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NEPHROLOGY FORUM

Mechanisms of diabetic hyperfiltration

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Case presentation

A 17-year-old Hispanic male was referred to the Adult Renal Clinic of Montefiore Medical Center because of progressive proteinuria and hypertension. The patient developed insulin-dependent diabetes mellitus (IDDM) at the age of 2 years while living in Puerto Rico. He had been a patient at several other health facilities in the Bronx and was referred at age 13 to the Pediatric Diabetes Service at Montefiore Medical Center. Prior to that time, his diabetic control had been poor, and he had been hospitalized several times for diabetic ketoacidosis.

Extensive, detailed medical records were available for him for ages 13 to 17. During that 4.5-year period, his glycemic control was somewhat improved, and only one hospitalization was needed to treat a minor skin infection. Compliance with the prescribed diet was very poor, however. Blood glucose measurements at home ranged between 120 and 300 mg/dl, most often in the hyperglycemic range, and the urinary glucose was 1+ to 3+ on every clinic visit. Hemoglobin A_{1C} was measured frequently during the 4.5 years, and was 2 to 3 times higher than the upper limit of normal on each occasion. The patient refused to administer insulin to himself and was injected by his mother or older sister; at times, however, he refused the injections. He attended a special school for physically handicapped children because of bilateral lenticular opacities; he was also thought to be mildly retarded. His height and weight were at the lower limits of normal for his age. Blood pressure was recorded in the range of 90–110 mm Hg systolic and 60–80 mm Hg diastolic at ages 13 to 15. Beginning at age 15.5, his blood pressure rose to the range of 140–156 mm Hg systolic and 88 to 108 mm Hg diastolic. At age 17, just prior to his first examination in the Adult Renal Clinic, his blood pressure was 145/88 mm Hg. Except for his short stature and early cataracts, no other significant abnormalities were noted on physical examination.

Review of the laboratory data for the prior 4.5 years revealed that serum electrolytes, liver chemistries, and hemogram were always within normal limits. The only consistent abnormality was an elevated serum cholesterol, which varied between 256 mg/dl and 350 mg/dl. From age 13 to 17, the BUN ranged between 11 mg/dl and 16 mg/dl, and the serum creatinine between 0.4 mg/dl and 0.9 mg/dl. Dipstick test results for urine albumin varied considerably but generally were negative from ages 13 to 16 years, although occasional tests during this time period showed 1+ to 2+ albumin. From age 16 to 17.5, the urine albumin was consistently 1+ to 2+. Twenty-four hour total protein excretion ranged from 200 to 800 mg.

Because his body weight was recorded on every clinic visit, it was possible to calculate the endogenous creatinine clearance from the equation:

$$C_{cr} = \frac{(140 - \text{age}) \text{ wt in kg}}{72 \times S_{cr}}$$

where C_{cr} refers to creatinine clearance and S_{cr} refers to serum creatinine. (Creatinine clearance also was measured on several occasions from 24-hour urine collections, but the urine collections were considered incomplete.) At age 14, his serum creatinine was 0.4 mg/dl and body weight was 40 kg; the calculated creatinine clearance was 175 ml/min. At age 15, the serum creatinine was 0.6 mg/dl and body weight was 45 kg; the calculated creatinine clearance was 130 ml/min. At age 16, serum creatinine was 0.8 mg/dl and weight was 50 kg; the calculated creatinine clearance was 108 ml/min. When he was referred to the Adult Renal Clinic at age 17, the serum creatinine was 0.9 mg/dl and body weight was 53.2 kg; calculated creatinine clearance was 101 ml/min. The fall in creatinine clearance from 175 ml/min to 101 ml/min occurred in spite of the increase in body weight and height. Thus, there was clear evidence that hyperfiltration was present from ages 14 to 15, but between 15 and 16 years of age, at approximately the same time that hypertension developed, his creatinine clearance fell progressively to less than 110 ml/min.

In the Adult Renal Clinic, physical examination revealed a blood pressure of 150/92 mm Hg. Aside from short stature and bilateral cataracts, the remainder of the physical examination was negative. Urinalysis showed 2+ albumin and 4+ glucose, but was negative on microscopic examination. Serum creatinine was 0.9 mg/dl; BUN, 16 mg/dl; serum cholesterol, 256 mg/dl; and glucose, 155 mg/dl. Enalapril therapy was initiated, 2.5 mg daily, and he was directed to follow a 2 g sodium diet. When seen one month later, the blood pressure was 110/78 mm Hg. The serum creatinine had risen to 1.1 mg/dl and the BUN to 20 mg/dl. A urine dipstick test was negative for albumin.

The patient has continued to be followed in the Adult Renal Clinic for 3 years. During that time, he has continued taking enalapril, 2.5 to 5.0 mg daily, except for brief periods when his prescription ran out. His blood pressure has varied from 118/55 mm Hg (while taking medication) to 160/110 mm Hg (while not taking medication). The urinary albumin has tended to parallel blood pressure, being trace to 1+ when the blood pressure was normal, to 2+ when hypertension was present. The serum creatinine has risen to 1.4 mg/dl and the BUN to 22 mg/dl. Serum electrolyte values remain normal. Total urinary protein excretion has risen to 1 g/day. Serum cholesterol remains elevated, above 300 mg/dl.

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Lipoprotein analysis revealed low-density lipoprotein, 222 mg/dl; high-density lipoprotein, 48 mg/dl; and triglycerides, 196 mg/dl. At age 20, his weight is 58 kg, and the calculated creatinine clearance is 69 ml/min.

Discussion

DR. NORMAN BANK (*Director, Renal Division, Montefiore Medical Center, and Professor of Medicine, Albert Einstein College of Medicine, Bronx, New York*): This young patient with IDDM illustrates the typical course of diabetic nephropathy with early renal hyperfiltration, progression to proteinuria, hypertension, and declining GFR. This progression has been described by a number of investigators [1–9]. Several factors might have contributed to the course of his disease, including poor metabolic control, lack of adherence to a suitable diet, hypercholesterolemia, and unsatisfactorily controlled hypertension. The prognosis is guarded, because progression to end-stage renal disease within the near future is a virtual certainty.

Hyperfiltration, a common finding in early IDDM, is thought to contribute importantly to renal injury [8, 9]. Understanding the one or more mechanisms responsible for hyperfiltration of early IDDM is important; the hope is that preventing hyperfiltration will delay the onset, slow the progression, or even prevent diabetic nephropathy. In this Forum, I will discuss the experimental findings relating to mechanisms of hyperfiltration. Many theories have been proposed and tested, often with a lack of agreement among investigators. It seems likely, in fact, that more than one mechanism contributes to diabetic hyperfiltration.

Careful measurements of renal function in young patients with IDDM reveal that glomerular filtration rate (GFR) and renal plasma flow (RPF) are often higher than in a matched group of normal subjects. For example, using ^{125}I-iothalamate and ^{131}I-Hippuran, Christiansen et al found that GFR was 27% higher and RPF 20% higher in a group of 13 diabetic patients than in normal controls [1]. The study group had had diabetes less than 7 years. These observations agree with findings by other investigators [2–7], who used a variety of techniques to measure renal function. In most studies, both GFR and RPF were elevated, although Ditzel and Junker found RPF to be slightly lower than in normal controls and GFR to be higher, resulting in a high filtration fraction [2]. Similarly, Mogensen and Andersen found GFR to be elevated in young diabetics, but not RPF [5]. I should note that certain pitfalls exist in measuring renal function accurately in diabetic patients. For example, autonomic dysfunction of the bladder could interfere with complete emptying, and increased extrarenal clearance of injected ^{51}Cr-EDTA could confound the measurement [10]. It seems unlikely, however, that these potential pitfalls can account for the preponderance of evidence pointing to an early functional abnormality in patients with IDDM, an alteration characterized by elevation of GFR and RPF.

Elevated GFR and RPF also have been found in studies of rats with experimental IDDM in which both whole-kidney and single-nephron GFR (SNGFR) are elevated within the first few weeks of induction of diabetes. Using micropuncture techniques, Hostetter et al studied rats with streptozotocin (STZ)-induced diabetes that were given insulin to partially ameliorate hyperglycemia. Such rats have elevated levels of glomerular filtration rate and plasma flow (SNGFR, Q_A) and reduced levels of the afferent arteriolar resistance (R_A), yielding normal values for the ultrafiltration coefficient (K_f) [11]. Jensen et al reported similar findings, except that intratubular pressure was lower than normal and resulted in a higher transglomerular hydraulic pressure gradient [12]. Michels et al also found SNGFR and Q_A to significantly exceed control values [13].

Table 1. Abnormalities in diabetes melitus that might contribute to renal hyperfiltration

Vasoactive hormones
Increased production of vasodilatory prostaglandins
Impaired responsiveness to thromboxane
Increased renal kalikrein production
Abnormal responsiveness to norepinephrine
Increased levels of atrial natriuretic hormone
Abnormal renin-angiotensin system
Insulinopenia and abnormal calcium metabolism
Increased poyol pathway metabolism
Hyperglycemia and extracellular fluid volume expansion

Data on the effect of insulin treatment on renal hemodynamics have been conflicting; some investigators found GFR and RPF to be reduced below normal if insulin was not administered [11, 13]; others found that strict metabolic control with insulin reduced hyperfiltration [14]. In our laboratory, hyperfiltration was present whether or not insulin was given [15–18]. With regard to glomerular capillary pressure (P_{GC}), findings have varied considerably among different laboratories. Values range from normal, to slightly elevated, to markedly elevated [11, 12, 14, 15, 18, 19]. The reason for these differences in hemodynamics and P_{GC} in experimental diabetes is not clear; possible explanations include differences in volume status, prior dietary intake of protein and salt, the type of anesthetic used for surgery, and arterial blood pressure at the time of micropuncture study. Untreated diabetic rats are polyuric and catabolic and fail to gain weight. Therefore, volume status is often uncertain. Insulin treatment can ameliorate the polyuria; nevertheless diabetic rats usually do not grow at the same rate as do normal rats. Because of this difference, whole-kidney GFR should be compared in rats of the same size and age. In lieu of such a protocol, some authors correct their data for body weight, that is, GFR/100 g body weight. When this maneuver is performed, GFR is clearly higher in diabetic than in normal rats. However, when expressed per gram of kidney weight, GFR in diabetic rats is often in proportion to the larger kidney weight. Single-nehpron measurements, which in most studies are not corrected either for kidney weight or body weight, consistently show striking absolute elevations of SNGFR, Q_A, and single-nephron blood flow (SNBF), a marked decrease in R_A, and no change in K_f [11, 15–18]. Efferent arteriolar resistance (R_E) also tends to be low, but this reduction, less marked than the fall in R_A, is not statistically significant [11]. What is certain from the findings in rats is that renal vascular resistance is low and, as a result, blood flow through the kidney is increased. The rise in plasma flow is largely responsible for the hyperfiltration, although a rise in P_{GC} also might contribute.

Table 1 outlines the proposed mechanisms of hyperfiltration in diabetes. I should say at the outset that other vascular beds throughout the body also show decreased resistance to blood flow in early IDDM, and that this finding strongly implies a generalized mechanism rather than one restricted to the kidney.

Vasoactive hormones

Vasodilatory prostaglandins contribute to the regulation of renal hemodynamics, most clearly under conditions in which the activity of vasocontrictor hormones is increased. Much interest has been focused on the possible role of vasodilatory prostaglandins in the hyperfiltration of early IDDM. Several laboratories have found that PGE_2 production by glomeruli or mesangial cells from diabetic rats is greater than normal [20–23]. Barnett et al found that glomeruli from rats with streptozotocin-induced diabetes of 3 weeks duration produced more PGE_2 under both basal and stimulated conditions than did normal glomeruli [20]. In contrast, glomeruli from BB rats with genetic diabetes produced normal amounts of PGE_2. In spite of the increased PGE_2 production, planar surface area of glomeruli decreased in response to added angiotension II, vasopressin, and norepinephrine in a fashion comparable to that seen in normal glomeruli. These results argued against the view that excess prostaglandins interfere with diabetic glomeruli's responsiveness to vasoconstrictor hormones. Schambelan et al also found that glomeruli from rats with STZ-induced diabetes produce more prostaglandins than do normal glomeruli [21]. Kreisberg and Patel showed that mesangial cells cultured from diabetic rat glomeruli produced predominantly PGI_2 instead of PGE_2, in contrast with normal mesangial cells [22]. The total amount of prostaglandins produced by diabetic mesangial cells was greater than that produced by normal cells, and this amount was attenuated by the addition of insulin to the culture medium. Craven and colleagues measured basal PGE_2 , 6-keto $PGF_{1\alpha}$, and thromboxane B_2 production by glomeruli isolated from streptozotocin diabetic rats 9 to 15 days after streptozotocin injection [23]. Levels of all three prostaglandins were elevated. Incubation of the diabetic glomeruli with insulin for 2 hours reduced prostaglandin production to normal. In contrast, glomeruli from rats that had been diabetic for 25 to 28 days did not produce more prostaglandins than did control glomeruli. Nevertheless, hyperfiltration in the intact animals was present at both 9 to 15 days and at 25 to 28 days. Thus, hyperfiltration could be dissociated from excess prostaglandin production.

