# Insulin-Like Growth Factor-I (IGF-I) and IGF-I Receptor Gene Expression in the Kidney of the Chronically Hypoinsulinemic Rat and Hyperinsulinemic Rat

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**Acute streptozotocin (STZ)-induced diabetes in rats causes a transient increase in insulin-like growth factor-I (IGF-I) in the kidney, followed by a rapid renal hypertrophy and constant renal hyperperfusion. However, renal IGF-I levels return to normal within 4 days. Thus, hyperperfusion, which is independent of renal hypertrophy of the chronically diabetic kidney, is not explained by increased renal IGF-I. We studied IGF-I and IGF-I receptor gene expression in the kidney of rats with long-standing STZ-induced diabetes. IGF-I mRNA level in the chronically diabetic kidney was approximately 50% of that in control rats, whereas IGF-I receptor mRNA was increased approximately threefold. Ten days' treatment with insulin 65 days after induction of diabetes resulted in a glucose-dependent decrease in IGF-I receptor mRNA. Chronic hyperinsulinemia with near normoglycemia did not change gene expression of either IGF-I or IGF-I receptor. The studies suggest that glucose levels per se, independent of insulin levels, play an important role in the regulation of IGF-I receptor gene expression in the chronically diabetic kidney. Furthermore, kidney hyperperfusion in chronic diabetes is coupled with the increase in IGF-I receptor mRNA, despite normal kidney IGF-I levels.**



NEPHROPATHY IS ONE of the major complications of both insulin-dependent and non–insulin-dependent diabetes mellitus.[1] Early nephropathy is associated with an expansion of the mesangium, which results in a reduction of the glomerular capillary luminal space and, as a consequence, of the filtration surface and rate. Insulin-like growth factor-I (IGF-I) level was shown to increase in rat kidney tissue immediately after induction of diabetes and seems to be the main cause of kidney hypertrophy,[2] as well as of the significant increase in glomerular filtration rate and kidney plasma flow.[3,4]

In rats, streptozotocin (STZ)-induced diabetes generally results in a decrease of kidney IGF-I mRNA levels within 2 days. Thus, the rapid increase in rat kidney IGF-I levels in acute STZ-induced diabetes[5] was related to an increase in IGF-I receptor mRNA and IGF-binding protein-I mRNA.[6-8] The change in IGF-I receptor mRNA and IGF-binding protein-I mRNA was mainly related to the hypoinsulinemia that follows induction of STZ-induced diabetes.[8] The increase in kidney IGF-I and renal flow is an early phenomenon in diabetes, but despite the fact that in the rat kidney IGF-I level tends to decrease to normal within days, diabetic nephropathy develops only after months of disease onset.[9] A decrease in serum IGF-I induced by the somatostatin analog octreotide in the chronic phase of diabetes results in decreased renal mass, decreased hyperperfusion, and decreased proteinuria in both rats and humans.[9,10]

The aim of the present study was to examine whether the increase in IGF-I and IGF-I receptor mRNA found in the kidney of STZ-induced diabetic rats persists also in the chronic phase. In addition, this study examines the effect of late insulin treatment in chronic STZ-induced diabetic rats on the expression of IGF-I and IGF-I receptor. The influence of chronic hyperinsulinemia with normoglycemia on IGF-I, IGF-I receptor, and IGF-binding protein-I gene expression was also measured to enable differentiation between the influence of chronic hyperinsulinemia per se versus chronic hyperglycemia on IGF-I and IGF-I receptor gene expression.

## MATERIALS AND METHODS

After an overnight fast, male Sprague-Dawley rats (176 ± 7 g) were administered a single injection of STZ (100 mg/kg body weight intraperitoneally in 0.01 mol/L citrate buffer, pH 4.4; Sigma, St Louis, MO) or vehicle. Administration of STZ resulted in the induction of hyperglycemia in 85% of the animals 24 hours after treatment. Rats were given free access to laboratory chow and water. Metabolic status of the animals was monitored by twice-weekly estimation of levels of urinary glucose (Diastix; Ames, Elkhart, IN) and ketones (Ketostix; Ames). Only rats with urine glucose levels greater than 100 mmol/L and no ketones in the urine were used in the study. Body weight was measured weekly.

