Renal Hypertrophy in Hyperglycemic Non-Obese Diabetic Mice Is Associated with Persistent Renal Accumulation of Insulin-Like Growth Factor I

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Abstract. The non-obese diabetic mouse is a model of spontaneous insulin-dependent diabetes as a result of autoimmune destruction of pancreatic β cells, similar to the disease seen in human Type I diabetes. This mouse strain develops glomerular lesions reminiscent of those seen in human disease. The study presented here investigated the changes in renal insulin-like growth factor (IGF) system in hyperglycemic non-obese diabetic mice. Female non-obese diabetic mice and their age- and sex-matched controls were euthanized 4 days, 2 wk, and 4 wk after the onset of glycosuria. Kidney weight increased in diabetic mice, beginning at 2 wk after the onset of glycosuria. This renal hypertrophy was associated with an increase in renal extractable IGF-I protein. However, a decrease in IGF-I mRNA was observed at the same time. Serum IGF-I levels remained stable after 2 wk of diabetes and decreased at 1 month. No change was detected in renal IGF-I receptor mRNA levels. Renal cortical IGF binding protein (IGFBP)-1 mRNA levels were increased. Ligand blot analysis revealed a significant increase in serum and renal 30-kd IGFBP and a decrease in serum and kidney IGFBP-3 and IGFBP-4 at 30 days of diabetes. Insulin therapy prevented the increases in kidney weight, renal IGF-I, and 30-kd IGFBP, but did not reverse the decreased serum IGF-I levels observed at 1 month of diabetes.

In summary, renal hypertrophy in non-obese diabetic mice is associated with a persistent accumulation of renal IGF-I and IGFBP-1. These changes were partially reversed with insulin therapy, which did not correct the hyperglycemia, suggesting an important role for insulin deficiency in mediating these changes in the IGF system. These findings suggest that the IGF system may play a potential role in the development of diabetic nephropathy. (J Am Soc Nephrol 8: 436-444, 1997)

Nephropathy is a major complication of diabetes mellitus (DM) and is one of the leading causes of death among diabetic patients (1). The mechanisms responsible for the development of renal functional and structural changes in DM are poorly understood. An increase in GFR and enlargement in kidney size are the characteristic renal changes in early human DM (2–4). Established diabetic nephropathy is characterized by thickening of the glomerular basement membrane, mesangial cell proliferation, accumulation of mesangial extracellular matrix with obstruction of the glomerular capillary lumen, and loss of glomerular filtration and function (5,6). In addition, diabetic nephropathy is characterized by microalbuminuria and proteinuria (7,8).

Insulin-like growth factor I (IGF-I) is a potent mitogenic polypeptide structurally related to IGF-II and proinsulin (9,10). These peptides play a role in the growth and development of many tissues during both fetal and extraterine life (11,12). The biological actions of IGF-I are mediated by a specific receptor, which has tyrosine kinase properties and activates a signal transduction cascade, leading to the activation of different genes (13). IGF-I receptor has a diffuse intrarenal distribution (14–16). The biological actions of the IGF are thought to be modulated by a family of IGF binding proteins (IGFBP), which includes six members (IGFBP-1 to -6) (17). Circulating IGFBP bind the majority of IGF in serum and transport the IGF out of the circulation to their target tissues. However, the IGFBP may either potentiate or inhibit the local effects of IGF-I (18,19).

IGF-I may play a causative role in kidney hypertrophy. In the streptozotocin (STZ)-induced DM model, renal hypertrophy is preceded by a transient rise in the concentration of extractable renal tissue IGF-I, which reaches a peak 24 to 48 h after the injection of STZ, and returns to baseline within 72 h from the intervention (20,21). Administration of growth hormone to hypophysectomized rats leads to an increase in kidney IGF-I (22,23), and IGF-I administration to hypophysectomized rats results in a significant increase in kidney weight (24). Although STZ-treated animals develop kidney disease that resembles human diabetic nephropathy, some dissimilarities...
with human disease do occur (25). In STZ-induced diabetes, the typical diabetic glomerulosclerosis associated with increasing azotemia, as seen in humans, does not develop, even in long-term follow-up models. In addition, β cell regeneration may also appear (26,27). In contrast, the non-obese diabetic (NOD) mouse model resembles human diabetic nephropathy (NOD) may also appear (26,27). In contrast, the non-obese diabetic JCL-ICR-strain mice (29). The NOD mice develop hypoinsulinenia secondary to autoimmune destruction of pancreatic β cells in association with insulitis and autoantibody production (30). NOD mice develop spontaneous diabetes between the ages of 100 and 200 days (31). As in humans, NOD mice develop proteinuria and significant glomerular lesions, including a prompt increase in glomerular surface area and an increase in mesangial sclerosis (32). The aim of the study presented here was to investigate the changes in renal IGF-I in hyperglycemic NOD mice.