Studies of cyclooxygenase inhibition in experimental diabetic animals have yielded conflicting results. Craven et al found that indomethacin reduced hyperfiltration in rats 9 to 15 days after induction of diabetes but did not do so 25 to 28 days after induction of diabetes [23]. These researchers suggested that increased prostaglandins had been contributing to hyperfiltration during the first 2 weeks of diabetes, but that after that, hyperfiltration appeared to be independent of the prostaglandin production rate. Similarly, Moel and coworkers found that aspirin normalized hyperfiltration in rats with diabetes of 8 days duration but raised GFR in rats 16 weeks after the induction of diabetes [24]. Jensen and colleagues reported that indomethacin reduced SNGFR markedly in rats 3 months after the induction of STZ diabetes [19]. The animals in that study did not have hyperfiltration prior to indomethacin administration, however, nor did they manifest any of the other glomerular hemodynamic abnormalities usually found in diabetic rats with hyperfiltration. The animals had been receiving insulin prior to the study, and the added insulin might have reduced prostaglandin production and decreased GFR to normal before the rats were given indomethacin. Kirschenbaum and Chaudhari found that GFR in rats with diabetes of 28 days duration was elevated, but that continuous administration of indomethacin during the 28 days prevented a rise in GFR [25]. Glomerular prostacyclin production, measured after 7 days of diabetes, was increased, whereas thromboxane production was decreased. They postulated that hyperfiltration was mediated by an imbalance of prostanoid biosynthesis. In our laboratory, the effect of acute intravenous administration of indomethacin was studied in rats 7 to 10 days after the onset of STZ-induced diabetes [16]. These animals were clearly hyperfiltering prior to indomethacin infusion. There was no significant effect on either whole-kidney GFR or SNGFR over the 3 hours after administration of the indomethacin, even though PGE_2 in the urine and early proximal tubular fluid fell markedly. We concluded that vasodilatory prostaglandins were not responsible for hyperfiltration. These autocoids are not stored, so inhibition of their synthesis causes acute depletion. If vasodilatory prostaglandins played a central role in hyperfiltration, GFR should have fallen quickly with cyclooxygenase inhibition.

The role of increased vasodilatory prostaglandins in mediating hyperfiltration in human diabetes also is not clear. Esmatjes and colleagues inhibited cyclooxygenase by infusing lysine acetylsalicylate into 7 humans with recent-onset IDDM who had hyperfiltration [26]. Both GFR and RPF fell in all but one subject. However, both GFR and RPF remained significantly higher than in the control subjects. The cyclooxygenase inhibitor had no effect in normal subjects. For 3 days prior to renal hemodynamic studies, Hommel et al gave indomethacin to 8 patients with IDDM and found that GFR fell in 7 [27]. These patients did not clearly have hyperfiltration prior to indomethacin administration, the average GFR being 120 ml/min/1.73 m^2. Moreover, they most likely had significant glomerulopathy, because albuminuria was present in all of them. In contrast, Christiansen et al found no effect of 3 days of indomethacin administration on either GFR or RPF in 9 patients with early IDDM [28].

A summary of the experimental results of cyclooxygenase inhibition on the hyperfiltration of diabetes in humans and animals is presented in Table 2. The reasons for the conflicting results are uncertain, but the duration of diabetes, the degree of glucose control, volume status at the time of study, and the duration of cyclooxygenase inhibition might have contributed to the discrepancies. Volume contraction is known to stimulate both prostaglandin and angiotensin II production. Renal hemodynamic control becomes dependent on the interplay of these two vasoactive systems in volume-contracted states, whereas this is not the case in euvolemic or volume-expanded states. Because volume status varies considerably in diabetes, differences in salt and water balance possibly contributed to the inconsistency of findings with cyclooxygenase inhibition. The contribution of vasodilatory prostaglandins to the hyperfiltration of IDDM thus remains an unsettled question.

A rapidly growing body of information indicates that vascular endothelium plays an important role in the regulation of smooth muscle tone. Constricting (for example, *endothelin*) and relaxing (such as *endothelium-derived relaxing factor*, EDRF) substances are both released by endothelial cells in response to various agonists that react with receptors on cell surfaces. The vasoactive products are thought to act locally on the underlying vascular smooth muscle cells and thus produce changes in tone.

Table 2. The effect of cyclooxygenase inhibition on hyperfiltration of diabetes[a]

Human studies			
GFR	RPF	Duration of Rx	Ref.
↓	↓	Acute	26
↓	—	3 days	27
→	→	3 days	28

Rat studies			
GFR	SNGFR	Duration of diabetes	Ref.
↓	↓	9–15 days	23
→	→	25–28 days	23
↓	—	8 days	24
↑	—	16 weeks	24
↓	↓	12 weeks	19
↓	—	4 weeks	25
→	→	2–3 weeks	16

[a] Abbreviations: GFR, glomerular filtration rate; RPF, renal plasma flow; Rx, cyclooxygenase inhibitor; SNGFR, single-nephron glomerular filtration rate.

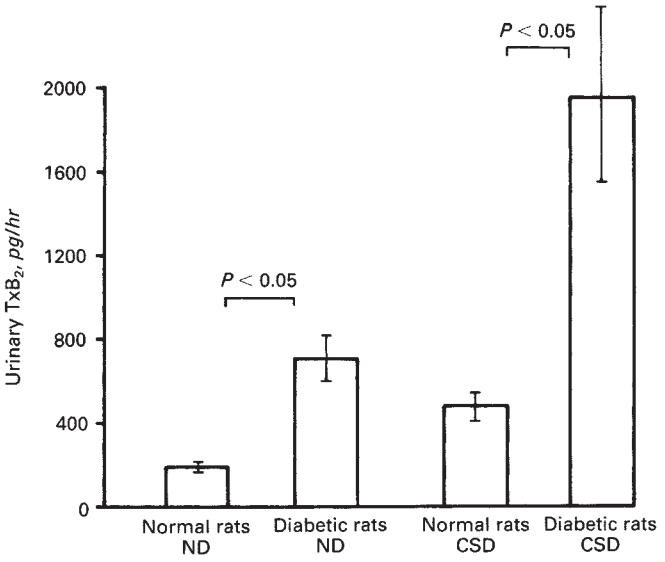

Fig. 1. *Urinary thromboxane (TxB_2) excretion in normal and diabetic rats.* ND, normal diet; CSD, cholesterol-supplemented diet.

A disturbance in the balance of these constricting/relaxing products could play a role in the hyperfiltration of IDDM [29].

Studies of aortic rings from diabetic rabbits and rats show that relaxation in response to acetylcholine or histamine (activators of EDRF) is subnormal [30, 31]. Scanning electron microscopy of the endothelium in the rat studies revealed swollen cells with raised or sloughed nuclei [31]. Tesfamariam and colleagues confirmed that acetylcholine-induced relaxation of aortae is impaired in rabbits with alloxan-induced diabetes, but that this abnormality is corrected by the addition of indomethacin [32]. Thromboxane synthesis was increased in diabetic aortae with an intact endothelium; the findings therefore suggested that the increased thromboxane prevented normal relaxation when EDRF was stimulated by acetylcholine. The same group of investigators found that aortae from normal rabbits exposed to high glucose concentrations in vitro behaved in the same manner as did aortae from diabetic animals [33]. Thus, hyperglycemia per se appeared to be responsible for the increased thromboxane synthesis and thus also for the failure of a normal relaxation response to EDRF.

Katayama et al reported that urinary thromboxane B_2 excretion is higher in diabetic patients than in controls [34, 35]. We also found that urinary thromboxane B_2 excretion is higher in rats with STZ-induced diabetes than in normal rats and that the level rises even further when serum cholesterol is elevated by feeding a diet containing 4% cholesterol (Fig. 1, unpublished observations). In spite of the marked increase in thromboxane B_2 excretion, there was little or no effect on hyperfiltration. In contrast, when normal rats are made hypercholesterolemic, the renal blood vessels constrict significantly; this constriction is mediated by increased thromboxane A_2 production [36]. We further studied the failure of diabetic rats to respond to increased thromboxane A_2 production by infusing a thromboxane agonist (U 46619) into the aorta above the renal arteries. As Figure 2 shows, the decrease in RBF, GFR, and SNGFR was smaller in diabetic rats than in normal controls. When glycemic control was improved by the administration of long-acting ultralente insulin daily, the response to U 46619 returned to normal.

These observations suggest that renal blood vessels of diabetic rats are partially resistant to the vasoconstrictor effect of both endogenous and exogenously administered thromboxane. The reason for this resistance is uncertain, but Wilkes and coworkers reported that glomerular thromboxane A_2 receptors are reduced in glomeruli from diabetic rats [37]. Yanagisawa et al recently reported that insulin markedly accentuates the contractions of coronary arteries from normal pigs stimulated by a stable analogue of thromboxane A_2 in vitro [38]. Insulin did not affect the contractions evoked by several other hormones. Thus it is possible that either the absence of insulin or decreased thromboxane A_2 receptors in diabetes impairs renal vascular responsiveness to thromboxane A_2. The cause of the abnormality in receptors and the impaired response to thromboxane A_2 in the absence of insulin is not known at present. It seems likely, however, that these disturbances will prove important in the hyperfiltration of diabetes.

Few studies of the role of *kallikrein* in diabetes have been carried out, but Jaffa and colleagues found that renal tissue levels and urinary excretion of kallikrein are reduced in untreated rats with STZ-induced diabetes [39]. This reduction was caused by decreased renal synthesis of pro-kallikrein, and this abnormality was corrected by insulin treatment. These researchers also studied the role of kallikrein in the same rat model, but this time using animals treated with insulin [40]. Kallikrein excretion was *higher* in the study rats than in controls, and this increase correlated with a higher GFR and RPF. The GFR was reduced to normal when the diabetic rats were treated with aprotinin, an inhibitor of kallikrein production, on the day before and on the day of study. We infused aprotinin acutely into the renal artery of rats with STZ-induced diabetes and hyperfiltration using a dose known to inhibit urinary active and total kallikrein by more than 90% [16]. We

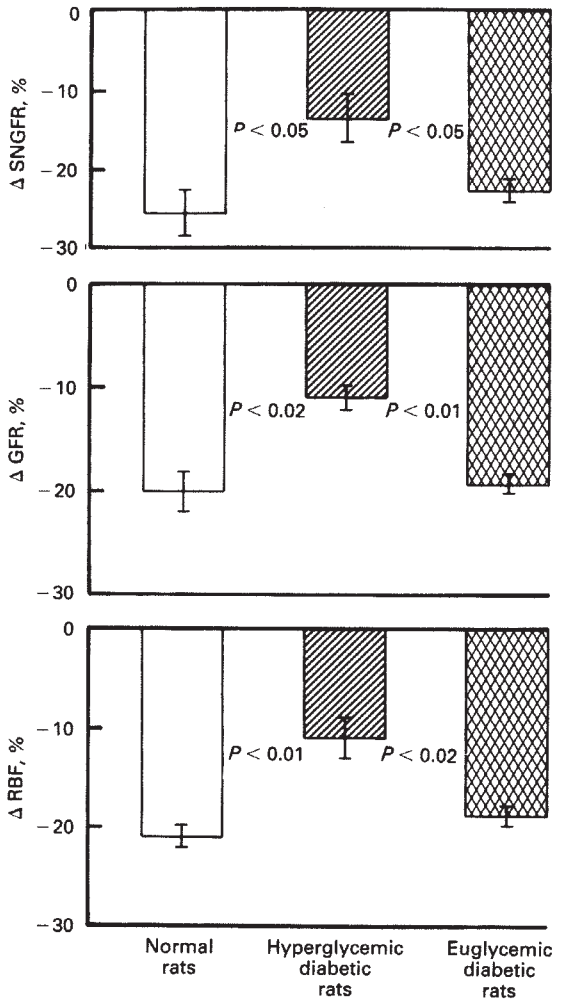

Fig. 2. *Renal hemodynamic response to intrarenal infusion of thromboxane agonist U 46619 in normal and diabetic rats.*

found no significant effect on urine flow, sodium excretion, GFR, or SNGFR. Thus, as is the case with prostaglandin inhibitors, different results are obtained with kallikrein inhibitors, depending on whether insulin is administered and whether the inhibitor is given acutely or chronically. The failure of acute inhibition of kallikrein to ameliorate the hyperfiltration in this rat model [16] implies that bradykinin is not the proximate cause of hyperfiltration. If it were, acute inhibition should have promptly corrected the hyperfiltration.