Two separate studies were performed. In the first one, rats were killed 75 days (chronic diabetes) or 10 days (acute diabetes) after induction of diabetes. Rats with chronic diabetes were separated into four groups. One group of diabetic rats (n = 11) was sham-operated 10 days before killing. Another group of diabetic rats (n = 12) was treated with 7-mm sustained-release insulin implants, starting 10 days before death. A third group (n = 12) of age-matched control rats was sham-operated 10 days before death, and a fourth group of age-matched normal rats (n = 12) was treated with 7-mm insulin implants starting 10 days before killing. Rats with acute diabetes were separated into two groups. One group was sham-operated (n = 6), and the other was implanted with 7-mm insulin implants (n = 8) 3 days after the induction of diabetes.

In the second study, normal rats were treated with an insulin implant for 42 days. One group of normal rats (n = 10) was treated with an insulin implant, and the other group of rats (n = 7) served as a control. Insulin-treated rats received at the beginning only a 2-mm segment of the 7-mm sustained-release insulin implant, and control rats underwent sham-implantation under the same conditions. Two weeks after the first implantation, insulin-treated rats were reimplanted with a 3.5-mm implant. This schedule was determined after extensive preliminary testing and generally does not produce hypoglycemia in normal rats. The insulin implant (Linplant; Linshin Canada, Scarborough, Ontario, Canada) was implanted in all the rats in the back of the neck. These implants are

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made by high-pressure compression of a power admixture of insulin and recrystallized palmitic acid.[11] They are designed to deliver approximately 2 U/d/7-mm pellet for more than 40 days. When an implant is broken into 2- to 3-mm segments, there will be little change in the total insulin release rate. A 3-mm segment will release approximately half the amount of insulin per day.[11] Implantation of rats was performed using a 12-gauge hypodermic needle under brief ether anesthesia.

At the end of the study, rats were anesthetized with ether. Blood was drawn from the aorta. Both kidneys were removed, weighed, and immediately frozen in nitrogen liquid and thereafter kept in −70°C for RNA preparation (right kidney) and tissue IGF-I measurement (left kidney).

Plasma glucose concentration was measured by the glucose oxidase method (Boehringer, Mannheim, Germany). Plasma insulin levels were determined by radioimmunoassay using human insulin as standard (Sorin Biomedica, Saluggia, Italy).

To measure the concentration of IGF-I in serum, circulating IGF-I was first dissociated from its carrier protein by treatment with 0.5N HCl, followed by chromatography through a C-18 Sep-Pak column (Waters, Milford, MA). IGF-I radioimmunoassays were performed as described previously.[12] Tissue concentration of IGF-I was measured after homogenization of whole frozen kidney in 1 mol/L acetic acid. After centrifugation, the supernatant was lyophilized, reconstituted in bovine serum albumin–borate buffer, and assayed as described earlier.

Total RNA was prepared from tissues of individual rats by a RNAzol kit (Biotecx Laboratories, Houston, TX) according to the method reported by Chomczynski and Sacchi,[13] and was quantified by absorbance at 260 nm. Integrity of the RNA and accuracy of spectrophotometric determinations were assessed by visual inspection of the ethidium bromide–stained 28S and 18S ribosomal RNA bands after agarose formaldehyde gel electrophoresis of 10-μg aliquots as described previously.[14]

IGF-I, IGF-I receptor, and IGF-binding protein-I riboprobes were generous gifts from Derek LeRoith of the National Institutes of Health (Bethesda, MD).

The antisense RNA probe used to detect IGF-I receptor mRNA has been previously described.[15] This transcript contains 40 bases of vector sequence and 265 bases complementary to 15 bases of 5′-untranslated sequence and to the region encoding the signal peptide and the first 53 amino acids of the IGF-I receptor α-subunit. On hybridization of this RNA probe with IGF-I receptor mRNA and subsequent RNase digestion, a protected band of 265 bases was obtained.

