The role of the IGF axis in IGFBP-1 and IGF-I induced renal enlargement in Snell dwarf mice

M van Kleffens, D J Lindenbergh-Kortleve, J G Koster[1], J W van Neck, A Flyvbjerg[2], R Rasch[3], S L S Drop and **S C van Buul-Offers[1]**

Laboratory of Pediatrics, Subdivision of Molecular Endocrinology, Erasmus University Rotterdam, The Netherlands

[1]Department of Pediatric Endocrinology, University Medical Center, Utrecht, The Netherlands

[2]Medical Department M, Medical Research Laboratory M, Institute of Experimental Clinical Research, Aarhus Kommune Hospital, Aarhus, Denmark

[3]Department of Cell Biology, Institute of Anatomy, University of Aarhus, Aarhus, Denmark

(Requests for offprints should be addressed to D J Lindenbergh-Kortleve, Laboratory for Pediatrics, Room Ee 1500, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands; Email: lindenbergh@kgk.fgg.eur.nl)

Abstract

Insulin-like growth factor (IGF) binding protein-1 (IGFBP-1) is generally believed to inhibit IGF action in the circulation. In contrast, IGFBP-1 has been reported to interact with cell surfaces and enhance IGF-I action locally in some tissues. Renal IGFBP-1 levels are found elevated in various conditions characterized by renal growth (e.g. diabetes mellitus, hypokalemia).

To test whether IGFBP-1 is a renotropic factor, IGFBP-1 was administered alone or in combination with IGF-I to Snell dwarf mice, an *in vivo* model without compensatory feedback effects on growth hormone (GH) secretion. In three control groups of Snell dwarf mice, placebo, GH or IGF-I was administered. Compared with placebo, kidney weight increased in all treated groups, however, with different effects on kidney morphology. Administration of IGF-I, alone or in combination with IGFBP-1, tended to increase glomerular volume, while no changes were seen in the other groups. Administration of IGFBP-1 or IGFBP-1+IGF-I both caused dilatation of the thin limbs of Henle's loop, while GH or IGF-I administration had no visible effect. Furthermore, IGF-I administration resulted in an increased mean number of nuclei per cortical area and renal weight, whereas GH, IGF-I+IGFBP-1 or IGFBP-1 caused a decreased renal nuclei number.

In situ hybridization and immunohistochemistry showed specific changes of the renal IGF system expression patterns in the different groups. Particularly, IGFBP-1 administration resulted in extensive changes in the mRNA expression of the renal IGF system, whereas the other administration regimen resulted in less prominent modifications. In contrast, administration of IGFBP-1 and IGFBP-1+IGF-I resulted in identical changes in the protein expression of the renal IGF system.

Our results indicate that IGFBP-1, alone or in combination with IGF-I, demonstrated effects on the renal tubular system that differ from the effects of IGF-I.

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Introduction

The growth promoting effects of the growth hormone (GH)–insulin-like growth factor (IGF) axis on body and organ growth seem to be highly dependent on the distribution of the IGF binding proteins (IGFBPs). IGFBP-1, generally believed to inhibit IGF action in the circulation, has also been reported to interact with cell surfaces and enhance local IGF-I action (Jones & Clemmons 1995, Lee *et al.* 1997). In various conditions characterized by renal growth, renal IGFBP-1 levels have been reported to be elevated (e.g. diabetes mellitus, hypokalemia) (Flyvbjerg 1993, Hsu *et al.* 1997, van Neck *et al.* 1997).

In man, circulating levels of IGFBP-1 are raised at short term in diabetes (Flyvbjerg 1993, Fervenza *et al.* 1997). In experimental diabetes in rodents, serum levels of IGFBP-1 are elevated as well, together with decreased IGF-I levels (Luo & Murphy 1992, Bach *et al.* 1994). In contrast, renal IGF-I concentrations are elevated despite an unchanged local production (Flyvbjerg *et al.* 1990, Landau *et al.* 1995, Raz *et al.* 1998). However, in the renal cortex of diabetic rodents, IGFBP-1 mRNA is strongly increased (Landau *et al.* 1995). Taken together, it is tempting to speculate that renal IGF-I is increased due to entrapment by IGFBP-1.

Potassium depletion also is characterized by a selective renal growth (Gustafson *et al.* 1973, Flyvbjerg *et al.* 1991).

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In the hypertrophic kidneys of these animals, increased amounts of IGFBP-1 mRNA and protein were observed (Hsu *et al.* 1997, Rohan *et al.* 1997). This increase in renal IGFBP-1 mRNA expression in potassium-deficient rats was not affected by IGF-I infusion (van Neck *et al.* 1997).

In order to test the renotropic effects of IGFBP-1 in an *in vivo* model, without compensatory feedback effects on GH secretion, IGFBP-1 was administered, alone or in combination with IGF-I, to Pit-1 deficient Snell dwarf mice. We have demonstrated that treatment of homozygous Snell dwarf mice with GH and IGF-I, for a period of 4 weeks, caused an increase in body length and body weight, whereas IGFBP-1 administration had no effect (van Buul-Offers *et al.* 2000). Furthermore, GH, IGF-I and IGFBP-1 treatment also had a pronounced effect on kidney size. Although IGFBP-1 inhibited IGF-I induced total body growth, it further stimulated IGF-I induced kidney growth (van Buul-Offers *et al.* 2000).

The aim of the present study was to investigate in further detail the renotropic effects of IGFBP-1, alone or in combination with IGF-I. In three control groups of Snell dwarf mice, placebo, GH or IGF-I was administered. We analyzed the effects of the different administrations on kidney morphology and kidney-specific mRNA and protein expression of the different members of the IGF system.

Materials and Methods

Test substances

E. coli-derived recombinant human IGF-I was kindly provided by Eli Lilly Co. (Indianapolis, IN, USA). Recombinant human GH was from Amersham-Pharmacia (Uppsala, Sweden). Human IGFBP-1 was purified as described previously (van Buul-Offers *et al.* 2000) from amniotic fluid from pregnant women (midterm) which was obtained for diagnostic purposes and approved by the institutional Ethics committee.

Animal experimentation

The experimental design has been described in detail previously (van Buul-Offers *et al.* 2000). Briefly, in two independent long-term experiments, groups of Snell dwarf mice (males and females, aged 6–8 weeks and with identical mean lengths and weights at the start of the experiments) received GH (8·3 μg/day), IGF-I (30 μg/day), IGFBP-1 (105 μg/day), IGF-I+IGFBP-1 (30+105 μg/day) or PBS (control) for 4 weeks. In order to avoid hypoglycemia, 10% glucose was added to the drinking water, starting 1 week before the experiment. The dwarf mice ($n=5$ per group in each experiment) were injected s.c. in the neck with 0·1 ml hormone solution or vehicle, three times daily, 5 days a week. In this article we discuss the findings of the first experiment. However, the second experiment resulted in comparable findings. The study complied with Dutch regulations for care and use of laboratory animals.

Tissue isolation

Animals were killed 2 h after the last injection. Kidneys were removed and weighed to the nearest milligram, fixed overnight in 4% paraformaldehyde, and embedded in paraffin. Sections (4 μm) were cut and mounted on aminopropyl trioxysilane-coated slides. Slides were dried at 37 °C for 3 days. In order to obtain optimal comparison between the different treatments, all organs were fixed, embedded in paraffin, and mounted on slides similarly and at the same time. Kidney sections of all groups have been mounted on the same slide. As a result, the experimental parameters of *in situ* hybridization and immunohistochemistry experiments per probe or antibody, respectively, were the same for all administration groups.

Morphology

To determine kidney size, the central sections from series of sagittal sections of kidneys were measured with a ruler. To count the number of nuclei per square, sections were placed under a light microscope with a grid. Per section 3 cortical areas were counted (=nuclei in a total area of 0·25 mm^2) of five animals per treatment group (=75 counts in total).

Cell proliferation

Human proliferating cell nuclear antigen (PCNA) was detected using anti-human PCNA (Roche Diagnostics, Almere, The Netherlands) in a dilution of 1:1000 and detected using the Vectastain peroxidase-conjugated elite ABC kit (Vector Laboratories, Burlingame, CA, USA).

Cell proliferation was estimated by determining the number of PCNA positive nuclei in central sections from series of sagittal sections of kidneys under a light microscope with a grid. Three sections were counted per animal of three animals per treatment group.