Materials and Methods

Animals

 Twelve-week-old female NOD/Alt mice were purchased from Jackson Laboratories (Bar Harbor, ME). Animal breeding complied with the NIH Guide for the Care and Use of Laboratory Animals (Bethesda, MD). The cumulative incidence of overt diabetes in these animals is over 80% in female mice by 100 days. Animals were housed in standard laboratory cages and fed ad libitum normal mouse chow. Animals had free access to unlimited supplies of tap water. The onset of diabetes was determined by the appearance and persistence of glycosuria. This was checked twice a week in all animals by using chemstrips (Ketostix; Bayer Diagnostics, Basingstoke, Hampshire, UK). When the urine glucose test was positive, tail-vein blood samples were checked with Glucometer Elite (Bayer Diagnostics, Puteaux Cedex, France). The normal range (99% confidence level) of blood glucose levels for NOD mice is from 3.0 mmol/L to 9.9 mmol/L. Diabetes was diagnosed when blood glucose levels were above the normal range in 2 consecutive days. The day that glycosuria was first noted was considered as Day 1 of diabetes. Diabetic mice were euthanized 4 days, 2 wk, and 4 wk after the onset of diabetes. Normoglycemic (as determined by the lack of glycosuria and normal glucose levels at the day of sacrifice) age-matched female NOD mice were used as controls. Another group of diabetic NOD mice were treated with SC injections of Ultralente human insulin (Novo Nordisk, Als, Bagsvaerd, Denmark) on an every-other-day basis from the beginning of glycosuria. Insulin dosage was gradually increased to 4 U, which kept blood glucose levels below 25 mmol/L (450 mg/dL). The insulin-treated group was euthanized after 30 days of diabetes. Mice were anaesthetized with ether and decapitated. Trunk blood was collected and serum was separated and frozen in −20°C to measure later IGF-I levels. Kidneys were removed carefully and immediately frozen in liquid nitrogen, and were then transferred to −70°C.

Determination of Serum and Kidney IGF-I.

 Serum IGF-I was measured after extraction with acid-methanol (30 μL serum and 750 μL acid-methanol). The mixture was incubated for 2 h at room temperature, centrifuged, and 25 μL of the supernatant was diluted 1:200 before analysis. Serum IGF-I was measured by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA) and recombinant human IGF-I as standard (Amer-}

sham International, Amersham, Bucks, UK). Monoiodinated IGF-I (125I-(tyr35)-IGF-I) was obtained from Novo-Nordisk A/S, Bagsvaerd, Denmark. When the serum extract was exposed to Western ligand blot (WLb), no IGFBP could be identified. Furthermore, semilog linearity of biosynthetic IGF-I and serum extracts was seen, indicating antigen similarity and no IGFBP interference in the RIA. Intra- and interassay variations were below 5 and 10%, respectively.

 Kidney IGF-I protein levels were determined by homogenizing frozen tissue in 0.1 N acetic acid with a Sybron polytron (Brinkman, Westbury, NY) and IGFBP were removed using ODS silica columns before the assay of IGF-I. The homogenate was assayed for IGF-I concentrations with the 125I-RIA kit (Incstar, Stillwater, MN). The sensitivity of the assay was approximately 20 μg/L. The intra-assay and interassay variances were 4.5 and 7.2%, respectively.

Tissue Extraction for IGFBP

 Approximately 50 mg of thawed kidney tissue was placed in 1.5-mL polypropylene tubes and weighed. The tissue was homogenized for 2 min in 0.5 mL of 20 mmol/L Tris, 2% Triton X-100 buffer (pH 7.4; Sigma, St. Louis, MO) using a micropestle (Research Products International, Mount Prospect, IL). After 0.13 mL of Laemmli buffer was added, each tube was boiled for 5 min and incubated overnight at 4°C. Aliquots of extracts were stored at −70°C. The protein content of the extracts was measured using a protein assay (Pierce Rockford, Rockford, IL) with bovine serum as standard.