The riboprobe used to measure IGF-I mRNA levels was described previously.[16] This probe allows detection of both IGF-I mRNA species encoding the IGF-Ia and IGF-Ib prohormones. Only levels of IGF-Ia mRNA that constitute greater than 90% of the total IGF-I message and correlate with IGF-Ib mRNA levels were measured in this study.

Rat IGF-binding protein-I mRNA was measured using an antisense probe derived from a rat IGF-binding protein-I cDNA clone isolated from a dexamethasone-treated $H_4$-II-E-C3 hepatoma cell library (G.T. Ooi, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, unpublished data, 1991).

RNA level was measured with a plasmid containing a 250-bp mouse β-actin gene fragment inserted downstream of SPG phage promoter (Ambion-Ribonuclease Protection Assay Kit, Austin, TX).

Solution hybridization–RNase protection assays were performed as previously described.[17] Briefly, 20 μg total RNA was hybridized with $1 \times 10^6$ dpm $^{32}$P-labeled antisense RNA probes. Hybridization was performed at 45°C for 16 hours in a buffer containing 40 mmol/L PIPES, pH 6.4, 0.4 mol/L NaCl, 1 mmol/L EDTA, pH 8, and 80% formamide. After hybridization, RNA samples were digested with RNase A and $T_1$, and the protected hybrids were extracted with phenol-chloroform, ethanol-precipitated, and electrophoresed on 8% polyacrylamide–8-mol/L urea denaturing gel. Multiple autoradiograms from each gel were scanned by a densitometer connected to an Apple Macintosh computer (Cupertino, CA).

All results are presented as the mean ± SE. Student's $t$ test was used to determine the existence of significant variations between groups. $P$ less than .05 was considered significant.

**Table 1. Serum Glucose and Insulin Levels and Kidney IGF-I and IGF-I Receptor mRNA Levels in C, C + I, D, and D + I Rats (Mean ± SE)**

| Animals | Glucose (mmol/L) | Insulin (μU/mL) | IGF-I Receptor mRNA (% from control) | IGF-I mRNA (% from control) |
|---|---|---|---|---|
| D (n = 11) | 26 ± 4.6† | 2.4 ± 0.22† | 280 ± 8† | 52 ± 3* |
| D + I (n = 12) | 4.4 ± 0.41 | 44 ± 3.2 | 68 ± 4 | 106 ± 4.2 |
| C (n = 12) | 7.1 ± 0.2‡ | 16 ± 0.9* | 100 ± 3‡ | 100 ± 4.6 |
| C + I (n = 8) | 4.8 ± 0.36 | 34 ± 3.1 | 66 ± 3.6 | 92 ± 5.2 |

NOTE. Levels of glucose and insulin in serum and of IGF-I and IGF-I receptor mRNA in kidney were determined at time of death 75 days after administration of STZ.

Abbreviations: C, control; C + I, control insulin-treated; D, diabetic; D + I, diabetic insulin-treated.

*$P$ < .05, †$P$ < .01; D $v$ D + I.

‡$P$ < .05.

## RESULTS

Diabetes resulted in a marked decrease in body weight (248 ± 4.4 g [n = 11] $v$ 462 ± 3.3 [n = 12], $P$ < .01) associated with a significant increase in serum glucose and a decrease in serum insulin and IGF-I levels (62 ± 3.8 $v$ 206 ± 6.1 nmol/L, $P$ < .05).

Table 1 lists serum glucose and insulin levels and kidney IGF-I and IGF-I receptor mRNA levels 75 days after induction of diabetes.