*Probe preparation (*in situ *hybridization)*

Specific mouse IGFBP cRNA probes were transcribed from IGFBP-2, -3, -4, -5, -6 cDNAs as described by Schuller *et al.* (1994). The mouse cDNA Sph1-Sac1 fragment was used as template for the IGFBP-1 cRNA probe and was cloned into pTZ18R or pTZ19R (Pharmacia, Uppsala, Sweden) for the antisense and sense probes respectively. Similarly, mouse IGF-I and -II cDNAs (kindly provided by Dr G. I. Bell, Howard Hughes Institute, Chicago, IL, USA) fragments were subcloned into pTZ18 and pTZ19 (EcoRI for IGF-I and

BamHI/Sac1 for IGF-II). A 265 bp EcoRI/Sma1 fragment of the rat type I IGF receptor cDNA ligated in PGEM3 (Promega, Madison, WI, USA) was kindly provided by Dr H. Werner and Dr D. LeRoith (National Institutes of Health, Bethesda, MD, USA) and was used as template for the type I IGF receptor probe. Digoxigenin-11-UTP labeled RNA probes were prepared according to the manufacturer's prescription (Boehringer Mannheim GmbH, Biochemica, Mannheim, Germany), using T7 or SP6 RNA polymerase.

In situ *hybridization*

A non-radioactive *in situ* hybridization was performed, as described previously (Lindenbergh-Kortleve *et al.* 1997). In short, kidney sections from control and the four administration groups were mounted on the same glass slide and processed batch-wise. Hybridization was performed overnight at 55 °C in a humid chamber in a hybridization solution containing 50% deionized formamide, 10% dextran sulfate, 2 × SSC, 1 × Denhardt's solution, 1 μg/ml tRNA, 250 μg/ml herring sperm DNA and the respective probes at a concentration of 100 ng/ml. After various washes (50% formamide/2 × SSC, 50% formamide/1 × SSC and 0·1 × SSC) sections were incubated with RNase T1 (2 U/ml in 1 mM EDTA/2 × SSC) for 15 min at 37 °C. The digoxigenin-labeled hybrids were detected by antibody incubation performed according to the manufacturer's recommendations (Boehringer Mannheim GmbH, Biochemica, Mannheim, Germany). PVA (polyvinylalcohol, MW 31–50 kDa, Aldrich Chemical Co. Milwaukee, WI, USA) enhanced staining with BCIP (5-bromo-4-chloro-3-indolyl phosphate), NBT (4-nitroblue tetrazolium chloride) and levamisol was stopped when the desired intensity of the blue precipitate was reached. This was dependent on the relative abundance of the respective mRNAs. Sections were counterstained with Nuclear red solution and mounted with Euparal (Chroma Gesellschaft, Stuttgart, Germany). Control sections for morphological analysis were either stained with PAS, or with hematoxylin and eosin. *In situ* hybridized sections were analyzed blindly by two independent investigators, in at least five separate observations. Negative controls for *in situ* hybridization were performed using sense probes, which never gave any significant staining.

Immunohistochemistry

Polyclonal antisera to mIGFBP-1, -2, -3, -4, -5 and -6 were used that were previously described (van Kleffens *et al.* 1999). Briefly, synthetic peptides (ID-DLO, Lelystad, the Netherlands), corresponding to parts of the variable region of the various mouse IGFBPs (IGFBP-1 and IGFBP-2: amino acid position (aa)174–189; IGFBP-3: aa200–213; IGFBP-4: aa155–170; IGFBP-5: aa176–191; IGFBP-6: aa128–143), were coupled to the carrier keyhole limpet hemocyanin (KLH; Pierce, Rockford, IL, USA) and injected into rabbits. The resulting antisera were used in an immunohistochemical staining, making use of the peroxidase–anti-peroxidase (PAP) method. Specificity of the antisera was determined using ELISA, Western blotting and by verifying the staining patterns in normal versus gene-deletion mutant mice of the respective protein (van Kleffens *et al.* 1999). The anti mIGFBP-1 and -3 polyclonal antisera were diluted 1:150, while the anti mIGFBP-2, -4, -5 and -6 polyclonal antisera were used in a dilution of 1:250. Staining patterns were analyzed in comparison to their respective pre-immune sera, diluted 1:10. Unlabeled goat anti-rabbit immunoglobulins (Dako, Glostrup, Denmark; 1:50) were used as second antibody and rabbit PAP (Dako; 1:100) as a linker. Rabbit polyclonal antisera against type I IGF-receptor and goat polyclonal antisera against IGF-I and IGF-II (all with cross-reactivity to human, mouse and rat, according to the manufacturer) were obtained from Research Diagnostics Inc. (Flanders, NJ, USA) and used at a dilution of 1:200. The specificity of the type I IGF-receptor antibody was assured by blocking the antibody–receptor binding using a control peptide for competition consisting of amino acids 31–50 of the human type I IGF-receptor as obtained from Research Diagnostics Inc. (Flanders). Staining was performed with diaminobenzidine (DAB; Fluka Chemika, Buchs, Switzerland; 0·75 mg/ml). Nuclei were made visible with Mayer's hematoxylin. Sections from control and all administration groups were mounted on the same glass slide and processed batch-wise.

Estimation of glomerular volume

A 2 mm thick horizontally cut slice from the middle of the kidney (containing the papilla) was fixed in 4% paraformaldehyde and embedded in Technovit. Then, 2 μm thick sections were cut on a rotation microtome and stained with *p*-aminosilacylic acid and hematoxylin. Mean glomerular tuft volume (V_G) was determined from the mean glomerular cross-sectional area (A_G) by light microscopy as previously described (Flyvbjerg *et al.* 1999). Profile areas were traced using a computer-assisted morphometric unit (Image Tool: University of Texas Health Science Center, San Antonio, TX, USA). A_G was determined as the average area of a total of 40–80 glomeruli (tuft omitting the proximal tubular tissue within Bowman's capsule) and V_G was calculated as $V_G = \beta/\kappa \times (A_G)^{3/2}$, where $\beta = 1{\cdot}38$, which is the shape coefficient for spheres (the idealized shape of glomeruli), and $\kappa = 1{\cdot}1$, which is a size distribution coefficient (Flyvbjerg *et al.* 1999).

Statistical analysis

The differences in kidney weight and glomerular volume after the different administration regimen were analyzed and expressed as mean ± S.E.M. For comparison of multiple