Western Ligand Blotting for IGFBP in Serum and Kidney

 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and WLb were performed according to the method of Hosenlopp et al. (33) as previously described (34). Thawed extracts were boiled for 1 min. at 13,000 rpm. An aliquot of the supernatant equivalent to 200 μg of tissue protein or 2 μL of serum was subjected to SDS-PAGE (10% polyacrylamide) under nonreducing conditions. The electrophoresed proteins were transferred by electroelution onto nitrocellulose paper (Schleicher & Schuell, Munich, Germany) and the membranes were incubated overnight at 4°C with approximately 500,000 cpm 125I-IGF-I (specific activity 2000 Ci/mmol) in 10 mL 10 mmol/L Tris-HCl buffer (TBS) containing 1% BSA and 0.1% Tween (pH 7.4). Membranes were washed with TBS and dried overnight, after which the nitrocellulose sheets were autoradiographed with Kodak X-AR film (Eastman Kodak, Rochester, NY) and exposed to Du Pont-New England Nuclear enhancing screens (New England Nuclear, Boston, MA) at −80°C for 3 to 7 days. Specificity of the IGFBP bands was ensured by competitive coincidence with unlabeled IGF-I purchased from Bachem (Bubendorf, Switzerland). WLb were quantitated by densitometry using a Shimadzu CS-9001 PC dual-wavelength flying spot scanner (Kyoto, Japan).

mRNA Analysis

 Frozen tissues in 4 M guanidinium isothiocyanate were loaded on CsCl and total RNA was isolated following the methods as described elsewhere (35). The precipitated RNA was resuspended in sterile H2O and quantitated by absorbance at 260 nm. The integrity and equivalent loading of total RNA was assessed by visual inspection of the ethidium bromide-stained 28S and 18S RNA bands after electrophoresis through 1.25%/2.2 M formaldehyde gels. The levels of IGF-I and IGF-I receptor mRNA were measured using an antisense mouse RNA probes, kindly provided by Dr. D. LeRoith from the National Institutes of Health, Bethesda, MD. The IGF-I is a 428-base pair (bp) EcoR1-BamH1 fragment that was subcloned from a genomic library

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into the pGem2 vector. This construct was then linearized with EcoRI and transcribed with T7 RNA polymerase (Promega) in the presence of 32P-UTP (Amersham, Arlington Heights, IL).

IGF-I receptor is a 430-bp EcoRI-BamHI fragment that was subcloned from a genomic library into the pBluescript SK+ (Stratagene, La Jolla, CA) vector. This construct was then linearized with EcoRI and transcribed with T3 RNA polymerase (Promega) in the presence of 32P-UTP (Amersham).

IGF-I and IGF-I receptor mRNA levels were determined using the solution hybridization/RNase protection assay. The solution hybridization/RNase protection assay was performed as described elsewhere (36). In brief, 20 μg of total RNA were hybridized with 400,000 cpm of each 32P-labeled riboprobe for 16 h at 45°C in 75% formamide-0.4 M NaCl, followed by digestion with 40 mg/mL RNase A and 2 mg/mL RNase T1. Protected hybrids were precipitated, denatured, and electrophoresed through 8% urea-polyacrylamide gels. Gels were exposed to Kodak X-Omat AR film (Eastman Kodak) at ~70°C with two intensifying screens, and the protected band (242 bp) on the autoradiography that corresponds to the IGF-I receptor was analyzed. The autoradiograms were quantitated using a Phosphorimager (Fujix BAS-1000; Fuji Photofilm Co., Japan).