Chronic diabetes caused a threefold increase in IGF-I receptor gene expression (Fig 1) and a twofold decrease in IGF-I gene expression (Fig 2). Quantitation by computer-

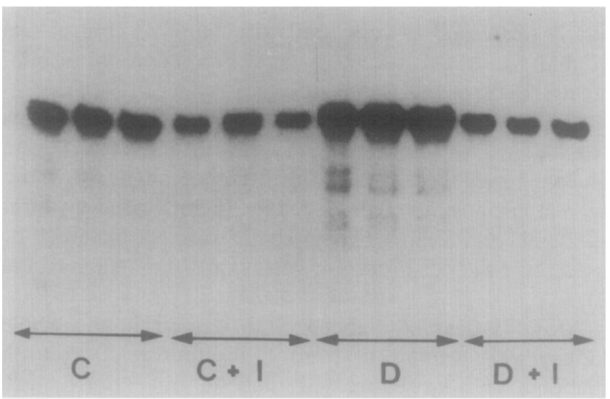


**Fig 1. IGF-1 receptor gene expression in kidney of control (C), control insulin-treated (C + I) diabetic (D), and diabetic insulin-treated (D + I) rats. Diabetes was induced 75 days before killing, and insulin pellets were implanted 10 days before killing. Results obtained with 3 representative animals from each group are shown.**

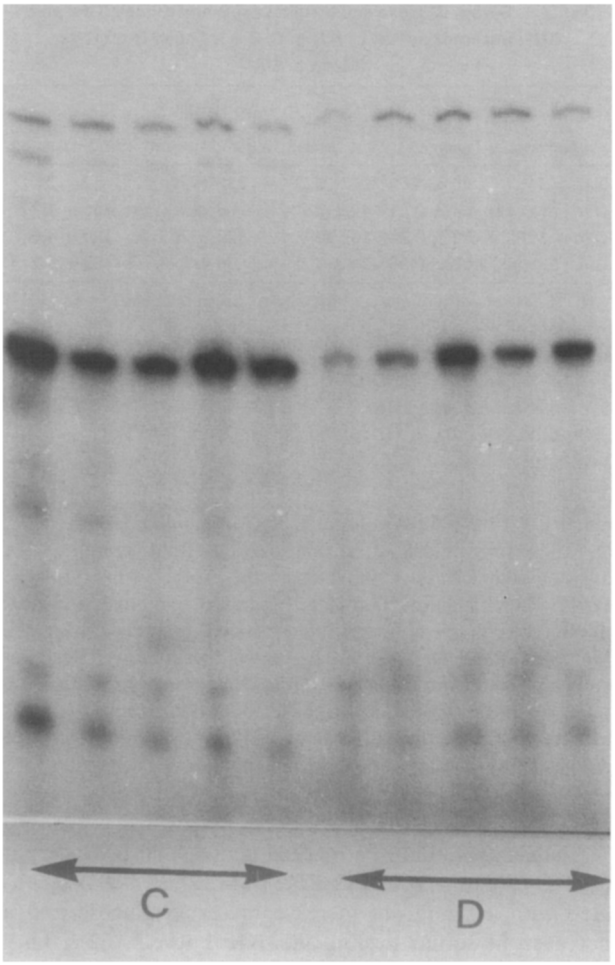


**Fig 2. IGF-I gene expression in kidney of control (C) and diabetic (D) rats. Diabetes was induced 75 days before killing. Results obtained with 5 representative animals from each group are shown.**

assisted densitometry demonstrated that β-actin mRNA level was not different between the groups. Insulin treatment in diabetic rats caused a 20-fold increase in serum insulin levels, resulting in glucose levels that were significantly decreased as compared with those of control rats (Table 1). IGF-I receptor gene expression in insulin-treated diabetic rats was significantly reduced (Fig 1).

Ten days of insulin treatment in normal nondiabetic rats resulted in a twofold increase in serum insulin levels. Serum glucose levels were reduced to a degree similar to those seen in insulin-treated diabetic rats. In both cases, hyperinsulinemia and relative hypoglycemia were accompanied by a decrease in IGF-I receptor gene expression (Table 1, Fig 1).

