype 2 (AT sub 2) angiotensin receptor mediates renal production of nitric oxide in conscious rats. *J Clin Invest* 100:264-269, 1997
- Zhang C, Mayeux PR: Angiotensin II signaling activates the NO-cGMP pathway in rat proximal tubules. *Life Sci* 63:L75-L80, 1998
- Fernandez-Alfonso MS, Gonzalez C: Nitric oxide and the renin-angiotensin system. Is there a physiological interplay between the systems? *J Hypertens* 17:1355-1361, 1999
- Murakami K, Tsuchiya K, Naruse M, Naruse K, Demura H, Arai J, Nihei H: Nitric oxide synthase I immunoreactivity in the macula densa of the kidney is angiotensin II dependent. *Kidney Int Suppl* 63:208-210, 1997
- Deng X, Welch WJ, Wilcox CS: Role of nitric oxide in short-term and prolonged effects of angiotensin II on renal hemodynamics. *Hypertension* 27:1173-1179, 1996
- Hennington BS, Zhang H, Miller MT, Granger JP, Reckelhoff JF: Angiotensin II stimulates synthesis of endothelial nitric oxide synthase. *Hypertension* 31:283-288, 1998
- Trovati M, Massucco P, Mattiello L, Costamagna C, Aldieri E, Cavalot F, Anfossi G, Bossia A, Ghigo D: Human vascular smooth muscle cells express a constitutive nitric oxide synthase that insulin rapidly activates, thus increasing guanosine 3'5'-cyclic monophosphate and adenosine 3'5'-cyclic monophosphate concentrations. *Diabetologia* 42:831-839, 1999
- Zeng G, Quon M: Insulin-stimulated production of nitric oxide is inhibited by wortmannin: direct measurement in vascular endothelial cells. *J Clin Invest* 98:894-898, 1996
- Zavaroni I, Platti PM, Monti LD, Gasparini P, Barilli LA, Massironi P, Ardigo D, Valsecchi G, Delsignore R, Reaven GM: Plasma nitric oxide concentrations are elevated in insulin-resistant healthy subjects. *Metabolism* 49:959-961, 2000
- Consentino F, Hishikawa K, Katusic ZS, Luscher T: High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 96:25-28, 1997
- Miller JA, Floras JS, Zinman B, Skorecki KL: Effect of hyperglycaemia on arterial pressure, plasma renin activity and renal function in early diabetes. *Clin Sci (Lond)* 90:189-195, 1996
- Osei SY, Price DA, Laffel LMB, Lansang MC, Hollenberg NK: Effect of the angiotensin II antagonist, eprosartan, on hyperglycemia-induced activation of intrarenal renin-angiotensin system in healthy humans. *Hypertension* 36:122-126, 2000
- De'Oliveira JM, Price DA, Fisher ND, Allan DR, McKnight JA, Williams GH, Hollenberg NK: Autonomy of the renin system in type II diabetes mellitus: dietary sodium and renal hemodynamic responses to ACE inhibition. *Kidney Int* 52:771-777, 1997
- Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511-2515, 1994
- Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest* 94:1172-1179, 1994
- McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR: Impaired endothelium-dependent and -independent vasodilation in patients with type 2 diabetes mellitus. *Diabetologia* 35:771-776, 1992
- Pieper GM, Meier DA, Hager SR: Endothelial dysfunction in a model of hyperglycemia and hyperinsulinemia. *Am J Physiol* 269:H845-H850, 1995
- Tesfamariam B, Cohen RA: Free radicals mediate endothelial cell dysfunction caused by elevated glucose. *Am J Physiol* 263:H321-H326, 1992
- Brodsky SV, Morrishow AM, Dharia N, Gross SS, Goligorsky MS: Glucose scavenging of nitric oxide. *Am J Physiol Renal Physiol* 280:F480-F486, 2001
- Laffel L, Price D, Hollenberg N: Glycemic control and plasma renin activity predict renal hemodynamic response to captopril in normotensive, normoalbuminuric patients with IDDM (Abstract). *J Am Soc Nephrol* 9:117A, 1998
- Vleming LJ, van der Pijl JW, Lemkes HH, Westendorp RG, Maassen JA, Daha MR, van Es LA, van Kooten C: The DD genotype of the ACE gene polymorphism is associated with progression of diabetic nephropathy to end-stage renal failure in IDDM. *Clin Nephrol* 51:133-140, 1999
- Schmidt S, Strojek K, Grzeszczak W, Bergis K, Ritz E, Excess of DD: homozygotes in haemodialysed patients with type II diabetes: the Diabetes Nephropathy Study Group. *Nephrol Dial Transplant* 12:427-429, 1997
- Jeffers BW, Estacio RO, Reynolds MV, Schrier RW: Angiotensin-converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus and its relationship with diabetic nephropathy. *Kidney Int* 52:473-477, 1997
- Rogus JJ, Moczulski D, Freire MB, Yang Y, Warram JH, Krolewski AS: Diabetic nephropathy is associated with AGT polymorphism T235: results of a family-based study. *Hypertension* 31:627-631, 1998
- Fogarty DG, Harron JC, Hughes AE, Nevin NC, Doherty CC, Maxwell AP: A molecular variant of angiotensinogen is associated with diabetic nephropathy in IDDM. *Diabetes* 45:1204-1208, 1996
- Johannessen J, Tarnow L, Parving H, Nerup J, Pocot F: CCTTT-repeat polymorphism in the human NOS2-promoter confers low-risk of diabetic nephropathy in type 1 diabetic patients. *Diabetes Care* 23:560-562, 2000