Norepinephrine is one of the major vasoactive hormones that constrict renal blood vessels. Therefore, a decrease of circulating levels, of synthesis at nerve endings, or of receptors might lead to impaired renal vascular tone. Christensen reported that plasma catecholamine concentration (mainly norepinephrine) was normal in long-term diabetic patients without neuropathy [41]. Beretta-Piccoli et al found the response of plasma norepinephrine to upright posture in diabetic subjects to be normal in most cases [42]. Likewise, epinephrine levels in diabetic subjects were normal. No difference in endogenous creatinine clearance was found when patients were grouped according to low versus normal plasma norepinephrine concentrations [42].

Studies of isolated vascular smooth muscle sensitivity and reactivity to norepinephrine in diabetic animals have yielded variable and inconsistent results. A number of different vascular beds have been studied, including the isolated aorta [43, 44] mesenteric arteries [45], and blood vessels of the hind limb [46, 47]. Both increased and decreased sensitivity and maximum responsiveness of these vessels to norepinephrine have been reported. Most studies report increased sensitivity to norepinephrine (decreased threshold), which is consistent with a postganglionic neuroeffector sympathetic defect, analogous to denervation hypersensitivity [47]. Some of the variability in vascular responses to norepinephrine can be explained by differences in the species studied, the duration and severity of diabetes and the particular vascular bed tested. Responses also vary depending on whether insulin has been administered. For the most part, isolated blood vessels, aortic rings, and cells in culture are highly unphysiologic preparations, because they lack innervation and blood flow is absent. Moreover, the contractile response depends, among other things, on the calcium concentration in the bathing medium. For example, Owen and Carrier demonstrated that aortic rings from diabetic rats were supersensitive to norepinephrine but that the response depended on the calcium concentration in the bathing medium [48]. In addition, rings from rats that had been diabetic for 28 to 35 days yielded greater maximum contractile force at all calcium concentrations than did rings from rats that had had diabetes for only 14 to 20 days. MacLeod and McNeill found that the aortae from "100-day" diabetic rats were supersensitive to norepinephrine, but this sensitivity was not observed 180 days after the onset of diabetes [44]. At 360 days, however, sensitivity and responsiveness to norepinephrine again were increased. On the other hand, Ramanadham and coworkers found that the thoracic aorta from rats with 4 weeks of STZ-induced diabetes exhibited decreased responsiveness to norepinephrine [43]. Morff studied arterioles of the cremaster muscle from rats with STZ-induced diabetes and found that larger arterioles responded normally to norepinephrine after 2 to 16 weeks of diabetes [49]. Smaller arterioles exhibited hypersensitivity early in the course of diabetes, but the response returned to normal at a later stage in the disease. From these several studies, it appears that the stage of diabetes as well as the vessel size and location influence the vascular response to norepinephrine. In patients with diabetes, Christlieb measured blood pressure sensitivity and reactivity to infused norepinephrine and found both to be increased [50]. In agreement, Beretta-Piccoli and Weidmann found that the baseline plasma norepinephrine was similar in diabetic and normal subjects, and that the pressor and threshold doses to infused norepinephrine were lower in diabetics [51]. Christlieb found reduced plasma levels of norepinephrine in rats with alloxan diabetes [52]. Reduced tissue levels of catecholamines have been reported for the tail artery of rats with STZ-induced diabetes [43, 53]. Most of the evidence thus indicates that blood vessels from diabetic animals are hyperreactive to exogenous norepinephrine, perhaps because of impaired endogenous synthesis or release of norepinephrine. Unfortunately, none of the studies in humans or animals has correlated the degree of sensitivity and reactivity to exogenous norepinephrine with renal hemodynamics. Although it is possible that diabetes might

have impaired renal nerve synthesis or storage of catecholamines, or reduced receptors, further studies are needed to clarify these issues.

The administration of *atrial natriuretic peptide* (ANP) causes renal vasodilation and increases GFR. Therefore it is reasonable for us to consider excess endogenous ANP as a mediator of chronic hyperfiltration. Atrial natriuretic peptide is elevated in the plasma of diabetic animals [54] and humans [55], and the levels vary inversely with the degree of metabolic control of glucose [56]. Ortola et al studied the relationship between GFR and ANP in diabetic rats [54]. In animals with moderately controlled diabetes (average blood glucose, 326 mg/dl), ANP was 280 pg/ml, whereas in animals with tightly controlled diabetes (average blood glucose, 85 mg/dl), ANP was 158 pg/ml. The GFR was elevated in the moderately controlled group and was completely normal in the tightly controlled group. When these authors infused an antiserum directed against ANP into hyperfiltering diabetic rats, they found a prompt decline in GFR and RPF to normal with no alteration of blood pressure or blood glucose. Their observations provide strong support for the view that ANP is the mediator of hyperfiltration in diabetic rats. Atrial natriuretic peptide is elevated in volume-expanded conditions, and its exogenous administration causes systemic vasodilation, suppression of plasma renin activity [57, 58], decreased aldosterone production [59], and decreased plasma aldosterone [57]. Infusion of ANP in normal rats raises glomerular capillary pressure [60].

Some of the hemodynamic and hormonal abnormalities of volume-expanded diabetic animals can be mimicked by ANP administration. However, several significant differences exist between the effects of exogenous ANP on renal hemodynamics and the hyperfiltration in diabetes. First, in normal dogs, Maack et al found that intravenous ANP lowered RPF at the same time that GFR and sodium excretion rose [57, 61]. Decreased RPF is not characteristic of diabetic hyperfiltration. Dunn et al [60] and Huang et al [62] found similar results when they infused ANP intravenously into normal rats. The GFR rose by 20% to 45%, whereas renal and glomerular plasma flow remained stable. The rise in GFR could be entirely accounted for by a rise in P_{GC} [60]. Glomerular plasma flow is typically elevated in rats with early STZ-induced diabetes. Schnermann et al found that stop-flow pressure (an indirect measure of glomerular capillary pressure) rose by 10 mm Hg when either atrial extract or synthetic atriopeptin II was infused into normal rats [63]. The increase was due to afferent arteriolar dilation, with little or no dilation of the efferent arteriole. Marin-Grez et al measured afferent and efferent arteriole diameters directly by intravital microscopy and found that ANP dilated the afferent arteriole but decreased the diameter of efferent arterioles by 10% [64]. This profile of glomerular hemodynamics is also not characteristic of diabetes, in which both afferent and efferent arteriolar resistances are low, plasma flow is increased markedly, and P_{GC} is increased moderately or not at all. Finally, low-dose infusion of ANP in normal humans, at rates that raised plasma ANP from 28 to 360 pg/ml (levels comparable to those of endogenous ANP in diabetic rats), slightly *decreased* GFR and RPF at the same time that sodium excretion rose and plasma renin activity (PRA) and angiotensin II levels decreased [65]. Because the effects of exogenous ANP on glomerular hemodynamics differ significantly from those in diabetic rats, the role of ANP as the major mediator of diabetic hyperfiltration is questionable. Atrial natriuretic peptide might contribute to hyperfiltration, however, in that it antagonizes the vasoconstrictor actions of both angiotensin II and norepinephrine [55, 66].

Because *angiotension II* is a major constricting hormone of blood vessels in the kidney, considerable attention has been focused on abnormalities in the *renin-angiotensin system* as a possible contributor to hyperfiltration in IDDM. Christlieb showed in rats with alloxan-induced diabetes that renal and plasma renin activity are reduced. Angiotensin II prompted an increased blood pressure response, but norepinephrine did not [52]. Increased blood volume might have been the cause of the lower PRA. In diabetic humans with poor glucose control, Christlieb and colleagues found blood volume to be somewhat higher than after improved metabolic control, although PRA did not change significantly [67]. Ballerman et al found that plasma renin concentration (PRC) was lower in diabetic rats than in normal controls, but insulin therapy normalized PRC [68]. In addition to a reduction in PRA, angiotensin II receptor density is reduced in diabetic glomeruli [18, 68]. The receptor density varies directly with dietary salt intake and correlates inversely with PRC [68], as in normal rats. However, the absolute density remains low under all conditions studied [68]. These observations support the concept that a combination of low circulating angiotensin II and decreased glomerular angiotensin II receptors is responsible for hyperfiltration in IDDM. Several studies suggest, however, that the decrease in angiotensin II receptors is not sufficent to prevent the vascular response to exogenously administered angiotensin II. In our laboratory, administration of angiotensin II into the renal artery of rats with STZ-induced diabetes resulted in a fall in SNGFR and Q_A, and a rise in afferent arteriolar resistance without any change in P_{GC}; these results were quantitatively similar to those in normal control rats that were chronically expanded due to the ingestion of large amounts of sodium chloride [16]. We also found that restriction of dietary sodium intake reduced GFR and RPF from supernormal to normal levels [17] (Fig. 3). This effect apparently was mediated by stimulation of the endogenous renin-angiotensin system, because acute infusion into the renal artery of saralasin, a receptor antagonist of angiotensin II, abruptly returned SNGFR and Q_A to supernormal levels (Fig. 4). Thus, the renal angiotensin II receptors apparently were capable of responding to stimulated levels of endogenous angiotensin II, in spite of the fact that receptor density would be expected to fall further as endogenous angiotensin II rose [68]. In 10 patients with IDDM and 10 matched controls, Bjorck measured the response of GFR and RPF to intravenous infusion of angiotensin II at 0.5 to 1.5 ng/kg/min [69]. He found that GFR and RPF, which were elevated in the diabetic subjects, fell equally in the two groups, indicating that vascular responsiveness to exogenous angiotensin II was not impaired in the diabetic humans. Taken together, these observations suggest that the reduced glomerular angiotensin II receptor density does not in itself account for diabetic hyperfiltration. Some studies of non-renal vascular beds in diabetic animals disclosed increased sensitivity and responsiveness of blood pressure and muscle arterioles to exogenously administered angiotensin II [50, 70]. Beretta-Picolli and Weidmann reported that the angiotensin II pressor dose was lower in nonazotemic diabetic patients than in normal subjects [51]. Diabetic hyperfiltration thus does not appear to be due to the

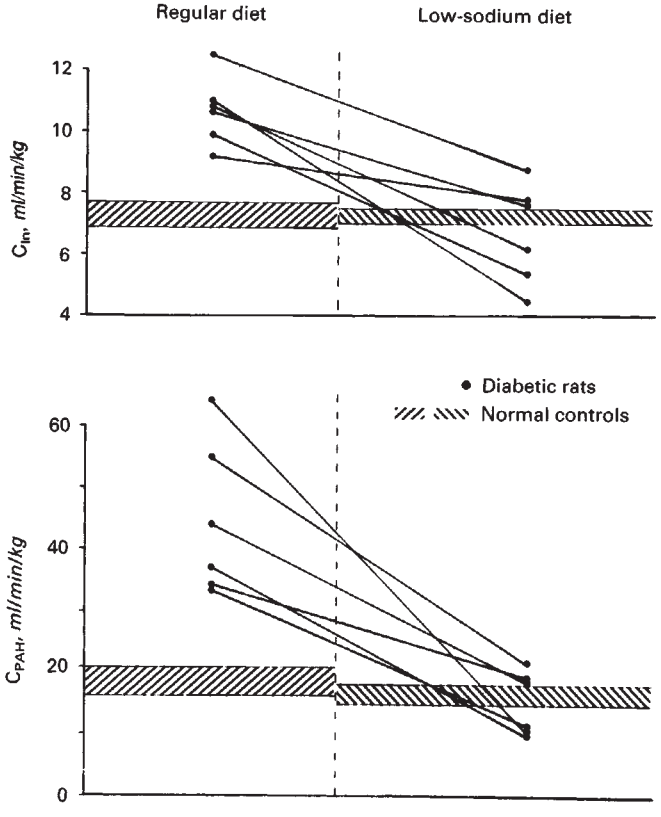

Fig. 3. *The effect of dietary sodium restriction on GFR (C_{In}) and RPF (C_{PAH}) in diabetic and normal rats.* Second clearance measurements made 3–5 days after switching to low-sodium diet (Ref. 17).

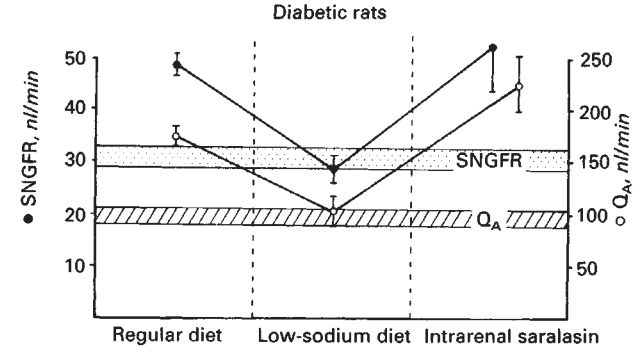

Fig. 4. *Changes in SNGFR and Q_A in diabetic rats fed a low-sodium diet and infused with saralasin acutely. Horizontal zones* are the ranges for normal rats.

kidney's impaired responsiveness to angiotensin II. The possibility remains, however, that low circulating and/or renal tissue levels of angiotensin II, perhaps related to ECF volume expansion, contribute to diabetic hyperfiltration.