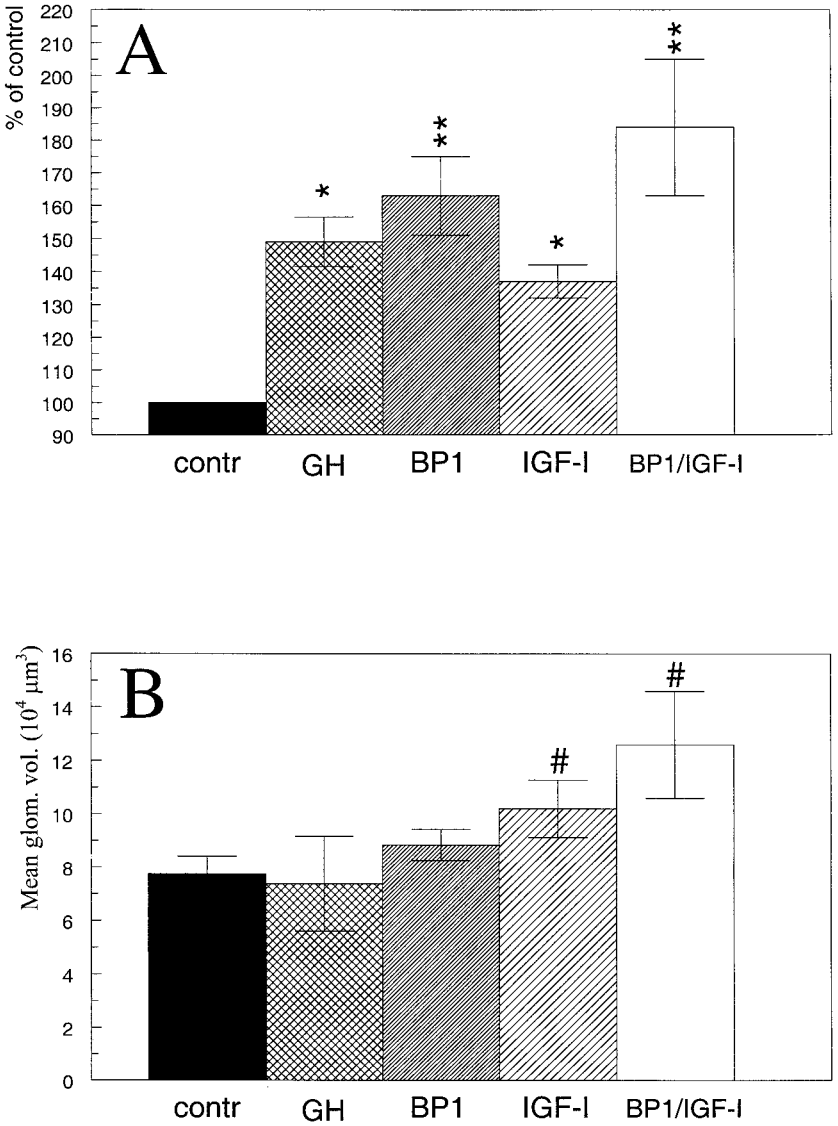

Figure 1 (A) Mean kidney weight (per pair of kidneys and expressed as percentage of the renal weight of control animals) and (B) mean glomerular volume (10^4 µm^3) of Snell dwarf mice injected for 28 days with (from the left) placebo; GH, 8·3 µg/day; IGFBP-1, 105 µg/day; IGF-I, 30 µg/day; and IGF-I+IGFBP-1, 30+105 µg/day. Values are means ± S.E.M.S ($n=5$). Differences between groups were determined by Scheffé's (**$P \leq 0{\cdot}05$) or Student's *t*-test (*$P \leq 0{\cdot}05$; $^{\#}P \leq 0{\cdot}12$).

groups we used ANOVA as described by Scheffé (1959). Student's *t*-test was applied for testing differences between two groups. A *P* value equal or less than 0·05 was regarded as significant.

Results

Kidney weight

Figure 1A shows the weights of the kidneys after 4 weeks of treatment, expressed as a percentage of the kidney weight of control animals. All treatment regimen significantly increased renal weight. The remarkable effects on the kidneys obtained with IGFBP-1 alone were significantly higher than with IGF-I alone ($P \leq 0{\cdot}05$), whereas the combination of IGFBP-1 and IGF-I seemed to demonstrate an additive effect on renal weight increase.

Renal morphology

To investigate possible differences in renal morphology between untreated, phenotypically normal heterozygous

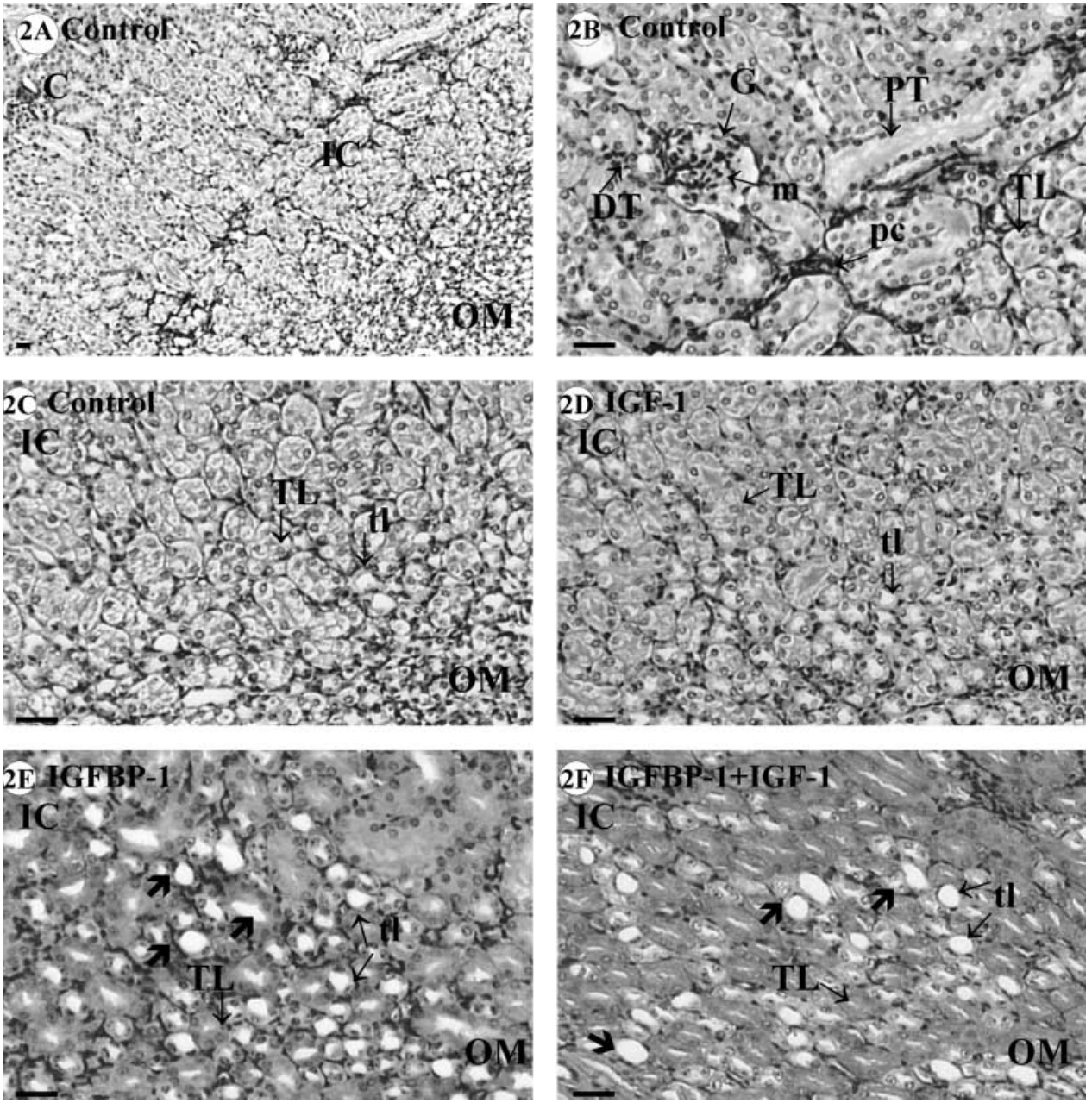

Figure 2 Morphology of the kidneys of Snell dwarf mice after different administration regimen. (A) PBS control: overview of the cortical and medullary area. (B) PBS control: cortex. (C) PBS control: outer medulla. (D) IGF-I administration: outer medulla. (E) IGFBP-1 administration: outer medulla. (F) IGFBP-1+IGF-I administration: outer medulla. Black arrows: dilatations of the thin limbs of Henle's loop. Abbreviations: C=cortex, IC=inner cortex, DT=distal tubule, G=glomerulus, m=mesangial cells, OM=outer medulla, pc=peritubular capillary, PT=proximal tubule, tl=thin limb of Henle's loop, TL=thick limb of Henle's loop. Bar=25 μm.

(+/dw) and untreated homozygous Snell dwarf mice, PAS stained sections were examined. No differences in morphology could be detected. Although kidneys of homozygous Snell dwarf mice were smaller, they were proportional to their decreased body size (results not shown). To examine the effects of the different administration regimen on the glomeruli, the glomerular volume was measured in all groups. Compared with placebo control kidneys, glomerular volume did not change significantly, although a tendency to increase could be observed in both the IGF-I and IGFBP-1/IGF-I groups ($P=0{\cdot}12$ and $P=0{\cdot}11$ respectively) (Fig. 1B). Figure 2A–C