After 75 days of diabetes, IGF-I levels in the whole kidney were similar in control, insulin-treated control, diabetic, and insulin-treated diabetic rats (154.4 ± 5.2, 162 ± 6, 144.8 ± 4.8, and 157.2 ± 6.0 pg/mg tissue, respectively, NS).

We divided the insulin-treated normal and diabetic rats from Table 1 into two groups according to blood glucose levels greater than (n = 11) or less than 4 mmol/L (n = 9)

**Table 2. Serum Glucose and Insulin Levels and Kidney IGF-I and IGF-I Receptor mRNA Levels in the Insulin-Treated Rats of the First Study**

| Animals | Glucose (mmol/L) | Insulin (μU/mL) | IGF-I Receptor mRNA (% from control) | IGF-I mRNA (% from control) |
|---|---|---|---|---|
| Normoglycemia (n = 11) | 5.4 ± 0.3* | 35 ± 3.6 | 84 ± 3.8* | 108 ± 5.2 |
| Hypoglycemia (n = 9) | 2.8 ± 0.18 | 38 ± 3.8 | 44 ± 2.8 | 94 ± 4.4 |

NOTE. Insulin-treated rats were divided according to blood glucose levels at death of >4 mmol/L (normoglycemia) or <4 mmol/L (hypoglycemia). Mean ± SE.

*$P < .05$.

(Table 2). It can be seen that although we found a significant difference in blood glucose levels between the two groups, blood insulin levels did not differ significantly. From Table 2, we can also see that hyperinsulinemia with hypoglycemia resulted in an approximately twofold decrease in IGF-I receptor mRNA, whereas no change in IGF-I receptor mRNA could be demonstrated with hyperinsulinemia and near-normal glucose levels (Table 2).

Similar results were demonstrated in short-lived STZ-induced diabetes in rats in which IGF-I receptor mRNA was increased and returned to normal with insulin treatment that resulted in near normoglycemia (Fig 3), whereas IGF-I mRNA was decreased in diabetic rats and reversed to baseline levels by insulin (data not shown).

Figure 4 demonstrates serum glucose and insulin levels during 42 days of hyperinsulinemia in normal rats implanted with insulin pellets. Marked hyperinsulinemia was not associated with hypoglycemia. This enabled us to isolate the influence of chronic hyperinsulinemia per se on IGF-I, IGF-I receptor, and IGF-binding protein-I gene expression. Although hyperinsulinemia decreased IGF-binding protein-I mRNA (Fig 5), no such effect could be demonstrated on IGF-I receptor mRNA (Fig 6) or IGF-I mRNA (data not shown).

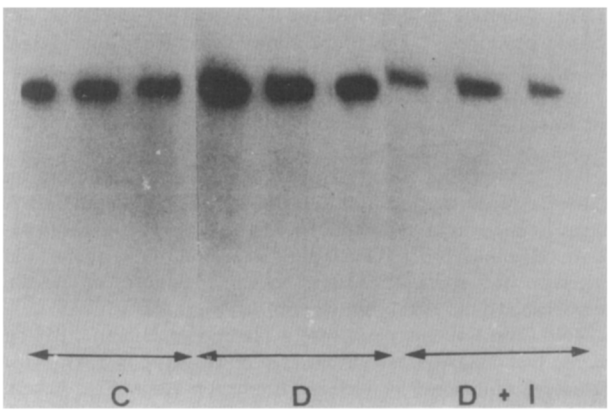


**Fig 3. IGF-I receptor gene expression in kidney of control (C), diabetic (D), and insulin-treated diabetic (D + I) rats. Diabetes was induced 10 days before killing. Insulin pellet was implanted 7 days before killing.**