Preparation of cRNA Probe

Rat IGFBP-1 cDNA was used to generate RNA probe as has been previously described (37). High-specific-activity 35S-labeled cRNA probe was synthesized in 10-μL reactions containing 100 μCi uridine 5’(α-thio-35S) thiotriphosphate (Amersham SJ 40383); 10 mM NaCl; 6 mM MgCl2; 40 mM Tris (pH 7.5); 2 mM spermidine; 10 mM dithiothreitol; 500 μM each of unlabeled ATP, CTP, and GTP; 25 μM each of unlabeled UTP; 0.5 μg linearized template; 15 U of the appropriate polymerase; and 15 U RNasin (Promega). After incubation at 42°C for 30 min, an additional 15 U of the appropriate polymerase and 15 U RNasin were added. The reaction was incubated at 42°C for an additional 30 min, at which time the DNA template was removed by digestion with DNaseI at 37°C for 10 min. Labeled cRNA was column-purified (Bio-Spin 6, Bio-Rad) to separate unincorporated nucleotides. Labeled probe was precipitated with 5 μL tRNA, 10 μL 5 M NaCl, 10 μL H2O, and 300 μL cold ethanol. The purified probe was then subjected to alkaline hydrolysis to produce fragments of an average length of 150 bases.

In Situ Hybridization

The left kidney was rapidly removed, frozen in liquid nitrogen, stored at ~70°C and used for in situ hybridization. Frozen sections were cut at a thickness of 10 μm, thaw-mounted on poly-L-lysine-coated slides, and stored at ~70°C until use. Sections were cut longitudinally so that all sections included the papilla, outer medulla, and cortex. All sections for a given experiment were prepared, washed, exposed, and analyzed together.

Before hybridization, sections were warmed to 25°C, fixed in 10% formaldehyde, and soaked for 10 min in 0.25% acetic anhydride/0.1 M triethanolamide hydrochloride/0.9% NaCl. Section were then dehydrated through an ethanol series, delipidated in chloroform, rehydrated, and air-dried. The 35S-labeled probe (107 dpm/mL or approximately 50 ng/mL) was added to a hybridization buffer composed of 50% formamide, 0.3 M NaCl, 20 mM Tris HCl (pH 8), 5 mM EDTA, 500 μg tRNA, 10% dextran sulfate, 10 mM dithiothreitol, and 0.02% each of BSA, ficoll, and polyvinylpyrrolidone. After the 35S-labeled probe in hybridization buffer was added to the sections, coverslips were placed over the sections and the slides were incubated in humidified chambers overnight (14 h) at 55°C. Slides were then washed several times in 4 × standard saline citrate (SSC) to remove cover slips and hybridization buffer, dehydrated, and immersed in 0.3 M NaCl, 50% formamide, 20 mM Tris HCl, and 1 mM EDTA at 60°C for 10 min. Sections were then treated with RNaseA (20 μg/mL) for 30 min at room temperature, followed by a 15-min wash in 0.1 × SSC at 55°C. Slides were air-dried and apposed to Hyperfilm-Betamax (Amersham, CEA, AB, Solna, Sweden) along with 14C-autoradiographic standards (ARC Inc., St. Louis, MO) for 1 to 4 days and then dipped in Kodak NTB2 nuclear emulsion, stored with desiccant at 4°C for 3 to 30 days, developed, and counterstained with Mayer’s hematoxylin and eosin for microscopic evaluation and verification of signal inside the cells.

Quantitative Densitometry

Quantification of mRNA levels was done as follows: after verification of a proper signal on film autoradiography and emulsion coating, the slides were exposed on a phosphorimager cassette for 7 days. The film autoradiographic images were digitized and quantified using a phosphorimager (Imagequant, Molecular Dynamics, Sunnyvale, CA) in which the gray scale values from the 14C standard curve were used in a third-degree polynomial function curve fit. Anatomically matched, standardized areas of the tubulointerstitial cortex, outer and inner stripe of the outer medulla, and inner medulla were defined by cursor control, and their transmittance was measured. Two to three measurements were taken for each structure in each kidney, and average values were expressed as “arbitrary optical density” (AOD). Data was normalized as a percentage of the age-matched control and expressed as mean ± SE for each group.

Statistical Analysis

The statistical significance of the differences between experimental groups was evaluated by t test. A P value of less than 0.05 was regarded as significant. Means ± SE are given.

Results

Clinical Data

The mice used in this study (N = 6 in each group) became diabetic at a mean age of 104±15 days. The control mice were euthanized at a mean age of 119±6 days (P = not statistically significant [NS]). Mean serum glucose in 4-day diabetic mice was 18.7 ± 2.2 mmol/L. Blood glucose increased to levels >28 mmol/L (“high” by glucometer) in most of the measurements, beginning at 2 wk of diabetes and on. However, none of the animals had ketonuria during the study period. Blood glucose levels in the insulin-treated group were not significantly different from the diabetic nontreated group.