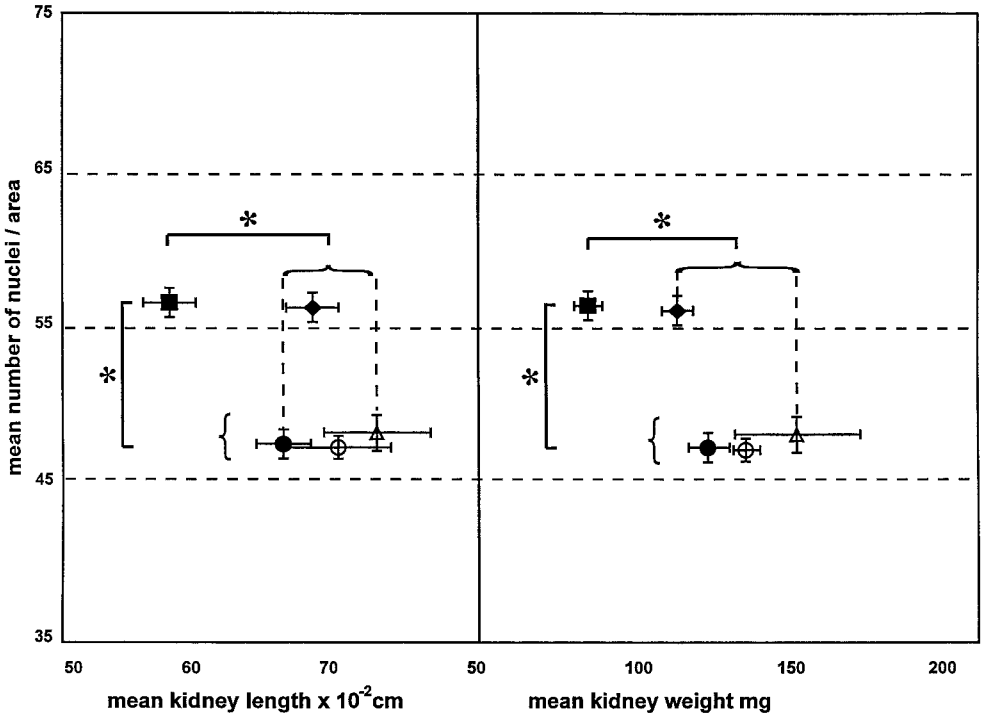

Figure 3 Mean kidney length (per kidney) and weight (per pair of kidneys) ± S.E.M. were plotted against the mean number of nuclei ± S.E.M. per cortical area, for all treatments. *Significant difference with $P=0{\cdot}05$. ■ PBS; ● GH; ♦ IGF-I; ○ IGFBP-1; △ IGF-I+IGFBP-1.

shows the morphology of the dwarf mouse kidney. After treatment with IGFBP-1 (Fig. 2E) and IGFBP-1+IGF-I (Fig. 2F), kidneys of Snell dwarf mice demonstrated pronounced dilatation of thin limbs of Henle's loop in the medulla. This dilatation could not be observed in IGF-I (Fig. 2D) and hGH (results not shown) treated mice. Regardless of the treatment, kidney weight increased simultaneously with kidney size. To determine if increased kidney weight reflected an increase in cell number, nuclei were counted in cortical areas. Kidney length and kidney weight were plotted against the number of cell nuclei per cortical area (Fig. 3). In glomeruli, no changes in numbers of nuclei between the different treatment regimen were observed (data not shown). In the tubular cortical region and compared with control, a decreased number of nuclei per cortical area was found for all treatments, except for IGF-I. After treatment of IGF-I, kidney length and weight were increased, while the cell number per area did not change. In contrast, treatment of Snell dwarf mice with hGH, IGFBP-1 or IGF-I+IGFBP-1 caused an increase in kidney length and weight, while the cell number per cortical area decreased simultaneously (Fig. 3). Also, staining against the cell proliferation marker PCNA revealed an increased cell labeling

Figure 4 Analysis of type 1 IGF receptor mRNA (blue), and type 1 IGF receptor, IGFBP-3 and -4 protein (brown) in kidneys of Snell dwarf mice injected for 28 days with PBS (control) (A, E, I, K); GH (B, F); IGFBP-1 (C, G, J, L); and IGF-I+IGFBP-1 (D, H). (A) Type 1 IGF receptor mRNA of the control group in the cortical region in proximal tubules, thick limbs of Henle's loop, distal tubules and glomeruli. (B) Type 1 IGF receptor mRNA in the GH group in the cortical region in proximal tubules, thick limbs of Henle's loop, distal tubules and glomeruli. (C) Type 1 IGF receptor mRNA in the IGFBP-1 group in the cortical region in proximal tubules, thick limbs of Henle's loop, distal tubules and glomeruli. (D) Type 1 IGF receptor mRNA in the IGFBP-1+IGF-I group in the cortical region in proximal tubules and thick limbs of Henle's loop. (E) Type 1 IGF receptor protein in the control group in the cortical region in proximal tubules, distal tubules and thick limbs of Henle's loop. (F) Type 1 IGF receptor protein in the GH group in the cortical region in proximal tubules, distal tubules and thick limbs of Henle's loop. (G) Type 1 IGF receptor protein in the IGFBP-1 group in the inner cortical region in proximal tubules and thick limbs of Henle's loop. (H) Type 1 IGF receptor protein in the IGFBP-1+IGF-I group in the inner cortical region in proximal tubules and thick limbs of Henle's loop. (I) IGFBP-3 protein in the control group in the inner cortical region in peritubular capillaries. (J) IGFBP-3 protein in the IGFBP-1 group in the inner cortical region in peritubular capillaries. (K) IGFBP-4 protein in the control group in the cortical region in proximal tubules and in thick and thin limbs of Henle's loop. (L) IGFBP-4 protein in the IGFBP-1 group in the cortical region in proximal tubules. BP1=IGFBP-1, BP-3=IGFBP-3, BP-4=IGFBP-4, DT=distal tubule, G=glomerulus, pc=peritubular capillary, PT=proximal tubule, t1rec=type 1 IGF receptor, tl=thin limb of Henle's loop, TL=thick limb of Henle's loop. Bar=25 μm.

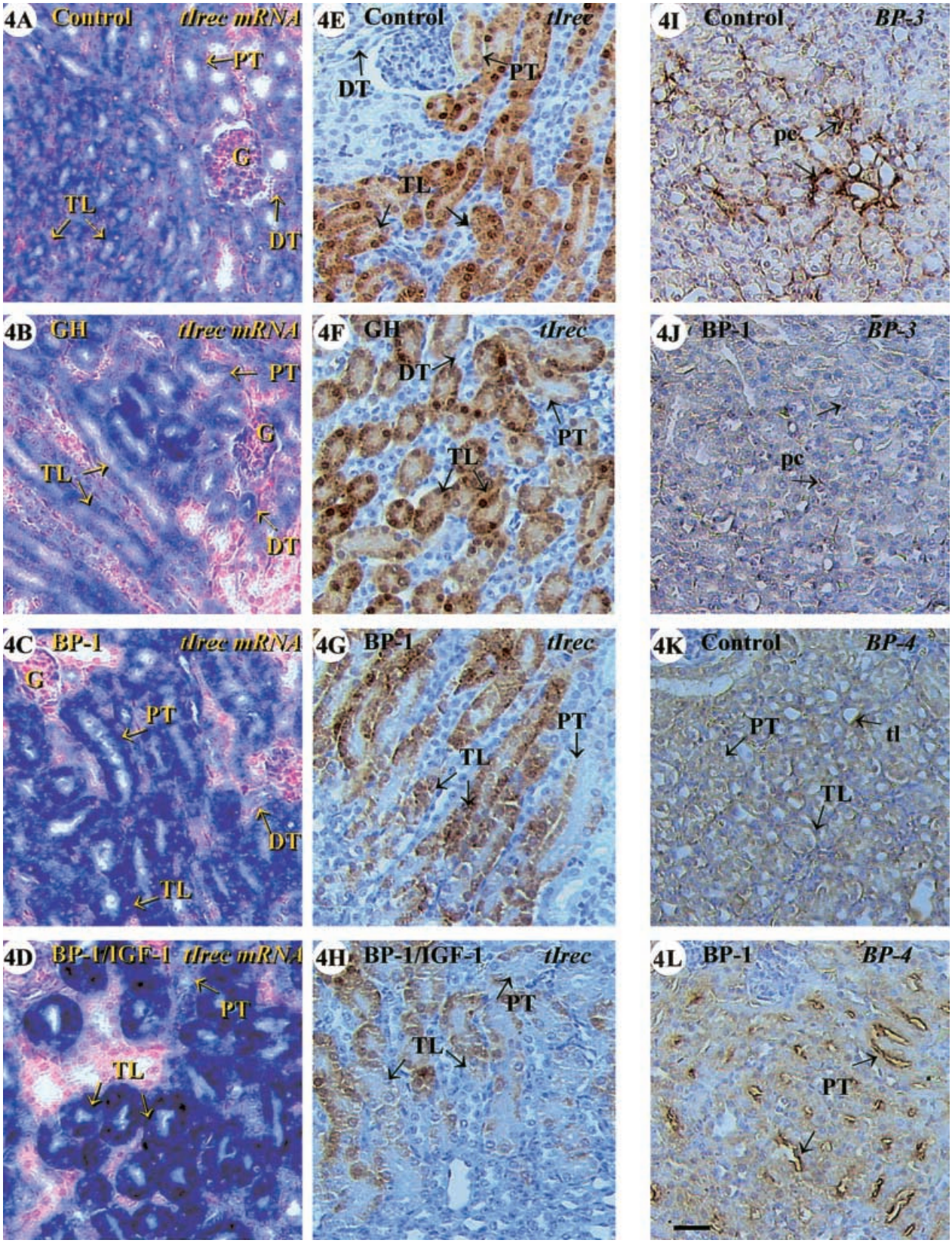
4A Control tIrec mRNA
PT
G
TL
DT
4E Control tIrec
DT
PT
TL
4I Control BP-3
pc
4B GH tIrec mRNA
PT
G
TL
DT
4F GH tIrec
DT
PT
TL
4J BP-1 BP-3
pc
4C BP-1 tIrec mRNA
G
PT
DT
TL
4G BP-1 tIrec
PT
TL
4K Control BP-4
PT
tl
TL
4D BP-1/IGF-1 tIrec mRNA
PT
TL
4H BP-1/IGF-1 tIrec
PT
TL
4L BP-1 BP-4
PT