Effects of insulin and calcium on vascular smooth muscle

Several observations indicate that insulin plays an important, direct role in smooth muscle and myocardial contraction. Therefore, insulin deficiency per se needs to be considered as a mechanism contributing to diabetic hyperfiltation. Pfaffman and colleagues found that contractions of aortae from diabetic rats

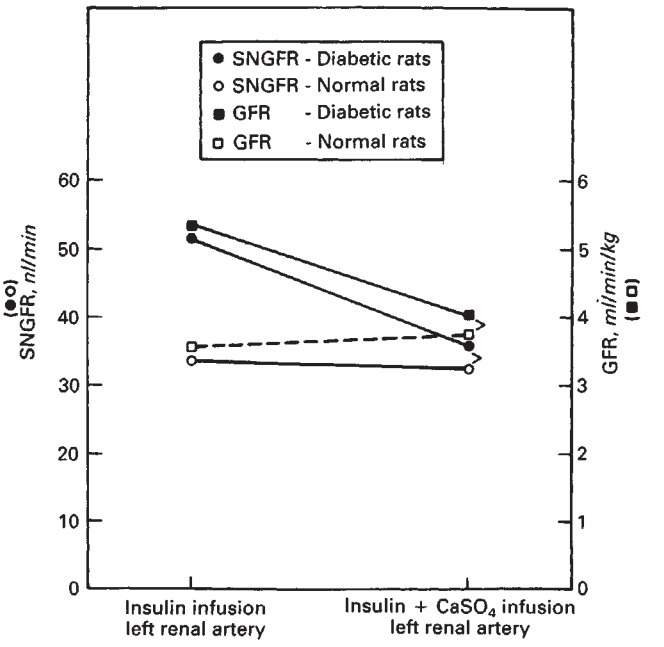

Fig. 5. *Acute effect of intrarenal infusion of insulin, followed by insulin + $CaSO_4$ in normal and diabetic rats.* (From Ref. 15.)

studied in vitro were markedly decreased in response to potassium chloride administration [71]. The decrease was completely reversed in animals treated with insulin. Using rat glomerular mesangial cells in culture, Kreisberg found that insulin was necessary for a contractile response to angiotensin II [72]. Evidence supports the view that insulin is important for entry of calcium into certain cells. For example, Cohen et al found that calcium suppressed renin release in isolated, perfused, normal rat kidneys, but that this suppression occurred only when insulin was present in the perfusate [73]. The effect of insulin was blocked by the addition of a calcium-channel blocker (verapamil) to the perfusate. Mueller reported that insulin restored vascular contraction in animals with alloxan-induced diabetes in response to various stimuli, all of which involve transmembrane calcium flux or mobilization of intracellular calcium [74]. Gotzsche reported the absence of myocardial contraction of isolated perfused diabetic rat hearts in response to isoproterenol. Also, calcium uptake is absent [75]. Providing insulin either to the animals or to the isolated in-vitro heart preparation reversed these abnormalities.

We studied the effect of intrarenal insulin and calcium infusion on hyperfiltration in diabetic rats [15]. Low concentrations of insulin and calcium, either singly or in combination, had no effect on GFR or SNGFR in normal rats (Fig. 5). In diabetic rats, infusion of low-dose insulin into the renal artery also had no effect on the elevated GFR or SNGFR. But when calcium was added to the insulin infusion, GFR and SNGFR both promptly fell to the normal range. This fall was accompanied by a normalization of P_{GC} and a decrease in $U_{Na}V$ and urine flow [15]. The effects of the combined infusion of calcium and insulin were reversed immediately when we added verapamil to the intrarenal infusion [15]. These observations strongly suggest that insulin deficiency impairs calcium uptake by renal vascular smooth muscle cells. Thus, insulin deficiency per se could play

an important role in the hyperfiltration of IDDM. In addition, vasoactive hormones that initiate calcium entry into vascular smooth muscle cells may lose their effectiveness in the absence of insulin.

The polyol pathway

A major area of interest with regard to the complications of diabetes is the cellular accumulation of sorbitol and the depletion of myoinositol [76]. These changes occur in several tissues that contain the enzyme aldose reductase. Glucose is converted to sorbitol via aldose reductase activity, and the sorbitol is then converted to fructose via activity of sorbitol dehydrogenase [77]. Myoinositol competes with glucose for cellular uptake, and it is also excreted in the urine in excessive amounts in diabetic patients [76]. However, cellular depletion of myoinositol occurs selectively and involves those tissues predisposed to developing diabetic complications, such as the lens, the retina, peripheral and autonomic nerves, and the kidney. The importance of myoinositol lies in the fact that it is a key precursor of cell membrane inositol phospholipids. In recent years, a large number of diverse calcium-mobilizing ligands have been found to initiate their actions by receptor-mediated hydrolysis of cell membrane phospholipids. Thus, the vasoconstricting effects of angiotensin II and norepinephrine on vascular smooth muscle, for example, are initiated by hormone-receptor activation of cell membrane phospholipase C (PLC). Plasma membrane phosphoinositides are hydrolyzed by PLC to several inositol phosphates and 1,2 diacylglycerol (DAG). Inositol triphosphate (IP_3) and DAG act in concert to raise intracellular calcium concentration and to activate protein kinase C (PKC). The latter is one of several protein kinases capable of phosphorylation of myosin light chains and thus of sustaining smooth muscle contraction [77]. In view of these well-recognized biochemical events, a reasonable hypothesis to explain diabetic hyperfiltration is that contractile cells within the kidney become depleted of myoinositol, and that cell membrane turnover of IP_3 and DAG are thereby impaired. This in turn would be expected to limit hormone-stimulated increases in intracellular calcium and activation of PKC. As a result, phosphorylation of myosin light chain would be impaired, as would sustained smooth muscle tone.

Although the sequence of events stemming from cellular myoinositol depletion provides a logical framework for this hypothesis, several recent observations require significant modification of the theory. Lee and coworkers found that retinal endothelial cells accumulate sorbitol in response to high glucose concentrations, indicating the presence of aldose reductase, but that PKC activity in membrane fractions actually increases markedly at the same time that the cytosolic fraction of PKC decreases [78]. The addition of sorbinil, an aldose reductase inhibitor, to the endothelial cell preparation blocked sorbitol accumulation but did not influence PKC activity. On the other hand, Na^+-K^+-ATPase was inhibited markedly in the presence of hyperglycemia, and this inhibition was prevented by sorbinil [78]. The same laboratory found that, because of increased formation of its precursors, diacylglycerol synthesis is actually increased in the presence of elevated glucose, not decreased as predicted by the myoinositol depletion hypothesis [79]. The increased diacylglycerol is thought to be responsible for the greater PKC activity in the cell membranes. Craven and DeRubertis, using isolated glomeruli from diabetic rats, found that despite a reduced inositol content and decreased polyphosphoinositide turnover, PKC was increased in particulate cell fractions and that this increase was prevented by insulin treatment [80]. They also found that glucose increased the production of diacylglycerol. Thus, considerable evidence militates against the hypothesis that a deficiency of diacylglycerol or PKC underlies impaired vascular smooth muscle tone in diabetes. Evidence remains, however, that hyperglycemia inhibits Na^+-K^+-ATPase activity, and that this alteration can be corrected by the inhibition of aldose reductase. It is possible that membrane depolarization occurs, secondary to impaired Na^+-K^+ transport, which effects transmembrane calcium flux. Further studies are needed to clarify the relationship between myoinositol, Na^+-K^+-ATPase, and smooth muscle function.

Aldose reductase has been found in various locations in the kidney both by staining methods [85, 86] and biochemical methods [87, 88]. Kikkawa and coworkers found both aldose reductase and sorbitol dehydrogenase activity in crude homogenates of cultured mesangial cells [88]. Incubation of mesangial cells in medium containing 55 mM glucose resulted in a large intracellular accumulation of sorbitol. The accumulation was blocked by the addition of an aldose reductase inhibitor. These in-vitro biochemical data strongly suggest that sorbitol concentrations rise in the glomeruli in the presence of elevated glucose, and that aldose reductase inhibitors correct this abnormality.

Evidence for an important role for the polyol pathway in influencing vascular tone was recently reported by Williamson et al [89]. These authors exposed rat blood vessels in a granulation tissue-skin chamber to high concentrations of glucose. They found that with 30 mM D-glucose in the chamber, but not L-glucose, blood flow increased markedly, as did tissue sorbitol concentrations. Addition of the aldose reductase inhibitor tolrestat to the chamber prevented the increase in both sorbitol concentration and blood flow. Renal hemodynamic studies have investigated the role of sorbitol accumulation and/or myoinositol depletion in the hyperfiltration of early diabetes. Goldfarb et al first reported that an aldose reductase inhibitor (sorbinil) or dietary myoinositol supplementation prevented hyperfiltration in diabetic rats [90]. In untreated diabetic rats, GFR was 11.0 ml/min/kg body weight, 9.4 ml/min/kg in a group fed 1.0% myoinositol, and 8.8 ml/min/kg in diabetic rats given sorbinil (25 mg/kg/day). Several other groups of investigators confirmed that aldose reductase inhibitors prevent hyperfiltration in diabetic rats. Craven and DeRubertis found that sorbinil reduced GFR from 7.5 to 4.5 ml/min/kg in diabetic rats, but that the drug had no effect in normal rats [91]. They also found that sorbinil normalized elevated PGE_2 and 6-keto-$PGF_{1\alpha}$ production in isolated diabetic glomeruli but had no effect on normal glomeruli. They postulated that sorbinil might prevent hyperfiltration by suppressing glomerular synthesis of vasodilatory prostaglandins. Frey et al also reported that sorbinil affects PGE_2 production [92]. In rats with STZ-induced diabetes, urinary PGE_2 was elevated during the first 2 weeks of diabetes in untreated rats but returned to normal when sorbinil was fed to the animals. The authors did not measure GFR. Williamson and colleagues did measure GFR using ^{57}Co-EDTA in diabetic rats and found GFR to be double that of normal control rats [93]. Treatment with either of two aldose reductase inhibitors

(sorbinil or tolrestat) prevented the hyperfiltration. Tilton and colleagues reported that 3 structurally different aldose reductase inhibitors blocked diabetic-induced increases in renal blood flow and GFR in rats [94]. In our laboratory, sorbinil fed to rats with STZ-induced diabetes in a diet rationed to 20 g/day prevented the increase in SNGFR, Q_A, and SNBF [18]. Plasma renin activity, blood glucose, and glomerular angiotensin II receptor density were not affected by sorbinil treatment [18]. Sorbinil did, however, alter glomerular hemodynamics in normal rats: single-nephron filtration fraction, stopped flow pressure, and R_A were slightly but significantly higher than in untreated normal rats [18]. These observations raise the possibility that sorbinil has an effect on vascular tone separate from its major role as an aldose reductase inhibitor. This possibility is raised by observations by Cohen and Klepser, who found glomerular Na^+-K^+-ATPase activity to be reduced in isolated glomeruli of rats in early stages of diabetes (less than 18 days), but not at later stages (more than 3 weeks) [95]. The in-vitro addition of sorbinil directly stimulated Na^+-K^+-ATPase activity in normal glomeruli but not in diabetic glomeruli. These researchers suggested that sorbinil might have a membrane-associated effect in normal glomerular tissue independent of its aldose-reductase inhibiting property. Thus, it is possible that the prevention of hyperfiltration induced by sorbinil, and perhaps other aldose reductase inhibitors, is mediated not only by correction of sorbitol and myoinositol tissue levels, but also by a membrane-associated effect on Na^+-K^+-ATPase and reduction in vasodilatory prostaglandins.

Not all investigators agree that aldose reductase inhibitors prevent hyperfiltration. Daniels and Hostetter administered ponalrestat (Statil) in a daily dose of 25 mg/kg body weight to rats with STZ-induced diabetes. Untreated diabetic rats had a significantly higher GFR than did normal controls, but ponalrestat had no significant effect on the hyperfiltration 3 to 5 weeks after the onset of diabetes [96]. The drug did reduce red blood cell and kidney sorbitol levels significantly, indicating its effectiveness as an aldose reductase inhibitor. The authors suggested that the reductions in GFR found by other investigators with aldose reductase inhibitors might be due to effects independent of their enzyme-inhibiting action, such as inhibition of prostaglandin synthesis or direct membrane effects. In spite of the controversial data, most evidence supports the view that hyperfiltration can be prevented by aldose reductase inhibition or myoinositol supplements.