Kidney Weights

Whereas no change in kidney weight was observed 4 days after the onset of glycosuria, there was an increase in kidney weight to 130 ± 10% and 127 ± 5% (P < 0.05) in diabetic mice at 2 and 4 wk since the onset of hyperglycemia, respectively, in comparison with age-matched controls. Insulin therapy for 1 month prevented the increase in kidney weight (104 ± 3% of age-matched controls; P = NS) (Figure 1A).

IGF-I Protein in Kidney and Serum

Renal extractable IGF-I protein was increased in diabetic mice, beginning at 14 days of diabetes (195 ± 14.9% of
control; \( P < 0.05 \). This increase persisted at 30 days of diabetes (239 \( \pm \) 24.1% of control; \( P < 0.05 \)). Insulin therapy for 1 month prevented the increase in kidney weight (Figure 1B). Serum IGF-I levels did not change during the first 2 wk of diabetes, but a significant decrease was observed after 4 wk in

Figure 1. Kidney weights (A), renal IGF-I extractable protein (B), and serum IGF-I (C) in (left to right) control mice and diabetic mice (DM) 4, 14, and 30 days after the appearance of glycosuria. Bar 5 (far right; 30d + DM Ins) represents diabetic mice treated for 30 days with 4 IU of ultralente insulin. Values are mean \( \pm \) SE \((N = 6\) for each group) and presented as percentages of nondiabetic controls. * \( P < 0.05 \).

Figure 2. Serum IGFBPs, as measured by WLB, in non-obese diabetic (NOD) control (Bar 1) and 4-, 14-, and 30-days diabetic mice (Bars 2 through 4) mice. Bar 5 represents: diabetic mice treated for 30 days with 4 IU of ultralente insulin. The autoradiographies were quantified by densitometry and depicted as arbitrary absorptiometry units (AU/mm²). (A) Thirty-kilodalton band, corresponding to IGFBP-1, -2, and -5. (B): Thirty-eight–to forty-two–kilodalton band, corresponding to IGFBP-3. (C) Twenty-four–kilodalton band corresponding to IGFBP-4. Values are mean \( \pm \) SE \((N = 6\) for each group). * \( P < 0.05 \) versus control.
diabetic animals (45 ± 24% of control; \( P < 0.05 \)). Insulin therapy did not reverse this decrease (Figure 1C).

**Serum IGFBP Measurements**

The WLB method yielded five different IGFBP bands in serum with apparent molecular weights of 38 to 42 kd (doublet), 30 kd, 28 kd, and 24 kd. The doublet band corresponded to the acid-stable IGF-binding subunit of IGFBP-3 and the 24-kd band to IGFBP-4, whereas the 30-kd band represents both IGFBP-1, IGFBP-2, and IGFBP-5, as these IGFBP in rats have similar molecular weights. The 28-kd IGFBP in serum may represent a glycosylated form of IGFBP-4. Serum IGFBP-1 levels increased in diabetic animals beginning at 14 days of diabetes, and increased further at 30 days (71 ± 22 and 124 ± 30 \( \text{AU/mm}^2 \) in controls; \( P < 0.05 \)). Insulin therapy prevented this accumulation (Figure 2A). A significant decrease in the band corresponding to IGFBP-3 was observed at 30 days of diabetes (31 ± 13 \( \text{AU/mm}^2 \) in controls; \( P < 0.01 \)) (Figure 2B). A similar decrease was observed in serum IGFBP-4 levels (14 ± 4 \( \text{AU/mm}^2 \) in controls; \( P < 0.05 \)) (Figure 2C). These changes were not reversed by insulin therapy.

**Kidney IGFBP Measurements**

The WLB method yielded four different IGFBP bands in kidney tissue with apparent molecular weights of 38 to 42 kd (doublet), 30 kd and 24 kd. There was an increase in kidney 30-kd IGFBP levels in the diabetic animals at 30 days of diabetes (83 ± 6 \( \text{AU/mm}^2 \) in controls; \( P < 0.01 \)). Insulin therapy prevented this accumulation (Figure 3A). A significant decrease in the band corresponding to IGFBP-3 was observed at 30 days of diabetes (6 ± 1 \( \text{AU/mm}^2 \) in controls; \( P < 0.01 \)) (Figure 3B). A similar decrease was observed in kidney IGFBP-4 levels (6 ± 1 \( \text{AU/mm}^2 \) in diabetic versus controls, respectively; \( P < 0.01 \)) (Figure 3C). These changes were not reversed by insulin therapy.