Table 1 (*A*) mRNA localization in control group (PBS). (*B*) Changes in mRNA expression after different administration regimen

	t1rec	IGF-I	IGF-II	BP-2	BP-3	BP-4	BP-5
(*A*) mRNA localization							
Structures							
Cortex							
Glomeruli: Mesangial cells						+	+
Bowman's capsule	+	+	+	+			
Proximal tubules	+	+	+	+		+	
Thick limbs of Henle's loop	+	+	+	+		+	
Distal/collecting tubules	+	+	+	+			+
Peritubular capillaries		+			+	+	+
Medulla							
Thick limbs of Henle's loop/collecting ducts	+	+	+	+			
Thin limbs of Henle's loop				+			
Peritubular capillaries		+			+	+	+
(*B*) Changes in mRNA expression							
Administration							
GH	↑			↑			
IGF-I				↑			
IGFBP-1	↑	↑	↑	↑			↑
IGFBP-1/IGF-I	↑			↑			

index in the IGF-I treatment group only (results not shown).

Renal expression of the members of the IGF system

mRNA localization Table 1A shows the renal mRNA expression patterns of the members of the IGF system of the control mice. These were similar to those described previously for kidneys obtained from normal BALB/c mice (Lindenbergh-Kortleve *et al*. 1997). mIGFBP-1 and mIGFBP-6 mRNA expression was very weak in kidneys of all administration groups and no differences were observed between the various administration modalities (results not shown). In Figs 4 and 5, representative mRNA expression patterns for the type I IGF-receptor, the IGFs and the IGFBPs of kidneys from GH, IGF-I, IGFBP-1 and IGFBP-1+IGF-I administered mice are shown, in order to illustrate the data used for Table 1B where differences, as compared with controls, are given, based on the screening of five animals for each treatment.

Control In kidneys of heterozygous normal and homozygous dwarf control mice, type I IGF receptor mRNA was localized in Bowman's capsule, distal and proximal tubules and thick limbs of Henle's loop (Fig. 4A). IGF-I mRNA was located in Bowman's capsule, proximal and distal tubules, peritubular capillaries of the inner cortex and the medulla and thick limbs of Henle's loop (Fig. 5A). Localization of IGF-II mRNA was found in Bowman's capsule, distal and proximal tubules and thick limbs of Henle's loop (Fig. 5B). IGFBP-2 mRNA was observed in the cortex in Bowman's capsule, thick limbs of Henle's loop, distal tubules and to a lesser extent in proximal tubules (Fig. 5C). In the medulla, IGFBP-2 hybridization signals were found in the thick and thin limbs of Henle's loop (results not shown). Localization of IGFBP-3 mRNA was confined to peritubular capillaries of the outer cortex and the medulla (results not shown). Hybridization signals for IGFBP-4 mRNA were found in mesangial cells of glomeruli, proximal tubules, cortical thick limbs of Henle's loop and in peritubular capillaries of both the cortex and outer medulla (results not shown). IGFBP-5 mRNA was localized in the cortex in mesangial cells of glomeruli, juxtaglomerular cells, distal tubules and in peritubular capillaries of the outer cortex and medulla (Fig. 5G).

GH administration In GH-administered dwarf mice, renal expression of the type I IGF-receptor mRNA was increased in distal tubules (Fig. 4B). Also IGFBP-2 mRNA expression was increased in the inner cortex in distal tubules (results not shown).

IGF-I administration IGF-system mRNA expression in kidneys of IGF-I administered dwarf mice was largely comparable to kidneys from control animals (results not shown). Only IGFBP-2 mRNA expression was more pronounced in distal tubules and cortical thick limbs of Henle's loop (results not shown).

IGFBP-1 administration IGFBP-1 administration revealed important changes in renal IGF-system mRNA expression. Type I IGF receptor mRNA expression was increased in the cortex in proximal tubules and in the thick limbs of Henle's loop (Fig. 4C). IGF-I mRNA expression was increased in the thick limbs of Henle's loop

in both cortex and medulla (Fig. 5D). Similarly, staining of IGF-II mRNA was enhanced in cortical thick limbs of Henle's loop (Fig. 5E). Increased IGFBP-2 mRNA signal was found in distal tubules of the cortex (results not shown). Furthermore, the number of epithelial cells from distal tubules that demonstrated IGFBP-5 mRNA expression was increased after IGFBP-1 treatment (Fig. 5J).

IGFBP-1+IGF-I administration Combined administration of IGFBP-1+IGF-I demonstrated elevated type I IGF receptor mRNA signals in the thick limbs of Henle's loop (Fig. 4D). IGFBP-2 mRNA expression was elevated in Bowman's capsule and in distal tubules (Fig. 5F).

Protein localization

Results of immunohistochemistry of kidneys of the different groups are summarized in Table 2A. Representative expression patterns for the type I IGF-receptor and the IGFBPs (Figs 4 and 5) of kidneys from GH, IGF-I, IGFBP-1 and IGFBP-1+IGF-I administered mice are shown, in order to illustrate the data used for Table 2B where differences, as compared with controls, are given based on the screening of five animals for each treatment. IGF-I, -II, IGFBP-1 and -6 could not be detected immunohistochemically.

Control The kidneys of control dwarf mice revealed type I IGF receptor protein localization in proximal tubules, distal tubules and in thick limbs of Henle's loop in cortex and medulla (Fig. 4E). IGFBP-2 protein was detected in proximal tubules and thick and thin limbs of Henle's loop (Fig. 5I). IGFBP-3 protein was localized in peritubular capillaries of both the cortex (Fig. 4I) and the outer medulla. IGFBP-4 localization was demonstrated in mesangial cells in the glomeruli, in proximal tubules and in thick and thin limbs of Henle's loop (Fig. 4K). IGFBP-5 staining was weak and could be confined to proximal tubules (Fig. 5H).

GH administration When comparing the GH infused group with the control group, renal type I IGF receptor staining was more intense in proximal tubules and cortical thick limbs of Henle's loop (Fig. 4F). All other members of the IGF system showed identical staining patterns as in kidneys of control animals (Table 2).

IGF-I administration Compared with controls, IGF-I administration did not result in any difference in staining patterns for the IGF system members that were tested as is illustrated for type I IGF receptor (results not shown).

IGFBP-1 administration Type I IGF receptor protein staining was decreased in proximal and distal tubules and in thick limbs of Henle's loop (Fig. 4G). Compared with the control group, IGFBP-3 staining was decreased in peritubular capillaries in both cortex and medulla (Fig. 4J). IGFBP-4 staining was increased in the brush border of proximal tubules (Fig. 4L). IGFBP-5 showed a more intense staining in distal tubules (Fig. 5K).

IGFBP-1+IGF-I administration The staining patterns of the IGF system components after combined IGFBP-1+IGF-I treatment largely resembled the patterns found after IGFBP-1 treatment, except for IGFBP-2 that was decreased in proximal tubules and the thick limbs of Henle's loop (Fig. 5L). Comparable to the IGFBP-1 administered group, type I IGF receptor protein staining was strongly decreased in proximal and distal tubules and in thick limbs of Henle's loop (Fig. 4H). IGFBP-3 staining was decreased in peritubular capillaries in both cortex and medulla, IGFBP-4 staining was increased in the brush border of proximal tubules and IGFBP-5 showed a more intense staining in proximal and distal tubules (results not shown).

Discussion

In our previous study we have demonstrated that treatment of homozygous Snell dwarf mice with GH and IGF-I, for a period of 4 weeks, caused an increase in body length and body weight (van Buul-Offers *et al.* 2000). GH, IGF-I and IGFBP-1 treatment also had a pronounced effect on kidney size. Although IGFBP-1 inhibited IGF-I induced total body growth, it further stimulated IGF-I induced kidney growth (van Buul-Offers *et al.* 2000). In this study we investigated the renotropic effects of IGFBP-1 in further detail.