Hyperglycemia and extracellular fluid volume

Does hyperglycemia cause vasodilation by an osmotic effect on the cells lining small blood vessels? Osmotic transfer of water from the intracellular compartment would be expected to shrink the lining cells, thereby enlarging the cross-sectional diameter of the lumen [97]. However, the effect of glucose appears to be greater than can be accounted for by its osmotic pressure. Thus, Williamson et al demonstrated that L-glucose does not dilate microvessels, whereas D-glucose does [89]. In clinical studies, Brochner-Mortensen [7] and Bell et al [56] found hyperfiltration to be present more often in poorly controlled diabetic patients than in those with improved blood glucose levels. Moreover, improved glycemic control by insulin reduces or even normalizes hyperfiltration [98–103]. Wiseman et al evaluated glycemic control in diabetic patients by monitoring the level of glycosylated hemoglobin (HbA_{1c}) [99]. With improved metabolic control, HbA_{1c} fell into the normal range. The GFR became normal in 4 of 6 patients. Similarly, Christiansen found a significant correlation between HbA_{1c} and GFR in 28 insulin-dependent diabetics who did not have significant albuminuria [100]. Christiansen et al also reported that during the first week of insulin treatment in newly diagnosed IDDM patients, GFR and RPF fell consistently, whereas kidney volume, evaluated by ultrasound, did not change [101]. In an earlier study, Mogensen and Andersen measured GFR, RPF, and kidney size (by x-ray) over a 3-month period in 6 newly diagnosed patients treated with insulin [4]. The GFR fell consistently in this time period, and renal size also decreased slightly but significantly. That the effect on renal hemodynamics was not due to insulin administration per se but rather to the fall in blood glucose was suggested by acute studies carried out by Christiansen et al [102]. Insulin infusion for 2 hours in diabetic subjects lowered GFR and RPF only if blood glucose was allowed to fall. If blood glucose was maintained constant, insulin administration had no effect on GFR or RPF. Richards and colleagues found that one month of rigorous control of blood glucose in diabetic patients significantly lowered blood pressure but produced no changes in PRA, aldosterone, or catecholamine levels [103]; these findings imply that hormonal changes were not responsible for the normalization of renal hemodynamics. Strict metabolic control also prevents hyperfiltration and a rise in P_{GC} in rats with STZ-induced diabetes [54, 104]. In animal and human studies in which the effect of "tight" metabolic control on diabetic hyperfiltration was examined, the weight of evidence consistently indicates that improved glycemic control is accompanied by a fall in the elevated GFR [4, 54, 99, 100, 102, 104].

The mechanism by which poor glycemic control contributes to hyperfiltration is not understood, but evidence indicates that acute changes in blood glucose produce only small effects [105–107]. Christiansen et al studied euglycemic diabetics by infusing glucose to raise blood glucose from 4.2 to 15.2 mmol/liter [105]. The GFR rose acutely but only slightly, from 128 to 132 ml/min, and RPF increased from 534 to 562 ml/min. Wiseman and coworkers infused dextrose into diabetic patients with and without hyperfiltration and into normal controls and found that raising blood glucose from 5–6 mmol/liter to 12.5 mmol/liter significantly increased GFR only in the hyperfiltering diabetic subjects (from 157 to 174 ml/min), not in the other 2 groups [106]. In the isolated perfused rat kidney, Kasiske and colleagues found that raising glucose to 500 mg/dl in the perfusate led to immediate vasodilation and a small but significant increase in GFR [107]. Indomethacin blocked this effect. Thus, considerable evidence suggests that either the level of blood glucose, or another factor associated or correlated with chronic hyperglycemia, can contribute to glomerular hyperfiltration in diabetes. In contrast, Ditzel et al [2] and Mogensen [108] were unable to find any correlation between spontaneous blood glucose and GFR. Moreover, in normal subjects whose blood sugar levels were raised to 600–700 mg/dl, GFR was not affected [108]; this finding concurs with Wiseman et al's report [106]. Acute elevation of blood glucose might not lead immediately to hyperfiltration. Chronically elevated blood glucose might influence GFR by indirect mechanisms, however.

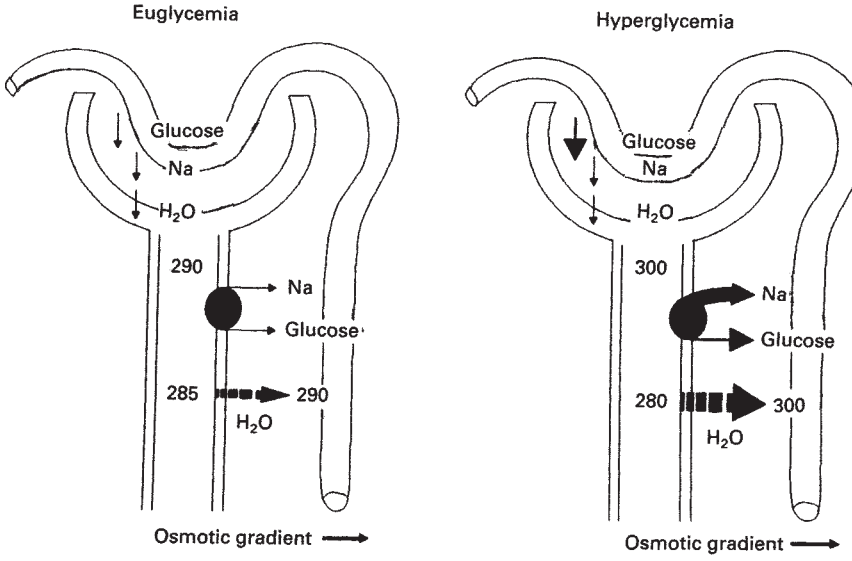

Fig. 6. *Schematic representation of effects of increased filtered load of glucose on proximal tubular sodium and water reabsorption.*

Recent observations point to the possible existence of important, indirect effects of hyperglycemia on GFR. Blantz et al studied tubuloglomerular feedback (TGF) in normal rats during perfusions of the loop of Henle with tubular fluid containing high concentrations of glucose [109]. They found a significant inhibition of TGF, in that SNGFR did not fall as much as when the loop was perfused with non-glucose-containing tubular fluid. Woods et al studied TGF in normal dogs infused intrarenally with glucose [110]. In dogs with filtering kidneys, GFR and RBF rose 18% to 19%, whereas in dogs with non-filtering kidneys (due to ureteral ligation), intrarenal infusion of glucose had no effect on RBF. These observations indicated that the filtered load of glucose, rather than the blood level of glucose, influenced renal hemodynamics. The authors postulated that filtered glucose reaching the distal nephron somehow suppresses TGF. Further evidence for a role for filtered glucose comes from studies of electrolyte reabsorption by the renal tubules of diabetic children [111]. Filtered sodium and tubular reabsorption of sodium were significantly higher in diabetic children than in matched controls, and the authors postulated that this increase was due to enhanced sodium-coupled glucose reabsorption (co-transport). They also suggested that the augmented renal tubular sodium reabsorption was important as a mechanism leading to elevated GFR in diabetes. Brochner-Mortensen and colleagues measured lithium clearance in young adults with IDDM [112]. Lithium is thought to be reabsorbed primarily in the proximal tubule, and thus its urinary clearance can be used as a measure of fluid leaving the end of the proximal tubule. The difference between filtered and excreted lithium is taken as a measure of proximal tubule sodium reabsorption. The researchers found evidence for increased filtered sodium and water, increased absolute and fractional reabsorption of sodium and water in the proximal tubule, and unaltered distal delivery of fluid. More recently, Wiseman et al measured sodium reabsorption in hyperfiltering diabetics during euglycemia and hyperglycemia induced by intravenous glucose infusion [106]. As GFR rose, total sodium reabsorption increased, whereas free-water clearance fell. The decrease in free-water clearance suggests that the increase in sodium reabsorption during hyperglycemia occurred proximal to the diluting segment. A relative reduction in the distal delivery of sodium might have limited free-water clearance. In accord with these findings are those of Hannedouche et al, who measured lithium clearance in type-1 diabetics and confirmed higher absolute and fractional reabsorption of sodium in the proximal tubule of diabetic versus control subjects as well as a decrease in distal sodium delivery [113]. Both groups of investigators postulated that decreased delivery of sodium to the macula densa might suppress TGF and thus contribute to hyperfiltration of diabetes.

Using microperfusion methods, we recently studied the effect of progressive elevation of glucose concentrations in the lumen of the proximal tubule on sodium and water absorption [114]. We found both in normal and diabetic rats that the addition of 100 mg/dl, 300 mg/dl, and 500 mg/dl of glucose to an isotonic electrolyte solution progressively stimulated sodium and water absorption. Rates of absorption plateaued at glucose concentrations of 300–500 mg/dl. The increased sodium reabsorption appeared to be mediated by the brush-border sodium/glucose co-transporter. In addition, the osmolality of the collected fluid fell to hypotonic levels and resulted in an exaggerated transtubular osmotic gradient. This gradient presumably acted as a passive driving force for increased water absorption. These observations are depicted in Fig. 6. Our findings support the view that poor glycemic control, by increasing the concentration of glucose in the proximal tubule, increases sodium and water absorption. This effect in turn could lead to extracellular fluid volume expansion. Total exchangeable body sodium is increased in diabetics in the absence of intrinsic renal disease [115, 116], although blood and plasma volumes are not always elevated. The effect of intraluminal glucose on sodium reabsorption is immediate; thus one can postulate that even short, intermittent periods of hyperglycemia in a relatively well-controlled diabetic can produce increases in total-body sodium that in turn could suppress the renin-angiotensin system. Elevation of plasma ANP also would be a consequence of this volume expansion. The combination of volume expansion, a suppressed renin-angiotensin system, and elevated ANP is distinctly unusual; in most other sodium-retaining conditions

Table 3. Hypothetical consequences of increased glucose-mediated proximal sodium and water reabsorption

Increase in total-body sodium
Extracellular fluid volume expansion
Suppression of renin-angiotensin system
Elevation of ANP
Hypoaldosteronism
Hypertension
Renal hypertrophy
Decreases in distal sodium delivery
Impaired free-water clearance
Impaired K^+ secretion
Impaired H^+ secretion

such as congestive heart failure, cirrhosis of the liver, and nephrotic syndrome, the renin-angiotensin system often is stimulated rather than suppressed. The triad of volume expansion, elevated ANP, and angiotensin suppression might play a critical role in hyperfiltration in early diabetes. Other possible consequences of glucose-mediated, stimulated proximal sodium and water absorption are listed in Table 3.

Conclusions

No single mechanism is likely to account for the hyperfiltration of early diabetes. Numerous abnormalities have been documented that could play a role, including reduced receptor density for angiotensin II and thromboxane A_2, increased urinary kallikrein, and increased prostaglandin production. However, experimental studies have not shown unequivocally that any of these abnormalities are critical for diabetic hyperfiltration. On the other hand, there is substantial evidence both in diabetic humans and in animals that improved glycemic control corrects or prevents hyperfiltration. Improved glycemic control leads to correction of a number of disturbances, including a reduction in volume, reduction of ANP, a decrease in serum osmolality, correction of tubulo-glomerular feedback, and correction of prostaglandin overproduction; in addition, the provision of insulin to achieve glycemic control also increases calcium entry into vascular smooth muscle cells. Thus, the unifying factors leading to a large number of abnormalities appear to be hyperglycemia and insulin deficiency itself. Poorly controlled glycemia is most likely the starting point for the cascade of signal-receptor-transduction abnormalities resulting in the hyperfiltration of diabetes.

Questions and answers

DR. JORDAN J. COHEN (*Dean, State University of New York at Stony Brook, Stony Brook, New York*): As you indicated, the degree of hyperfiltration in the early stages of diabetes can be quite striking, with glomerular filtration rates 70%, 80%, or even 100% higher than normal. You suggested that this hyperfiltration simply might be a reflection of chronic volume expansion resulting from a stimulation of glucose-dependent sodium reabsorption triggered by the high filtered load of glucose. This possibility would be strengthened if other forms of chronic volume expansion—notably primary aldosteronism, chronic DOCA administration, or SIADH—were associated with such extreme elevations of GFR. Do we know from any of these circumstances what the relationship is between the magnitude of the chronic volume expansion and the resulting degree of hyperfiltration? Also, I wonder how much volume expansion one might reasonably expect to see on a chronic basis from augmented glucose-dependent sodium reabsorption, given that "escape" from sodium retention would be anticipated to occur as a result of the dampening effects of other regulatory processes on sodium reabsorption.