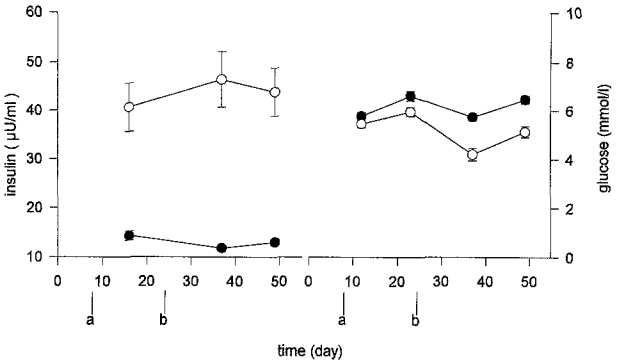


**Fig 4. Serum glucose and insulin levels during 42 days' follow-up study of normal rats implanted with one third of the insulin pellet at the beginning of the study (a) and half of the insulin pellet 14 days later (b). (○) n = 10 implanted rats; (●) n = 7 sham-operated rats. Mean ± SE.**

## DISCUSSION

Enlargement of the kidney is one of the major structural changes accompanying the onset of diabetes.[1] Several studies have demonstrated an increase in the expression of IGF-I receptor and IGF-binding protein-I genes in the kidney of STZ-induced diabetic rats shortly after induction of diabetes.[6,7] IGF-I receptors mediate the mitogenic effect of IGF-I on mesangial cells in culture[18] and are probably involved in other IGF-mediated actions such as increased glomerular filtration rate and kidney plasma flow in diabetic humans.[3,4] Thus, it appears that the increased IGF-I receptor gene expression plays a major role in the pathogenesis of diabetic nephropathy.

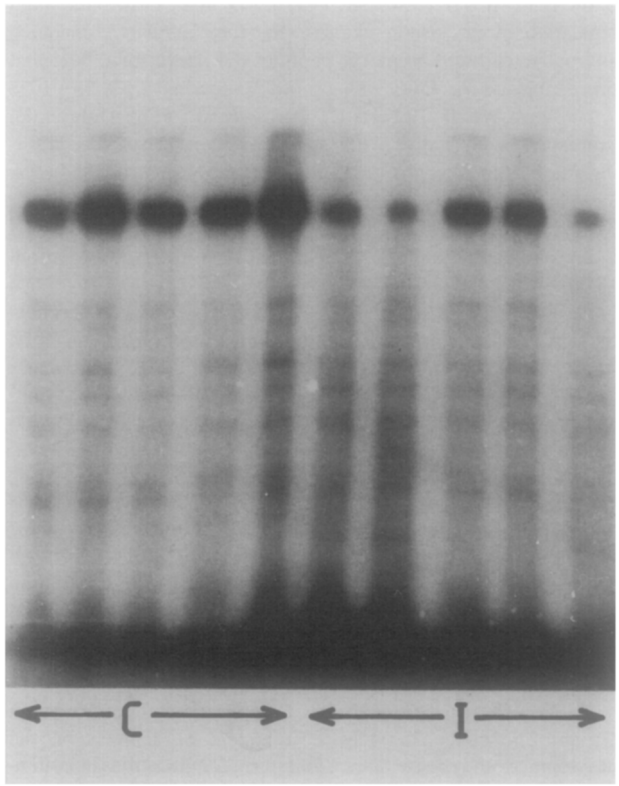


**Fig 5. Expression of IGF-binding protein-I (IGFBPI) in kidney of control (C) and chronically hyperinsulinemic (I) rats. Results obtained with 5 representative animals from each group are shown.**

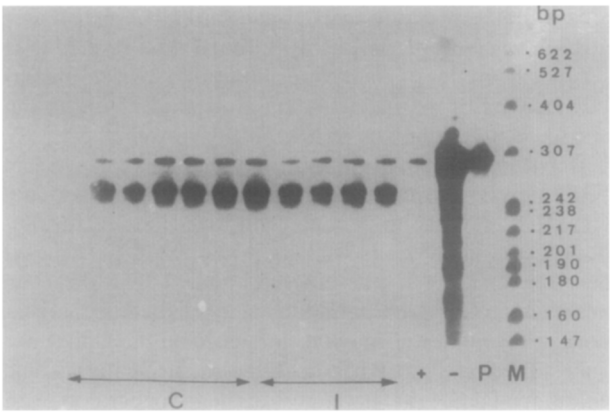


**Fig 6. Expression of IGF-I receptor gene in kidney of control (C) and chronically hyperinsulinemic (I) rats. Results obtained with 5 representative animals from each group are shown. bp, base pair; +, yeast RNA and probe with RNase; –, yeast RNA and probe without RNase; P, native probe; M, marker PBR 322 DNA digested with *Msp*I.**