Effects of GH, IGF-I and/or IGFBP-1 on kidney morphology

In the present study the effects of GH, IGF-I, IGFBP-1 and IGFBP-1+IGF-I administration on the morphology and the expression pattern of members of the IGF system in kidneys of Snell dwarf mice were analyzed. Although an increased wet weight of the kidneys was observed in all treatment groups, when compared with controls, the morphology was different. When IGFBP-1, alone or in combination with IGF-I, was administered, fluid filled dilatations of thin limbs of Henle's loop were observed. This increased kidney weight occurred with a decreased mean number of nuclei per cortical area. Thus, we may conclude that short-term IGFBP-1 or IGF-I+IGFBP-1 treatment affected renal intracellular fluid accumulation and not cellular proliferation, as supported by an unchanged proliferation labeling index. This in contrast with treatment with IGF-I alone resulting in enlarged kidney size, due to enhanced kidney cell growth.

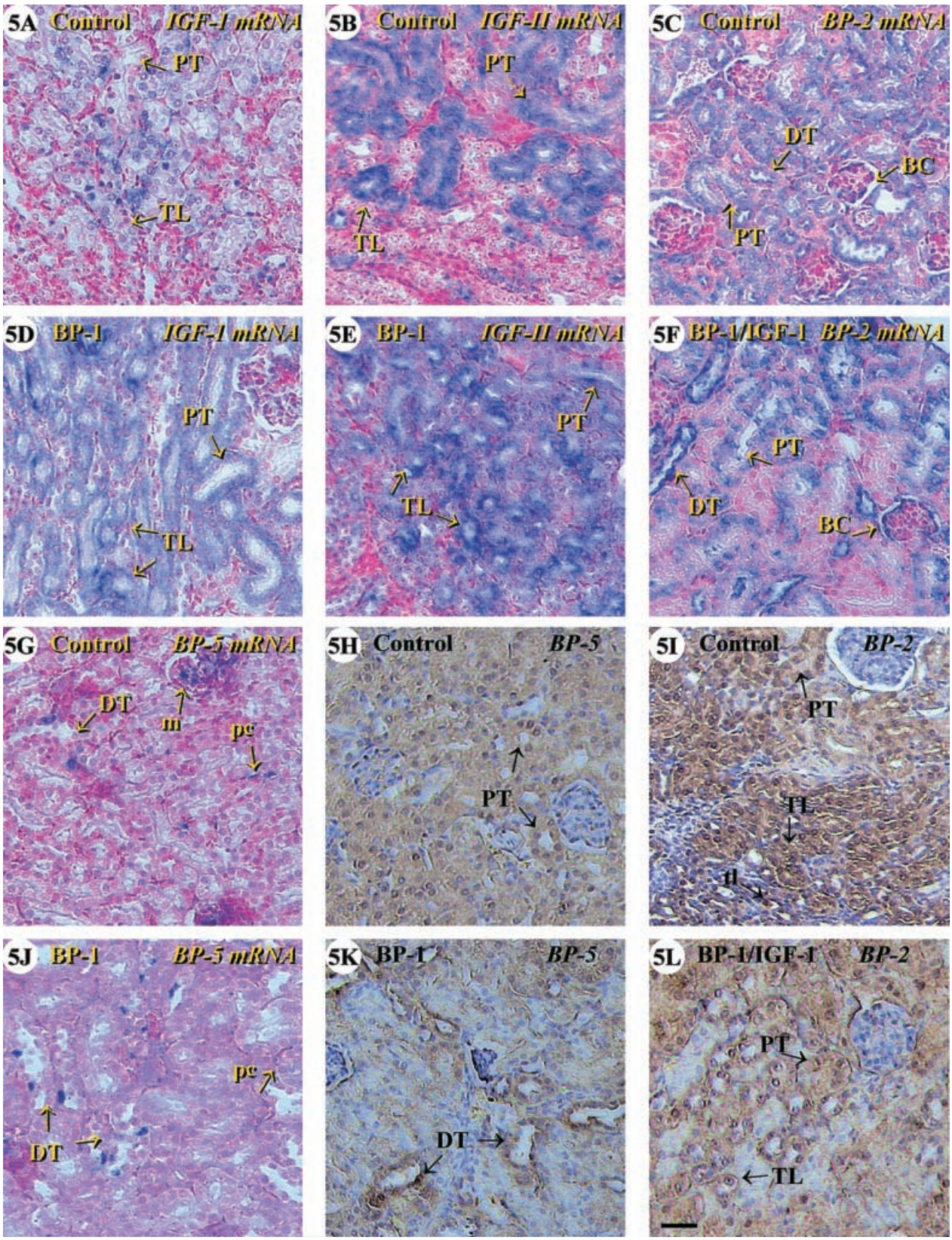
5A Control IGF-I mRNA
PT
TL
5B Control IGF-II mRNA
PT
TL
5C Control BP-2 mRNA
DT
BC
PT
5D BP-1 IGF-I mRNA
PT
TL
5E BP-1 IGF-II mRNA
PT
TL
5F BP-1/IGF-1 BP-2 mRNA
PT
DT
BC
5G Control BP-5 mRNA
DT
m
pc
5H Control BP-5
PT
5I Control BP-2
PT
TL
tl
5J BP-1 BP-5 mRNA
pc
DT
5K BP-1 BP-5
DT
5L BP-1/IGF-1 BP-2
PT
TL

Table 2 (*A*) Protein localization in control group (PBS). (*B*) Changes in protein expression after different administration regimen

	t1rec	BP-2	BP-3	BP-4	BP-5
(*A*) Protein localization					
Structures					
Cortex					
Glomeruli: Mesangial cells				+	
Bowman's capsule					
Proximal tubules	+	+		+	+
Thick limbs of Henle's loop	+	+		+	
Distal/collecting tubules	+			+	
Peritubular capillaries			+		
Medulla					
Thick limbs of Henle's loop/collecting ducts	+	+		+	
Thin limbs of Henle's loop		+		+	
Peritubular capillaries			+		
(*B*) Changes in protein expression					
Administration					
GH	↑				
IGF-I					
IGFBP-1	↓		↓	↑	↑(d)
IGFBP-1/IGF-I	↓	↓	↓	↑	↑

(d) in distal/collecting tubules.

This study is comparable with a previous study by van Buul-Offers and co-workers (1995), where the effects of IGFBP-3 and IGF-I, alone and in combination, were investigated on total body and organ growth. In contrast to IGFBP-1, IGFBP-3 inhibited IGF-I induced kidney growth, whereas administration of IGFBP-3 alone did not affect kidney size. In retrospect, we also observed no differences in morphology of the IGFBP-3 treated kidneys compared with the controls (data not shown). These findings might indicate different functions for IGFBP-1 and IGFBP-3 in the kidney.

The renal effects of IGFBP-1 administration in our present study seemed to be in contrast with observations in transgenic mouse models that overexpress IGFBP-1. In transgenic animals overexpressing IGFBP-1 under control of the metallothionein or phosphoglycerate promoter, no gross changes in kidney size and morphology were described (Dai *et al.* 1994, Murphy *et al.* 1995). It must be mentioned that in these transgenic animals elevated circulatory IGFBP-1 levels were not observed. In contrast, transgenic animals overexpressing IGFBP-1 under control of the liver-specific α1-antitrypsin promoter demonstrated a marked increase in the mesangial extracellular matrix. However, the total glomerular volume was not increased, suggesting that these IGFBP-1 transgenic animals developed glomerulosclerosis without glomerular hypertrophy (Doublier *et al.* 2000).