**Kidney IGF-I mRNA Levels**

Kidney IGF-I mRNA levels were determined using the solution hybridization/RNase protection assay. There was a significant decrease in kidney IGF-I mRNA levels at 4 and 30 days of diabetes (62 ± 0.1% and 33 ± 5% of controls, respectively; \( P < 0.05 \)) (Figure 4).

**Kidney IGFBP-1 mRNA Levels**

IGFBP-1 mRNA was determined in the renal cortex by in situ hybridization technique. The IGFBP-1 message in control animals was localized by autoradiography mainly to the outer stripe of outer medulla in control mice, as reported previously in rats (45). In 2-wk diabetic animals, there was a decrease in the message at the outer medulla (data not shown). Cortical IGFBP-1 mRNA signal increased in diabetic mice. Quantitation of autoradiogram intensity using a phosphorimager revealed an increase in cortical IGFBP-1 mRNA levels in diabetic animals at 14 days after the onset of glycosuria (180 ± 25% of controls; \( P < 0.01 \)) (Figure 5).

**IGF-I Receptor mRNA Levels**

To evaluate changes in IGF-I receptor mRNA levels after the appearance of diabetes, total RNA was prepared from kidneys 2 wk after the appearance of glycosuria. IGF-I receptor mRNA level was determined by solution hybridization/RNase
Figures 5. Renal cortical IGFBP-1 mRNA levels in control and 2-wk diabetic mice, as measured by in situ hybridization, as described in the Methods section. * P < 0.01 versus control.

Figure 6. Kidney IGF-I receptor mRNA levels in 2-wk diabetic and age-matched control NOD mice. The levels of mRNA were measured by solution hybridization/RNase protection assay using 20-μg aliquots of total RNA. Undigested antisense native RNA probe is shown on the leftmost lane and the same probe after the application of RNase is shown on the second lane from the left. Lanes 1 through 4 correspond to RNA from control kidneys and lanes 5 through 9 correspond to RNA from diabetic mice. The position of the 242-bp IGF-I receptor protected band is also shown. Film was exposed for 2 days at -70°C with two intensifying screens.

Discussion
Diabetic kidney disease is characterized by an early renal and glomerular hypertrophy. IGF-I has been shown to mediate renal hypertrophy in other non-diabetes models such as potassium depletion by chronic furosemide administration (38,39), unilateral nephrectomy (40), and partial kidney infarction (41). Previous studies (21,42,43) have found an early and transient accumulation of IGF-I protein in the kidneys of post-pubertal STZ-induced diabetic rats. STZ may be toxic to tubular cells, and glomerulosclerotic changes are difficult to document in the STZ model. Renal hypertrophy has been previously reported in the NOD mouse model, as well as in other animal models of spontaneous diabetes (44). This hypertrophy is followed by other typical changes as seen in human disease, such as glomerulomegaly, the appearance of albuminuria, and increased mesangial matrix and cellularity, leading eventually to glomerulosclerosis (32).