DR. BANK: I did not intend to imply that diabetic hyperfiltration can be accounted for solely by glucose-stimulated sodium and water reabsorption, as several other important abnormalities co-exist that could contribute to reduced vascular tone. However, sodium and water retention might account for decreased renin-angiotensin levels and the elevation of ANP in diabetics. It is difficult to compare degrees of sodium retention in diabetes versus primary hyperaldosteronism, because careful balance studies under similar controlled conditions have not been carried out to compare these two conditions. In fact, studies of diabetic patients vary considerably in their findings. The most consistent finding in diabetes has been an increase in total-body sodium, whereas intravascular volume has been reported to be increased in some studies but not in others. Capillary leakage of proteins might account for some of the discrepancies among published reports and also might result in expansion of interstitial volume at the expense of plasma volume. It is clear that both in diabetes and hyperaldosteronism, a new steady state of sodium and water balance must be reached. What the counterregulatory mechanisms are is not clear, although elevation of ANP could play a role in both conditions. Hypertension in primary hyperaldosteronism also could be a factor in the "escape" phenomenon. The level of increase in total-body sodium at the time that balance is restored could depend on these counterregulatory mechanisms. In addition, glucose-mediated sodium reabsorption is quantitatively much greater than aldosterone-mediated sodium reabsorption, because the former takes place in the proximal convoluted tubule, whereas the latter occurs in more distal segments of the nephron. But whether this difference results in greater total-body sodium in diabetes versus hyperaldosteronism is not clear. It is generally held that the volume expansion due to primary hyperaldosteronism, chronic DOCA administration, and SIADH is associated with hyperfiltration, but the increase in GFR is not as great as in diabetes.

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Evidence has implicated an increase in plasma ANP concentration in the phenomenon of mineralocorticoid escape [117].

DR. BANK: Atrial natriuretic peptide secretion is generally increased in volume-expanded states, and ANP might be implicated in the "escape" phenomenon seen with chronic mineralocorticoid administration. In diabetes, ANP has been found to be elevated, both in humans and experimental animals. Although not proven, the elevated ANP might play a role in limiting progressive volume expansion by inhibiting distal sodium reabsorption.

DR. JEROME P. KASSIRER (*Associate Physician-in-Chief, New England Medical Center, Boston, Massachusetts*): What is the clinical significance of the increase in filtration in early diabetes? Is there a more rapid decline in renal function in these patients as compared with those whose blood sugars are well controlled and who do not have hyperfiltration?

DR. BANK: There are few published studies in which diabetics with and without hyperfiltration were followed over a period of years to determine whether early hyperfiltration eventuated in the development of nephropathy [8, 9, 100]. Although the number of patients studied was small, there was a definite correlation between early hyperfiltration and the onset of proteinuria some years later. In my view, more patients need to be studied longitudinally to confirm these findings.

DR. COHEN: In type-II diabetes, insulin levels often are quite high. This fact might be useful in pin-pointing the role of insulin per se in hyperfiltration. Is hyperfiltration a feature of the early course of patients with type-II diabetes?

DR. BANK: Renal function data in early type-II diabetes has been more difficult to come by, because the onset of diabetes is more difficult to date. Moreover, type-II diabetes, which occurs mostly in older individuals, is often complicated by other illnesses, such as essential hypertension or cardiovascular disease, which could affect glomerular filtration rate. However, a recent abstract reported elevated GFR and renal blood flow in patients with poorly controlled type-II diabetes [118].

DR. PAUL KURTIN (*Director, Dialysis Unit, New England Medical Center*): To follow up on that question, are there any glucose-insulin clamp studies that separate out the effects of insulinopenia from those of hyperglycemia?

DR. BANK: In a 1981 study, insulin was infused into type-I diabetics, and glucose was either allowed to fall into the normal range or was "clamped" at a fixed level [102]. Glomerular filtration rates fell only when glucose was allowed to fall, but not when the blood glucose level was "clamped." This study suggests that insulin is not itself responsible for the hyperfiltration.

DR. JAMES STROM (*Department of Nephrology, St. Elizabeth's Hospital of Boston, Brighton, Massachusetts*): Could one correct the hyperfiltration by inducing negative sodium balance in the animals, for example, with diuretics?

DR. BANK: In our study of a low-sodium diet in diabetic rats [17], a natriuresis did occur during the first day that dietary sodium was reduced. This was not observed in normal controls. We did not administer a diuretic, and the mechanism for the sodium loss was not clear. It might have been due to ANP, which is elevated in diabetic rats. In any case GFR, when measured several days later, had fallen into the normal range. Perhaps a similar result would occur with administration of a diuretic, although we have not performed that experiment. We believe that the fall in GFR was mediated by stimulation of the renin-angiotensin system, as saralasin infusion caused GFR to rise again to hyperfiltration levels. I am not aware of any human studies in which either a low-sodium diet or diuretics have been tried for diabetic hyperfiltration.

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): You mentioned that ANP increases resistance in the efferent arteriole, just as angiotensin II does. If a maximum amount of angiotensin II is present at the efferent arteriole, does ANP have an additive effect?

DR. BANK: I have not seen such a study. It is well established that ANP generally acts as an antagonist to the renin-angiotensin system, and that it counteracts the vasoconstrictor effect of angiotensin II on vascular smooth muscle. An exception is the efferent arteriole, where both ANP and angiotensin II appear to cause constriction. The second messenger systems for these two hormones are different. Angiotensin II activates phospholipase C, which generates inositol phosphate isomers and DAG, and also increases the influx of extracellular calcium. Atrial natriuretic hormone, on the other hand, activates guanylate cyclase, and leads to a rise in intracellular cyclic GMP. One might anticipate, based on these considerations, that the effects of angiotensin II and ANP on the efferent arteriole might be additive.

DR. MADIAS: I would like to return to the sodium issue. In your experiments, placing hyperfiltering rats with streptozotocin-induced diabetes on a low-salt diet returned GFR to the normal range. I was wondering whether you have observations on animals being placed on salt restriction concomitantly with the induction of the diabetic state to assess whether prevention of volume expansion prevents the development of hyperfiltration and subsequent renal disease.

DR. BANK: That experiment has not been tried, to my knowledge.

DR. MADIAS: I find the experiment by Ortola and colleagues very impressive in suggesting that ANP might be important in the hyperfiltration of experimental diabetes. In discountenancing its potential role, you cited the disparate hemodynamic effects of ANP when it is administered to normal subjects as compared with the hemodynamic profile of the diabetic state. I would argue, however, that the hemodynamic effects of ANP might be different when administered to diabetic animals. Do we know what the hemodynamic effects of ANP are when it is administered to diabetic animals with euglycemia, a state in which plasma levels of endogenous ANP are normal?

DR. BANK: I am not aware of experiments in which ANP has been administered to euglycemic diabetic animals. Hyperfiltration and endogenous ANP levels become normal when diabetic rats are made euglycemic by long-acting insulin [54]. It would be interesting to infuse ANP under those conditions to determine whether hyperfiltration recurs. One would have to be careful to administer doses within the range found in hyperfiltering diabetic animals. Larger, pharmacologic doses in normal animals induce hyperfiltration, whereas physiologic doses of ANP have relatively small effects on GFR.

DR. RONALD PERRONE (*Divison of Nephrology, New England Medical Center*): You've indicated that you believe hyperglycemia to be the ultimate explanation for all of the hyperfiltration in early diabetes. Yet insulin pump therapy doesn't always normalize GFR and doesn't reverse renal hypertrophy [99]. Do you think that's because the insulin pump doesn't yield perfect glycemic control, or do you think other factors are involved?

DR. BANK: In most studies on glycemic control, both in humans and rats with diabetes, GFR did fall to or toward the normal range. The weight of evidence is that this takes time to occur, that is, days to weeks. There are fewer reports of renal hypertrophy being reversed by control of hyperglycemia. Some studies have shown regression of hypertrophy, whereas others have not. These discrepancies might be due in part to the inexact methods used for measuring renal size in humans. Alternatively, renal hypertrophy, once established, might be permanent or take a very long time to regress.

DR. KURTIN: I'd like to return to Dr. Cohen's first question.

Glucocorticoids do increase GFR. Children with minimal-change disease treated with glucocorticoids can have a GFR as high as 180–200 ml/min/1.73 m^2 (personal observation). Would you please comment on hyperfiltration that might be seen with glucocorticoids as compared with mineralocorticoids?

DR. BANK: Glucocorticoids are known to have a direct effect on renal vasculature, causing vasodilation and increases in GFR. The issue raised by Dr. Cohen was whether excess mineralocorticoids, that is, aldosterone and DOCA, which lead to volume expansion, cause marked elevations of GFR, as in diabetics. These agents do not, but on the other hand, volume expansion is probably not as great as in diabetes.

DR. KURTIN: Dr. Lance Dworkin has reviewed various hormonal effects on GFR. Glucocorticoids increase GFR via an increase in blood flow. This effect can be seen acutely and thus might not be a volume effect [119].

DR. HARRINGTON: I'd like to return to Dr. Kassirer's question. Why do we care about the hyperfiltration? You mentioned that approximately 45% of patients with diabetes develop renal failure, yet the remaining 55% do not. Is there a substantial difference in the degree of early hyperfiltration between those who progress and those who do not?

DR. BANK: As I mentioned, a few studies have followed diabetic patients from the early onset of diabetes over 7 to 10 years. The patients who had higher GFR values at the beginning of the observation period developed proteinuria and declining GFR, as compared with patients who initally had a more normal GFR. Because of the evidence I presented that improved glycemic control lowers GFR, we might assume that the hyperfiltering patients also had poor glycemic control. If that assumption is correct, then multiple abnormalities associated with poor glycemic control could have contributed to developing diabetic nephropathy, not necessarily hyperfiltration alone. In other words, the hyperfiltration might be a marker of poor glycemic control. The patient discussed today exemplifies this situation. He had hyperfiltration, but he also had very poor glycemic control and persistently elevated glycosylated hemoglobin. It is therefore difficult to attribute his progressive proteinuria and renal impairment to hyperfiltration alone. Another factor that pertains to this discussion is the marked difference between blacks and whites in the development of end-stage renal failure due to diabetes. It is not known whether the higher incidence in blacks is due to hereditary factors or to substandard medical care.

DR. RONALD LECHAN (*Division of Endocrinology, New England Medical Center*): Cytokines such as IL-6 have been identified in endothelial cells as well as in vascular smooth muscle cells. Could they be contributing to renal blood flow, and might they be affected by hyperglycemia?

DR. BANK: I have not seen any studies on vascular IL-6 effects on renal blood flow, nor on effects of hyperglycemia on IL-6.

DR. ANDREW S. LEVEY (*Division of Nephrology, New England Medical Center*): I would like to again refer to the question of hyperfiltration and its correlation with the progression to renal disease. Do all diabetics with early-onset disease manifest a phase of hyperfiltration, or does this only occur in the subpopulation that later develops nephropathy?

DR. BANK: The patients who had hyperfiltration developed evidence of nephropathy, whereas those with lower GFRs did not, at least during the time they were followed.

DR. LEVEY: That's a little different than what I meant. In the study by Mogensen and Christiansen, patients were initially selected on the basis of whether their renal disease had progressed; the two groups were then compared [120]. My question is, among a cohort of patients with new-onset diabetes, is the distribution of GFR normal or biomodal, and does a larger subgroup have hyperfiltration?

DR. BANK: To my knowledge, GFR has not been measured in a sufficiently large number of patients with recent-onset diabetes to allow us to define its distribution. The published data include a considerable spread in values, the mean being higher than in normal controls. There does not seem to be a bimodal distribution, but rather a normal distribution that is displaced toward higher values. The data have to be evaluated, however, in light of previous insulin therapy and glycemic control, because both could influence the GFR.

DR. HARRINGTON: Can you relate the rise in GFR to differences in blood sugar, hemoglobin A1C, or anything else? You might not be able to do such a study because as the hyperglycemia is corrected, the GFR also is corrected. You would, in fact, probably need to measure the GFR on day 1.

DR. BANK: That is correct. Most of the measurements reported in the literature have been in treated diabetics with variable degrees of glycemic control. Christiansen et al measured GFR in patients during the first week of insulin therapy [101]. In that study, all patients were hyperfiltering before receiving insulin, and GFR fell with insulin treatment. This suggests that in untreated diabetes, hyperfiltration is quite common, and that measurements made after treatment has been started will vary according to the level of glycemic control. In experimental rats with streptozotocin-induced diabetes, hyperfiltration is uniformly present when blood glucose ranges between 300 and 400 mg/dl, but filtration becomes normal after euglycemia has been achieved by insulin therapy.