In the present study, administration of GH or IGF-I to the Snell dwarf mice resulted in enlarged kidneys, confirming previous reports (Hirschberg 1993, van Buul-Offers *et al.* 1995). Overexpression of GH and/or IGF-I in transgenic mice affected renal morphology (Quaife *et al.* 1989, Wanke *et al.* 1991). This, however, differed from the observations in our study. Kidneys of GH transgenic mice showed glomerulosclerosis and tubular cysts (Brem *et al.* 1989) whereas in our study no effects on kidney

Figure 5 Analysis of IGF-I, -II, IGFBP-2 and -5 mRNA (blue) and protein (brown) in kidneys of Snell dwarf mice injected for 28 days with PBS (control) (A–C, G–I); IGFBP-1 (D, E, J, K); and IGF-I+IGFBP-1 (F, L). (A) IGF-I mRNA in the control group in the inner cortical region in peritubular capillaries, thick limbs of Henle's loop, and proximal tubules. (B) IGF-II mRNA in the control group in the inner cortical region in proximal tubules and thick limbs of Henle's loop. (C) IGFBP-2 mRNA in the control group in the outer cortical region in Bowman's capsule, distal tubules, and proximal tubules. (D) IGF-I mRNA in the IGFBP-1 group in the cortical region in proximal tubules and thick limbs of Henle's loop. (E) IGF-II mRNA in the IGFBP-1 group in the inner cortical region in proximal tubules and in the thick limbs of Henle's loop. (F) IGFBP-2 mRNA in the IGFBP-1+IGF-I group in the outer cortical region in distal tubules and Bowman's capsule. (G) IGFBP-5 mRNA in the control group in the cortical region in mesangial cells, peritubular capillaries, and distal tubules. (H) IGFBP-5 in the control group in the cortical region in proximal tubules. (I) IGFBP-2 in the control group in the cortical region in proximal tubules, thick and thin limbs of Henle's loop. (J) IGFBP-5 mRNA in the IGFBP-1 group in the cortical region in distal tubules. (K) IGFBP-5 in the IGFBP-1 group in the cortical region in proximal and distal tubules. (L) IGFBP-2 in the IGFBP-1+IGF-I group in the cortical region in proximal tubules and thick limbs of Henle's loop. BC=Bowman's capsule, BP1=IGFBP-1, BP-2=IGFBP-2, BP-5=IGFBP-5, DT=distal tubule, m=mesangial cells, pc=peritubular capillary, PT=proximal tubule, tl=thin limb of Henle's loop, TL=thick limb of Henle's loop. Bar=25 μm.

morphology were seen. These differences might be explained by the fact that in the transgenic animals, circulating levels of GH were pharmacologically high whereas in our (dwarf) model we used substitution doses (Brem *et al.* 1989). Supranormal levels of IGF-I in transgenic animals resulted in enlarged glomeruli (Quaife *et al.* 1989). The circulating IGF-I levels of these transgenic mice and of the IGF-I-treated dwarf mice in our study were comparable (540 and 417 μg/l respectively) (Mathews *et al.* 1988, van Buul-Offers *et al.* 2000), although the transgenic animals have constant circulatory IGF-I levels whereas in our study IGF-I was administered three times per day, for 4 weeks. Interestingly, also in our study, a tendency towards an increase in glomerular volume was observed.

We only can speculate about the mechanisms of the tubular effects of IGFBP-1, which is solely observed in groups that received IGFBP-1, either alone or in combination with IGF-I and is not seen in the IGF-I administration group. It can be hypothesized that, under normal circumstances, circulatory IGF-I, when it is predominantly bound to IGFBP-3, does not leave the glomerular capillary network. In contrast, when IGFBP-1 is present abundantly, IGFBP-1/IGF-I complexes will be formed and transported from the glomerular capillary network into the tubular system. Here, IGFBP-1 determines the tubular bioavailability for the IGFs. This hypothesis is favored by a body of experimental evidence. First, the renal morphological effects of prolonged IGF-I overexpression in transgenic animals only are limited to glomerular hypertrophy (Doi *et al.* 1990). In addition, IGF-I administration, both in the human and in experimental animals, had direct effects on glomeruli where it increased renal plasma flow and glomerular filtration rate with a concomitantly decreased vascular resistance (Feld & Hirschberg 1996*a*). Furthermore, in hypokalemia, increased renal IGFBP-1 mRNA and increased immunoreactive IGFBP-1 has been observed, which is spatially associated with tubular hyperplasia (Hsu *et al.* 1997, Rohan *et al.* 1997, van Neck *et al.* 1997). These results suggest that IGFBP-1/IGF-I might play a major role in distal nephron adaptation.

Whether the effects of IGFBP-1 are IGF-I dependent or independent needs to be further elucidated. In this respect, a role for IGFBP-1 has been suggested in a streptozotocin induced diabetes model (Landau *et al.* 1995, Feld & Hirschberg 1996*b*, Raz *et al.* 1998, Segev *et al.* 1999). Feld & Hirschberg (1996*b*) suggested that IGFBP-1 may have a stimulating effect on kidney growth, independent of IGF-I, through mitogenic actions via $\alpha_5\beta_2$-integrins. Our results indicate, however, that these putative IGF-independent functions of IGFBP-1 were not blocked when IGF-I was co-administered with IGFBP-1, suggesting that IGF-I binding to its own receptor does not interfere with the putative IGF-independent actions of IGFBP-1.

Effects on renal expression of IGF system components

Of all administration regimen, the effect of IGFBP-1 administration on expression patterns of IGF system mRNA and protein was most prominent. Furthermore, the administered compounds had different effects on mRNA expression and protein localization. Most remarkable was the finding that the effects of administration of IGFBP-1 or IGFBP-1+IGF-I had similar effect on IGF system protein levels. Also the morphological changes after IGFBP-1 or IGFBP-1+IGF-I administration were identical. These findings, again, might indicate that IGFBP-1 plays an important role in the kidney.

Our results show a considerable overlap in the localization of the IGF system mRNA and protein. However, compared with protein, a larger number of different members of the IGF system were detected at the mRNA level. Furthermore, mRNA expression in a given cell type was not necessarily followed by the detectable presence of the corresponding protein in that cell type. It is unclear whether these differences represent a protein expression below the detection limit of our immunohistochemical assay, or whether these reflect the nature of these proteins. After all, the IGFs and IGFBPs are secreted proteins. Thus, mRNA levels and localization may deviate from protein levels and localization. Therefore, protein patterns likely are more informative when trying to elucidate the function(s) of the IGFBPs.

Discrepancies between mRNA and protein of IGF system components in the kidney have been reported in several studies. For example, in normal rat tubules, hardly any IGF-I mRNA is detected (Matejka & Jennische 1992, Matejka *et al.* 1992), while the protein is clearly found in proximal tubules (Kobayashi *et al.* 1991). This suggests that the majority of IGF-I protein in the kidney might be trapped from the circulation (D'Ercole *et al.* 1984, Mathews *et al.* 1986, Feld & Hirschberg 1996*b*). In our study, IGF-I protein could not convincingly be detected in any of the groups. This might be due to the specificity of the rabbit anti-human polyclonal antiserum in mouse immunohistochemistry.

A complicating factor in matching our findings with the literature is that descriptions of changed IGF system expression patterns in renal disease models are scarce and, if existing, are often derived from rat studies where, compared with mice, differences in IGF system expression are observed, especially regarding IGFBP-1 expression (Chin *et al.* 1992, Price *et al.* 1995, Rabkin *et al.* 1995, Lindenbergh-Kortleve *et al.* 1997).

In our study, the effects of GH or IGF-I administration on kidney morphology and changes in IGF system expression seemed to be comparable, and less prominent than the effects of IGFBP-1 or IGFBP-1+IGF-I administration.

When we examine the similarities in IGF system protein patterns between the IGFBP-1 and the IGFBP-1+IGF-I group, both demonstrate increased IGFBP-4 levels

in the thick limbs of Henle's loop. These may play an important role in the onset of fluid accumulation and/or formation of dilatated thin limbs of Henle's loop. Furthermore, IGFBP-5 levels in the proximal tubules were found to be increased whereas IGFBP-3 levels in the peritubular capillaries and type I IGF receptor levels in the distal/collecting tubules were decreased. However, we can not exclude that the changes in IGF system protein expression are not sufficient to induce the observed fluid accumulation and/or dilatation of the thin limbs of Henle's loop. Therefore, we suggest that a complex network of interacting factors may be implicated in this process.

In summary, we have shown that treatment with IGFBP-1 and IGFBP-1+IGF-I of Snell dwarf mice causes specific morphological changes in the kidney. Furthermore, specific alterations on mRNA as well as on protein levels of the IGF system occur. We suggest that IGFBP-1, directly or by regulating IGF bioavailability, may affect renal fluid exchange and/or increase hydrostatic pressure that may cause dilatations of the thin limbs of Henle's loop. Hence, our study provides a framework for a further understanding of the role of IGFBP-1 in renal physiology.

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References

Bach MA, Chin E & Bondy CA 1994 The effects of subcutaneous insulin-like growth factor-I infusion in insulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* **79** 1040–1045.