The increase in IGF-I level in diabetic kidney within 12 hours of STZ injection can only be explained by the increase in kidney IGF-I receptor mRNA and IGF-binding protein-I mRNA, since it occurs in the presence of a parallel decrease in kidney IGF-I mRNA and in circulating IGF-I. However, kidney IGF-I returns to baseline levels 4 days after induction of diabetes. Thus, the hyperfiltration state and kidney hypertrophy that persist during the chronic phase of the disease cannot be explained by kidney IGF-I levels.

Werner et al[6] have shown that in the STZ-induced diabetic rat kidney, IGF-I receptor mRNA and IGF-I binding to kidney are increased 14 days after induction of diabetes, at the time when kidney IGF-I levels have returned to normal. In our study, a persistent increase in IGF-I receptor mRNA could still be demonstrated 75 days after induction of diabetes, a time when proteinuria has already developed in the diabetic rat kidney. We measured kidney IGF-I levels in our chronically diabetic rats and found them to be similar to kidney IGF-I levels in age-matched control rats. Thus, the persistent increase in IGF-I receptor mRNA 75 days after induction of diabetes that has been shown to correlate with increased binding of IGF-I to the kidney might play a role in the development of late complications in the kidney, through a constantly increased IGF-I receptor availability.

It was recently shown that the diabetes-induced increase in fibronectin gene expression in kidney tissue in vivo and the hyperglycemia-induced increase in cultured human endothelial cells is not reversed by treatment with insulin in vivo or by a reduction of glucose concentration in the cell media in vitro.[19] Our study demonstrates that, in contrast to fibronectin, regulation of IGF-I and IGF-I receptor mRNA is reversible with changes in glucose levels, even after

long-standing hyperglycemia. Changes in IGF-I receptor mRNA levels were shown to be more closely related to serum glucose levels than to serum insulin levels, whereas IGF-binding protein-I regulation is dependent on insulin and tends to be inhibited by chronic hyperinsulinemia.

Octreotide, a somatostatin analog that decreases serum IGF-I levels, prevents the hyperfiltration state and decreases kidney size in patients with long-standing insulin-dependent diabetes, as well as in chronic STZ-induced diabetes in rats.[9,10] This suggests that IGF-I plays an important role in the development of diabetic complications in the kidney, through its influence on the kidney not only in the first days of diabetes but also during the chronic phase. It might be that part of the influence of octreotide on renal perfusion and renal mass results also from its ability to change IGF-I receptor gene expression, since octreotide was shown to influence IGF-I and IGF-binding protein-I gene expression in liver in vivo and in vitro.[20-22] The increase in IGF-I receptor mRNA in the chronic phase of diabetes and the fact that good control of glucose levels or treatment with octreotide might halt the progression of the disease suggest that at least part of the influence of these drugs on the diabetic kidney is mediated by their ability to decrease IGF-I receptor gene expression.

Our finding that glucose levels play a major role in the regulation of IGF-I receptor mRNA, whereas insulin regulation of IGF-I receptor gene[15] mainly through its influence on glucose levels, can explain the clinical observation that insulin treatment that does not result in normoglycemia would not prevent the development and progression of diabetic nephropathy. This was demonstrated in the Diabetes Control and Complications Trial study,[23] in which treatment resulting in constant hyperinsulinemia with hyperglycemia did not prevent progression of kidney disease, whereas hyperinsulinemia with near normoglycemia did halt disease progression.

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