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References

1. CHRISTIANSEN JS, GAMMELGAARD J, FRANDSEN M, PARVING H-H: Increased kidney size, glomerular filtration rate and renal plasma flow in short-term insulin-dependent diabetics. *Diabetologia* 20:451–456, 1981
2. DITZEL J, JUNKER K: Abnormal glomerular filtration rate, renal plasma flow, and renal protein excretion in recent and short-term diabetics. *Br Med J* 2:13–19, 1972
3. MOGENSEN CE: Glomerular filtration rate and renal plasma flow in short-term and long-term diabetes mellitus. *Scand J Clin Lab Invest* 28:91, 1971
4. MOGENSEN CE, ANDERSEN MJF: Increased kidney size and glomerular filtration rate in untreated juvenile diabetes: Normalization by insulin-treatment. *Diabetologia* 11:221–224, 1975
5. MOGENSEN CE, ANDERSEN MJF: Increased kidney size and glomerular filtration rate in early juvenile diabetes. *Diabetes* 22:706–712, 1973
6. BROCHNER-MORTENSEN J, DITZEL J: Glomerular filtration rate and extracellular fluid volume in insulin-dependent patients with diabetes mellitus. *Kidney Int* 21:696–698, 1982

7. BROCHNER-MORTENSEN J: Glomerular filtration rate and extracellular fluid volumes during normoglycemia and moderate hyperglycemia in diabetics. *Scand J Clin Lab Invest* 32:311–316, 1973
8. MOGENSEN CE: Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy. *Scand J Clin Lab Invest* 46:201–206, 1986
9. MOGENSEN CE, CHRISTENSEN CK, CHRISTIANSEN JS, BOYE N, PEDERSEN MM, SCHMITZ A: Early hyperfiltration and late renal damage in insulin-dependent diabetes. *Pediatr Adol Endocrinol* 17:197–205, 1988
10. MORDEN G, BJORCK S, GRANERUS G, NYBERG G: Estimation of renal function in diabetic nephropathy. *Nephron* 47:36–42, 1987
11. HOSTETTER TH, TROY JL, BRENNER BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–415, 1981
12. JENSEN PK, CHRISTIANSEN JS, STEVEN K, PARVING H-H: Renal function in streptozotocin-diabetic rats. *Diabetologia* 21:409–414, 1981
13. MICHELS LD, DAVIDMAN M, KEANE WF: Determinants of glomerular filtration and plasma flow in experimental diabetic rats. *J Lab Clin Med* 98:869–885, 1981
14. JENSEN PK, CHRISTIANSEN JS, STEVEN K, PARVING H-H: Strict metabolic control and renal function in the streptozotocin diabetic rat. *Kidney Int* 31:47–51, 1987
15. BANK N, LAHORRA MA, AYNEDJIAN HS: Acute effect of calcium and insulin on hyperfiltration of early diabetes. *Am J Physiol 252* (Renal Fluid Electrolyte Physiol 23):E13–E20, 1987
16. BANK N, LAHORRA MAG, AYNEDJIAN HS, SCHLONDORFF D: Vasoregulatory hormones and the hyperfiltration of diabetes. *Am J Physiol 254* (Renal Fluid Electrolyte Physiol 23):F202–F209, 1988
17. BANK N, LAHORRA MAG, AYNEDJIAN HS, WILKES BM: Sodium restriction corrects hyperfiltration of diabetes. *Am J Physiol 254* (Renal Fluid Electrolyte Physiol 23):F668–F676, 1988
18. BANK N, MOWER P, AYNEDJIAN HS, WILKES BM, SILVERMAN S: Sorbinil prevents glomerular hyperperfusion in diabetic rats. *Am J Physiol 256* (Renal Fluid Electrolyte Physiol 25):F1000–F1006, 1989
19. JENSEN PK, STEVEN K, BLAEHR H, CHRISTIANSEN JS, PARVING H-H: Effects of indomethacin on glomerular hemodynamics in experimental diabetes. *Kidney Int* 29:490–495, 1986
20. BARNETT R, SCHARSCHMIDT L, KO Y-H, SCHLONDORFF D: Comparison of glomerular and mesangial prostaglandin synthesis and glomerular contraction in two rat models of diabetes mellitus. *Diabetes* 36:1468–1475, 1987
21. SCHAMBELAN M, BALKE S, SRAER J, BENS M, NIVEZ MP, WAHBE F: Increased prostaglandin production by glomeruli isolated from rats with streptozotocin-induced diabetes mellitus. *J Clin Invest* 75:404–412, 1985
22. KREISBERG JI, PATEL PY: The effects of insulin, glucose and diabetes on prostaglandin production by rat kidney glomeruli and cultured glomerular mesangial cells. *Prostaglandins Leukot Med* 11:431–442, 1983
23. CRAVEN PA, CAINES MA, DERUBERTIS FR: Sequential alterations in glomerular prostaglandin and thromboxane synthesis in diabetic rats: Relationship to the hyperfiltration of early diabetes. *Metabolism* 36:95–103, 1987
24. MOEL DI, SAFIRSTEIN RL, MCEVOY RC, HSUEH EW: Effect of aspirin on experimental diabetic nephropathy. *J Lab Clin Med* 110:300–307, 1987
25. KIRSCHENBAUM MA, CHAUDHARI A: Effect of experimental diabetes on glomerular filtration rate and glomerular prostanoid production in the rat. *Miner Electrolyte Metab* 12:352–355, 1986
26. ESMATJES E, FERNANDEZ MR, HALPERIN I, CAMPS J, GAYA J, ARROYO V, RIVERA F, FIGUEROLA D: Renal hemodynamic abnormalities in patients with short-term insulin-dependent mellitus: Role of renal prostaglandins. *J Clin Endocrinol Metab* 60:1231–1236, 1985
27. HOMMEL E, MATHIESEN E, ARNOLD-LARSEN S, EDSBERG B, OLSEN UB, PARVING H-H: Effects of indomethacin on kidney function in Type 1 (insulin-dependent) diabetic patients with nephropathy. *Diabetologia* 30:78–81, 1987
28. CHRISTIANSEN JS, FELDT-RASMUSSEN B, PARVING H-H: Short-term inhibition of prostaglandin synthesis has no effect on the elevated glomerular filtration rate of early insulin-dependent diabetes. *Diabetic Med* 2:17–20, 1985
29. WAKABAYASHI I, HATAKE K, KIMURA N, KAKISHITA E, NAGAI K: Modulation of vascular tonus by the endothelium in experimental diabetes. *Life Sci* 40:643–648, 1987
30. OYAMA Y, KAWASAKI H, HATTORI Y, KANNO M: Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. *Eur J Pharmacol* 131:75–78, 1986
31. MERAJI S, JAYAKODY L, SENARATINE MPJ, THOMSON ABR, KAPPAGODA T: Endothelium-dependent relaxation in aorta of BB rat. *Diabetes* 36:978–981, 1987
32. TESFAMARIAM B, JAKUBOWSKI JA, COHEN RA: Contraction of diabetic rabbit aorta caused by endothelium-derived PGH_2-TxA_2. *Am J Physiol* 257:H1327–H1333, 1989
33. TESFAMARIAM B, BROWN ML, DEYKIN D, COHEN RA: Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. *J Clin Invest* 85:929–932, 1990
34. KATAYAMA S, INABA M, MARUNO Y, OMOTO A, KAWAZU S, ISHII J: Increased thromboxane B_2 excretion in diabetes mellitus. *J Lab Clin Med* 109:711–719, 1987
35. KATAYAMA S, INABA M, MARUNO Y, OMOTO A, KAWAZU S, ISHII J, SAWADA M: Increased renal TxA_2 synthesis in diabetes mellitus: Simultaneous determination of urinary TXB_2 and 2,3-Dinor-TXB_2. *Prostaglandins Leukot Essent Fatty Acids* 39:47–51, 1990
36. KAPLAN R, AYNEDJIAN HS, SCHLONDORFF D, BANK N: Renal vasoconstriction caused by short-term cholesterol feeding is corrected by thromboxane antagonist or probucol. *J Clin Invest* 86:1707–1714, 1990
37. WILKES B, MENTO P, MACICA C, SOLOMON J: Reduced thromboxane (TX) receptors in diabetic glomeruli: relationship to hyperfiltration (*abstract*). *Am Soc Nephrol*, 1989, p 336a
38. YANAGISAWA-MIWA A, HIDEKI I, SUGIMOTO T: Effects of insulin on vasoconstriction induced by thromboxane A_2 in porcine coronary artery. *Circulation* 81:1654–1659, 1990
39. JAFFA AA, MILLER DH, BAILEY GS, CHAO J, MARGOLIUS HS, MAYFIELD RK: Abnormal regualtion of renal kallikrein in experimental diabetes. *J Clin Invest* 80:1651–1659, 1987
40. HARVEY JN, JAFFA AA, MARGOLIUS HS, MAYFIELD RK: Renal kallikrein and hemodynamic abnormalities of diabetic kidney. *Diabetes* 39:299–304, 1990
41. CHRISTIANSEN NJ: Plasma catecholamines in long-term diabetics with and without neuropathy and in hypophysectomized subjects. *J Clin Invest* 51:779–787, 1972
42. BERETTA-PICCOLI C, WEIDMANN P, ZIEGLER W, GLUCK Z, KEUSCH G: Plasma catecholamines and renin in diabetes mellitus. *Klin Wochenschr* 57:681–691, 1979
43. RAMANADHAM S, LYNESS WH, TENNER TE: Alterations in aortic and tail artery reactivity to agonists after streptozotocin treatment. *Can J Physiol Pharmacol* 62:418–423, 1984
44. MACLEOD KM, MCNEILL JH: The influence of chronic experimental diabetes on contractile responses of rat isolated blood vessels. *Can J Physiol Pharmacol* 63:52–57, 1985
45. LONGHURST PA, HEAD RJ: Responses of the isolated perfused mesenteric vasculature from diabetic rats: The significance of appropriate control tissues. *J Pharmacol Exp Ther* 235:45–49, 1985
46. FRIEDMAN JJ: Vascular sensitivity and reactivity to norepinephrine in diabetes mellitus. *Am J Physiol* 256:H1134–H1138, 1989
47. MUELLER SM, MUELLER TM, ERTEL PJ: Sympathetic and vascular dysfunction in early experimental juvenile diabetes mellitus. *Am J Physiol 243* (Heart Circ Physiol 12):H139–H144, 1982
48. OWEN MP, CARRIER GO: Calcium dependence on norepinephrine-induced vascular contraction in experimental diabetes. *J Pharmacol Exp Ther* 212:253–258, 1980
49. MORFF RJ: Microvascular reactivity to norepinephrine at different arteriolar levels and durations of streptozocin-induced diabetes. *Diabetes* 39:354–360, 1990
50. CHRISTLIEB AR: Vascular reactivity to angiotensin II and norepinephrine in diabetic subjects. *Diabetes* 25:268–274, 1976