In our study, we found that renal hypertrophy in NOD diabetic mice is associated with an increase in renal IGF-I protein content, which persists for up to 4 wk after the appearance of spontaneous glycosuria. This is a later change than the
transient one reported in the STZ model (20,21,43). The accumulation of renal IGF-I protein was associated with a decrease both in circulating IGF-I concentrations and in renal IGF-I mRNA levels. The discrepancy between the increase in renal IGF-I protein content and the decrease in IGF-I mRNA levels is not clear. A similar decrease in IGF-I mRNA levels was also reported in the STZ model in as early as a few hours, and persisted for up to 120 days after the induction of DM (43,45). Such a discrepancy could be explained by an increase in IGF-I mRNA transcript stability, or post-translational modifications in IGF-I protein stability. The latter has been shown to be modulated by the system of IGFBP. To date, six different IGFBP have been cloned and designated IGFBP-1 to -6 (46). Under normal circumstances, IGFBP-3 is the predominant carrier of IGF in the circulation, and one of its roles is to function as a carrier protein for IGF, thereby protecting IGF from degradation and sequestration and ensuring sufficient supply to target tissues. It seems evident today, however, that IGFBP may also act as modulators of IGF action at cellular level, both by enhancing and inhibiting the biological actions of IGF. IGFBP-1 to -5 are all expressed in the kidney. IGFBP-1 is mainly localized in the medulla and to a lesser extent in the cortex (45). However, cortical IGFBP-1 mRNA levels are markedly increased in the STZ diabetic rat model (43). IGFBP-2 mRNA is exclusively localized to the glomeruli (45), IGFBP-3 mRNA to the interstitial cells in the cortex (45), IGFBP-4 in the distal tubule in the cortex (45), and IGFBP-5 mRNA to the interstitial cells in the medulla (45). In the study presented here, IGFBP were measured by means of WLB, because specific rat IGFBP antibodies, at least in our studies, did not recognize IGFBP in tissue homogenates (data not shown). The WLB method yielded in kidney tissue four different bands of IGFBP with apparent molecular weights of 38 to 42 kd (doublet), 30 kd, and 24 kd. The doublet band corresponds to the acid-stable IGF-binding subunit of IGFBP-3, the 24-kd band to IGFBP-4, and the 30-kd band represents both IGFBP-1, IGFBP-2, and IGFBP-5, as these IGFBP in rats have similar molecular weights. Based on the distribution of renal IGFBP mRNA described above, the predominant IGFBP in cortex are IGFBP-1 and IGFBP-2, whereas IGFBP-1 and IGFBP-5 prevail in the medulla. In agreement with the pronounced rise in cortical renal IGFBP-1 mRNA described in this study, the 30-kd IGFBP band that was also increased in this study most probably represents IGFBP-1. The fall in IGFBP-3 and IGFBP-4 may be a result of a decreased synthesis in the kidney. This possibility seems, however, less likely, because unchanged levels of IGFBP-3 and IGFBP-4 mRNA were found in STZ-diabetes (45). A much more intriguing hypothesis is that a specific degradation of kidney IGFBP-3 and IGFBP-4 may occur in NOD diabetes, thereby increasing the IGF-I bioavailability in the kidney. This could occur through modulation of kidney-derived specific proteases with proteolytic actions against IGFBP (47). However, future studies are warranted to confirm or refute this hypothesis.

The increase in renal hypertrophy, renal IGF-I and IGFBP-1 could be prevented with insulin therapy. Serum IGF-I and IGFBP-3 and -4 levels decreased in diabetic mice, but this effect could not be reversed with insulin. Interestingly, insulin at this dosage did not normalize hyperglycemia, although it prevented ketosis. The inverse relationship between IGFBP-1 and insulin has been previously described in other models (48). These data favor a more important role for hypoinsulinemia than hyperglycemia in mediating the changes in renal IGF system.

Most of the biological actions of IGF-I are mediated by activation of the IGF-I receptor. The IGF-I receptor is a transmembrane tyrosine kinase that is related to the insulin receptor (14-16). IGF-I receptors are found in kidney membranes, glomerular mesangial cells, and tubules (15-16). Previous studies using the STZ model have shown an increase in IGF-I receptor mRNA levels 14 days after the induction of DM, accompanied by an increase in IGF-I binding (49). Such changes in IGF-I receptor abundance could cause renal IGF-I sequestration, and therefore explain the accumulation of this protein in diabetic kidneys. In our study, no change in renal IGF-I receptor mRNA levels was found. Renal hypertrophy has been also shown to be related to the increase in expression of other growth factors, primarily transforming growth factor β (50-52). An activation of several proto-oncogenes has been shown to take place early after induction of DM by STZ (53). Therefore it is possible that a sequential activation of several growth factors takes place.

In summary, we report an increase in renal IGF-I protein that persists for up to 4 wk after the appearance of diabetes in the NOD mouse model. This increase was not associated with a concomitant increase in renal IGF-I mRNA levels and could have been the result of an increase in renal IGFBP-1 content. Because the growth-hormone-IGF-I axis is thought to play a significant role in the development of glomerulosclerosis (54-56), further studies modulating renal IGF-I levels are needed to elucidate whether the development of such complication can be prevented.

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References