51. Beretta-Piccoli C, Weidmann P: Exaggerated pressor responsiveness to norepinephrine in nonazotemic diabetes mellitus. *Am J Med* 71:829–835, 1981
52. Christlieb AR: Renin, angiotensin, and norepinephrine in alloxan diabetes. *Diabetes* 23:962–970, 1974
53. Schmidt RE, Scharp DW: Axonal dystrophy in experimental diabetic autonomic neuropathy. *Diabetes* 31:761–770, 1982
54. Ortola FV, Ballerman BJ, Andersen S, Mendez RE, Brenner BM: Elevated plasma atrial natriuretic peptide levels in diabetic rats. *J Clin Invest* 80:670–674, 1987
55. Laragh JH, Atlas SA: Atrial natriuretic hormone: A regulator of blood pressure and volume homeostasis. *Kidney Int* 34:S64–S-71, 1988
56. Bell GM, Bernstein RK, Laragh JH, Atlas SA, James GD, Pecker MS, Sealey JE: Increased plasma atrial natriuretic factor and reduced plasma renin in patients with poorly controlled diabetes mellitus. *Clin Sci* 77:177–182, 1989
57. Maack T, Marion DN, Camargo MJF, Kleinert HD, Laragh JH, Vaughan ED, Atlas SA: Effects of auriculin (atrial natriuretic factor) on blood pressure, renal function, and the renin-aldosterone system in dogs. *Am J Med* 77:1069–1075, 1984
58. Henrich WL, McAllister EA, Smith PB, Campbell WB: Guanosine 3′,5′-cyclic monophosphate as a mediator of inhibition of renin release. *Am J Physiol 255* (Renal Fluid Electrolyte Physiol 24):F474–F478, 1988
59. Atarashi K, Mulrow PJ, Franco-Saenz R: Effect of atrial peptides on aldosterone production. *J Clin Invest* 76:1807–1811, 1985
60. Dunn BR, Ichikawa I, Pfeffer JM, Troy JL, Brenner BM: Renal and systemic hemodynamic effects of synthetic atrial natriuretic peptide in the anesthetized rat. *Circ Res* 59:237–246, 1986
61. Maack T, Atlas SA, Camargo MJD, Cogan MG: Renal hemodynamic and natriuretic effects of atrial natriuretic factor. *Fed Proc* 45:2128–2132, 1986
62. Huang C-L, Lewicki J, Johnson LK, Cogan MG: Renal mechanism of action of rat atrial natriuretic factor. *J Clin Invest* 75:769–773, 1985
63. Schnermann J, Marin-Grez M, Briggs JP: Filtration pressure response to infusion of atrial natriuretic peptides. *Pflugers Arch* 406:237–239, 1986
64. Marin-Grez M, Fleming JT, Steinhausen M: Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. *Nature* 324:473–476, 1986
65. Cottier C, Matter L, Weidmann P, Shaw S, Gnädinger MP: Renal response to low-dose infusion of atrial natriuretic peptide in normal man. *Kidney Int* 34(suppl 25):S72–S78, 1988
66. Kleinert HD, Maack T, Atlas SA, Januszewicz A, Sealey JE, Laragh JH: Atrial natriuretic factor inhibits angiotensin-norepinephrine-, and potassium-induced vascular contractility. *Hypertension* 6 (suppl I):I143–I147, 1984
67. Christlieb AR, Assal J-P, Katsilambros N, Wiliams GH, Kozak GP, Suzuki T: Plasma renin activity and blood volume in uncontrolled diabetes. *Diabetes* 24:190–193, 1975
68. Ballerman BJ, Skorecki KL, Brenner BM: Reduced glomerular angiotensin II receptor density in early untreated diabetes mellitus in the rat. *Am J Physiol* 247:F110–F116, 1984
69. Bjorck S: The renin angiotensin system in diabetes mellitus. *Scand J Urol Nephrol*, suppl 126:1–51, 1990
70. Hill MA, Larkins RG: Altered microvascular reactivity in streptozotocin-induced diabetes in rats. *Am J Physiol 257* (Heart Circ Physiol 26):H1438–H1445, 1989
71. Pfaffman MA, Ball CR, Darby A, Hilman R: Insulin reversal of diabetes-induced inhibition of vascular contractility in the rat. *Am J Physiol* 242:H490–H495, 1982
72. Kreisberg JI: Insulin requirement for contraction of cultured rat glomerular mesengial cells in response to angiotensin II: Possible role for insulin in modulating glomerular hemodynamics. *Proc Natl Acad Sci USA* 79:4190–4192, 1982
73. Cohen AJ, Laurens P, Fray JCS: Suppression of renin secretion by insulin: dependence on extracellular calcium. *Am J Physiol* 245:E531–E534, 1984
74. Mueller SM: Insulin treatment prevents vascular dysfunction in early juvenile alloxan-induced diabetes mellitus. *Am J Physiol* 247:H132–H138, 1984
75. Goetzsche O: Abnormal myocardial calcium uptake in streptozotocin-diabetic rats. *Diabetes* 34:287–290, 1984
76. Greene DA, Lattimer SA, Sima AAF: Sorbitol, phosphoinositides, and sodium-potassium-ATPase in the pathogenesis of diabetic complications. *N Engl J Med* 316:599–606, 1987
77. Kamm KE, Stull JT: Regulation of smooth muscle contractile elements by second messengers. *Annu Rev Physiol* 51:299–313, 1989
78. Lee T-S, MacGregor LC, Fluharty SJ, King GL: Differential regulation of protein kinase C and (Na, K)-adenosine triphosphatase activities by elevated glucose levels in retinal capillary endothelial cells. *J Clin Invest* 83:90–94, 1989
79. Lee T-S, Saltsman KA, Ohashi H, King GL: Activation of protein kinase C by elevation of glucose concentration: Proposal for a mechanism in the development of diabetic vascular complications. *Proc Natl Acad Sci USA* 86:5141–5145, 1989
80. Craven PA, DeRubertis PR: Protein kinase C is activated in glomeruli from diabetic rats: Possible mediation by glucose. *J Clin Invest* 83:1667–1675, 1989
81. Clements RS: The polyol pathway. A historical review. *Drugs* 32 (suppl 2):3–5, 1986
82. Jacobson M, Sharma YR, Cotlier E, Hollander JD: Diabetic complications in lens and nerve and their prevention by sulindac or sorbinil: Two novel aldose reductase inhibitors. *Invest Ophthalmol Vis Sci* 24:1426–1429, 1983
83. Greene DA, Lattimer SA: Impaired rat sciatic nerve sodium-potassium adenosine triphosphatase in acute streptozotocin diabetes and its correction by dietary myo-inositol supplementation. *J Clin Invest* 72:1058–1063, 1983
84. MacGregor LC, Matschinsky FM: Treatment with aldose reductase inhibitor or with myo-inositol arrests deterioration of the electroretinogram of diabetic rats. *J Clin Invest* 76:887–889, 1985
85. Ludvigson MA, Sorenson RL: Immunohistochemical localization of aldose reductase. II. Rat eye and kidney. *Diabetes* 29:450–459, 1980
86. Terubayashi H, Sato S, Nishimura C, Kador PF, Kinoshita JN: Localization of aldose and aldehyde reductase in the kidney. *Kidney Int* 36:843–851, 1989
87. Beyer-Mears A, Ku L, Cohen MP: Glomerular polyol accumulation in diabetes and its prevention by oral sorbinil. *Diabetes* 33:604–607, 1984
88. Kikkawa R, Umemura K, Haneda M, Arimura T, Ebata K, Shigeta Y: Evidence for existence of polyol pathway in cultured rat mesangial cells. *Diabetes* 36:240–243, 1987
89. Williamson JR, Ostrow E, Eades D, Chang K, Allison W, Kilo C, Sherman WR: Glucose-induced microvascular functional changes in nondiabetic rats are stereospecific and are prevented by an aldose reductase inhibitor. *J Clin Invest* 85:1167–1172, 1990
90. Goldfarb S, Simmons DA, Kern EFO: Amelioration of glomerular hyperfiltration in acute experimental diabetes mellitus by dietary myo-inositol supplementation and aldose reductase inhibition. *Trans Assoc Am Physicians* 99:67–72, 1986
91. Craven PA, DeRubertis FR: Sorbinil suppresses glomerular prostaglandin production and reduces hyperfiltration in the streptozotocin diabetic rat (*abstract*). *Clin Res* 36:517a, 1988
92. Frey J, Zager P, Jackson J, Eaton P, Scavini M: Aldose-reductase activity mediates renal prostaglandin production in streptozotocin diabetic rats. (*abstract*). *Kidney Int* 35:292, 1989
93. Williamson JR, Chang K, Tilton RG, Prater C, Jeffrey JR, Weigel C, Sherman WR, Eades DM, Kilo C: Increased vascular permeability in spontaneously diabetic BB/W rats and in rats with mild versus severe streptozotocin-induced diabetes. *Diabetes* 36:813–821, 1987
94. Tilton RG, Chang K, Pugliese G, Eades DM, Province MA, Sherman WR, Kilo C, Williamson JR: Prevention of hemodynamic and vascular albumin filtration changes in diabetic rats by aldose reductase inhibitors. *Diabetes* 37:1258–1270, 1989
95. Cohen MP, Klepser H: Glomerular Na^+-K^+-ATPase activity in

acute and chronic diabetes with aldose reductase inhibition. *Diabetes* 37:558–562, 1988
96. DANIELS BS, HOSTETTER TH: Aldose reductase inhibition and glomerular abnormalities in diabetic rats. *Diabetes* 38:981–986, 1989
97. GRAY SD: Effect of hypertonicity on vascular dimensions in skeletal muscle. *Microvasc Res* 3:117–124, 1971
98. MOGENSEN CE: Kidney function and glomerular permeability to macromolecules in early juvenile diabetes. *Scand J Clin Lab Invest* 28:79–90, 1971
99. WISEMAN MJ, SAUNDERS AJ, KEEN H, VIBERTI GC: Effect of blood glucose control on increased glomerular filtration rate and kidney size in insulin-dependent diabetes. *N Engl J Med* 312:617–621, 1985
100. CHRISTIANSEN JS: Early renal hyperfunction and hypertrophy in insulin-dependent patients: changes found at diagnosis and early in the course of diabetes, in *The Kidney and Hypertension in Diabetes Mellitus*, edited by MOGENSEN CE, Boston, Academic Publishers, 1988, p 157
101. CHRISTIANSEN JS, GAMMELGAARD J, TRONIER B, SVENDSEN PA, PARVING H-H: Kidney function and size in diabetics before and during initial insulin treatment. *Kidney Int* 21:683–688, 1982
102. CHRISTIANSEN JS, FRANDSEN M, PARVING H-H: The effect of intravenous insulin infusion on kidney function in insulin-dependent diabetes mellitus. *Diabetologia* 20:199–204, 1981
103. RICHARDS AM, DONNELLY T, NICHOLLS MG, IKRAM H, HAMILTON EJ, ESPINER EA: Blood pressure and vasoactive hormones with improved glycaemic control in patients with diabetes mellitus. *Clin Exp Hypertens [A]* 11(3):391–406, 1989
104. JENSEN PK, CHRISTIANSEN JS, STEVEN K, PARVING H-H: Strict metabolic control and renal function in the streptozotocin diabetic rat. *Kidney Int* 31:47–51, 1987
105. CHRISTIANSEN JS, CHRISTENSEN CK, HERMANSEN K, PEDERSEN EB, MOGENSEN CE: Enhancement of glomerular filtration rate and renal plasma flow by oral glucose load in well controlled insulin-dependent diabetics. *Scand J Clin Lab Invest* 46:265–272, 1986
106. WISEMAN MJ, MANGILI R, ALBERETTO M, KEEN H, VIBERTI GC: Glomerular response mechanisms to glycemic changes in insulin-dependent diabetics. *Kidney Int* 31:1012–1018, 1987
107. KASISKE BL, O'DONNELL MP, KEANE WF: Glucose-induced increases in renal hemodynamic function. Possible modulation by renal prostaglandins. *Diabetes* 34:360–364, 1985
108. MOGENSEN CE: Glomerular filtration rate and renal plasma flow in normal and diabetic man during elevation of blood sugar levels. *Scand J Clin Lab Invest* 28:177–182, 1971
109. BLANTZ RC, PETERSON OW, GUSHWA L, TUCKER BJ: Effect of modest hyperglycemia on tubuloglomerular feedback activity. *Kidney Int* 22:S206–S212, 1982
110. WOODS LL, MIZELLE HL, HALL JE: Control of renal hemodynamics in hyperglycemia: possible role of tubuloglomerular feedback. *Am J Physiol* 252:F65–F73, 1987
111. DITZEL J, BROCHNER-MORTENSEN J: Tubular reabsorption rates as related to elevated glomerular filtration in diabetic children. *Diabetes 32* (suppl 2):28–33, 1983
112. BROCHNER-MORTENSEN J, STOCKEL M, SORENSEN PJ, NIELSEN AH, DITZEL J: Proximal glomerulo-tubular balance in patients with Type I (insulin-dependent) diabetes mellitus. *Diabetologia* 27:189–192, 1984
113. HANNEDOUCHE TP, DELGADO AG, GNIONSAHE DA, BOITARD C, LACOUR B, GRÜNFELD J-P: Renal hemodynamics and segmental tubular reabsorption in early type I diabetes. *Kidney Int* 37:1126–1133, 1990
114. BANK N, AYNEDJIAN HS: Progressive increases in luminal glucose stimulate proximal sodium absorption in normal and diabetic rats. *J Clin Invest* 86:309–316, 1990
115. O'HARE JA, FERRISS JB, BRADY D, TWOMEY B, O'SULLIVAN DJ: Exchangeable sodium and renin in hypertensive diabetic patients with and without nephropathy. *Hypertension* 7:II43–II48, 1985
116. BERETTA-PICCOLI C, WEIDMANN P: Body sodium-blood volume state in nonazotemic diabetes mellitus. *Miner Electrolyte Metab* 7:36–47, 1982
117. BALLERMAN BJ, BLOCH KD, SEIDMAN JG, BRENNER BM: Atrial natriuretic peptide transcription, secretion, and glomerular receptor activity during mineralocorticoid escape in the rat. *J Clin Invest* 788:840–843, 1986
118. BRUTON JL, PERUSEK MC, LANCASTER JL, KOPP DT, TUTTLE KR: Effects of glycemia on basal and amino acid-stimulated (AA-S) renal hemodynamics and kidney size in non-insulin dependent diabetes (NIDD) (*abstract*). *JASN* 1:623, 1990
119. DWORKIN L, ICHIKAWA I, BRENNER BM: Hormonal modulations of glomerular function. *Am J Physiol* 244:F95–F104, 1983
120. MOGENSEN JS, CHRISTIENSEN CE: Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 311:89–93, 1984