Brem G, Wanke R, Wolf E, Buchmüller T, Müller M, Brenig B & Hermanns W 1989 Multiple consequences of human growth hormone expression in transgenic mice. *Molecular Biology and Medicine* **6** 531–547.

van Buul-Offers SC, Reijnen-Gresnigt R, Bloemen R, Hoogerbrugge C & van den Brande JL 1995 Co-administration of IGF-binding protein-3 differentially inhibits the IGF-I induced total body and organ growth of Snell dwarf mice. *Progress in Growth Factor Research* **6** 377–383.

van Buul-Offers SC, van Kleffens M, Koster JG, Lindenbergh-Kortleve DJ, Gresnigt MG, Drop SL, Hoogerbrugge CM, Bloemen RJ, Koedam JA & van Neck JW 2000 Human insulin-like growth factor (IGF) binding protein-1 inhibits IGF-I-stimulated body growth but stimulates growth of the kidney in snell dwarf mice. *Endocrinology* **141** 1493–1499.

Chin E, Zhou J & Bondy C 1992 Anatomical relationships in the patterns of insulin-like growth factor (IGF)-I, IGF binding protein-1, and IGF-I receptor gene expression in the rat kidney. *Endocrinology* **130** 3237–3245.

Dai Z, Xing Y, Boney CM, Clemmons DR & D'Ercole AJ 1994 Human insulin-like growth factor-binding protein-1 (hIGFBP-1) in transgenic mice: characterization and insights into the regulation of IGFBP-1 expression. *Endocrinology* **135** 1316–1327.

D'Ercole A, Stiles AD & Underwood LE 1984 Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *PNAS* **81** 935–939.

Doi T, Striker LJ, Gibson CC, Agodoa LY, Brinster RL & Striker GE 1990 Glomerular lesions in mice transgenic for growth hormone and insulin-like growth factor-I. I. Relationship between increased glomerular size and mesangial sclerosis. *American Journal of Pathology* **137** 541–552.

Doublier S, Seurin D, Fouqueray B, Verpont M-C, Callard P, Striker LJ, Striker J, Binoux M & Baud L 2000 Glomerulosclerosis in mice transgenic for human insulin-like growth factor binding protein-1. *Kidney International* **57** 2299–2307.

Feld SM & Hirschberg R 1996*a* Growth hormone, the insulin-like growth factor system, and the kidney. *Endocrine Reviews* **17** 423–480.

Feld SM & Hirschberg R 1996*b* Insulin-like growth factor-binding proteins in the nephrotic syndrome. *Pediatric Nephrology* **10** 355–358.

Fervenza FC, Tsao T, Hoffman AR & Rabkin R 1997 Regional changes in the intrarenal insulin-like growth factor-I axis in diabetes. *Kidney International* **51** 811–818.

Flyvbjerg A 1993 The role of insulin-like growth factor I in initial renal hypertrophy in experimental diabetes. In *Growth Hormone and Insulin-like Growth Factor I in Human and Experimental Diabetes*, pp 271–306. Eds A Flyvbjerg, H Ørskov & KGMM Alberti. Chichester: John Wiley & Sons Ltd.

Flyvbjerg A, Bornfeldt KE, Marshall SM, Arnqvist HJ & Ørskov H 1990 Kidney IGF-I mRNA in initial renal hypertrophy in experimental diabetes in rats. *Diabetalogia* **33** 334–338.

Flyvbjerg A, Dørup I, Everts ME & Ørskov H 1991 Evidence that potassium deficiency induces growth retardation through reduced circulating levels of growth hormone and insulin-like growth factor-I. *Metabolism* **40** 769–775.

Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ & Scarlett JA 1999 Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. *Diabetes* **48** 377–382.

Gustafson AB, Shear L & Gabuzda GJ 1973 Protein metabolism *in vivo* in kidney, liver, muscle and heart of potassium deficient rats. *Journal of Laboratory and Clinical Medicine* **82** 287–296.

Hirschberg R 1993 Effects of growth hormone and IGF-I on glomerular ultrafiltration in growth hormone deficient rats. *Regulatory Peptides* **48** 241–250.

Hsu FW, Tsao T & Rabkin R 1997 The IGF-I axis in kidney and skeletal muscle of potassium deficient rats. *Kidney International* **52** 363–370.

Jones JI & Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16** 3–34.

van Kleffens M, Groffen CA, Dits NF, Lindenbergh-Kortleve DJ, Schuller AG, Bradshaw SL, Pintar JE, Zwarthoff EC, Drop SL & van Neck JW 1999 Generation of antisera to mouse insulin-like growth factor binding proteins (IGFBP)-1 to -6: comparison of IGFBP protein and messenger ribonucleic acid localization in the mouse embryo. *Endocrinology* **140** 5944–5952.

Kobayashi S, Clemmons DR & Ventkatachalam MA 1991 Colocalization of insulin-like growth factor-binding protein with insulin-like growth factor I. *American Journal of Physiology* **261** F22–F28.

Landau D, Chin E, Bondy C, Domene H, Roberts ST jr, Grønbaek H, Flyvbjerg A & LeRoith D 1995 Expression of insulin-like growth factor binding proteins in the rat kidney: effects of long-term diabetes. *Endocrinology* **136** 1835–1842.

Lee PD, Giudice LC, Conover CA & Powell DR 1997 Insulin-like growth factor binding protein-1: recent findings and new directions. *Proceedings of the Society for Experimental and Biological Medicine* **216** 319–357.

Lindenbergh-Kortleve DJ, Rosato RR, van Neck JW, Nauta J, van Kleffens M, Groffen C, Zwarthoff EC & Drop SLS 1997 Gene expression of the insulin-like growth factor system during mouse kidney development. *Molecular and Cellular Endocrinology* **132** 81–91.

Luo J & Murphy LJ 1992 Differential expression of the insulin-like growth factor binding proteins in spontaneously diabetic rats. *Journal of Molecular Endocrinology* **8** 155–163.

Matejka GL & Jennische E 1992 IGF-I binding and IGF-I mRNA expression in the post-ischemic regenerating rat kidney. *Kidney International* **42** 1113–1123.

Matejka GL, Eriksson PS, Carlsson B & Jennische E 1992 Distribution of IGF-I mRNA and IGF-I binding sites in the rat kidney. *Histochemistry* **97** 173–180.

Mathews LS, Nordtedt G & Palmiter RD 1986 Regulation of insulin-like growth factor I gene expression by growth hormone. *PNAS* **83** 9343–9347.

Mathews LS, Hammer RE, Behringer RR, D'Ercole AJ, Bell GI, Brinster RL & Palmiter RD 1988 Growth enhancement of transgenic mice expressing human insulin-like growth factor-I. *Endocrinology* **123** 2827–2833.

Murphy LJ, Rajkumar K & Molnar P 1995 Phenotypic manifestations of insulin-like growth factor binding protein-1 (IGFBP-1) and IGFBP-3 overexpression in transgenic mice. *Progress in Growth Factor Research* **6** 425–432.

van Neck JW, Flyvbjerg A, Schuller AGP, Rosato RR, Groffen C, van Kleffens M, Lindenbergh-Kortleve D, Dørup I & Drop SLS 1997 IGF, type I IGF receptor and IGF-binding protein mRNA expression in kidney and liver of potassium-depleted and normal rats infused with IGF-I. *Journal of Molecular Endocrinology* **19** 59–66.

Price GJ, Berka JL, Edmondson SR, Werther GA & Bach LA 1995 Localization of mRNAs for insulin-like growth factor binding proteins 1 to 6 in rat kidney. *Kidney International* **48** 402–411.

Quaife CJ, Mathews LS, Pinkert CA, Hammer RE, Brinster RL & Palmiter RD 1989 Histopathology associated with elevated levels of growth hormone and insulin-like growth factor-I in transgenic mice. *Endocrinology* **124** 40–48.

Rabkin R, Brody M, Lu LH, Chan C, Shaheen AM & Gillett N 1995 Expression of the genes encoding the rat renal insulin-like growth factor-I system. *Journal of the American Society of Nephrology* **6** 1511–1518.

Raz I, Rubinger D, Popovtzer M, Gronbaek H, Weiss O & Flyvbjerg A 1998 Octreotide prevents the early increase in renal insulin-like growth factor binding protein 1 in streptozotocin diabetic rats. *Diabetes* **47** 924–930.

Rohan RM, Unterman TG, Liu L & Hise MK 1997 Expression of the insulin-like growth factor system in the hypokalemic rat kidney. *American Journal of Physiology* **272** F661–F667.

Scheffé H 1959 The S-method of multiple comparisons: general case. In *The Analysis of Variance*, pp 68–73. Ed H Scheffé. New York: John Wiley and Sons Ltd.

Schuller AGP, Groffen C, van Neck JW, Zwarthoff EC & Drop SLS 1994 cDNA cloning and mRNA expression of the six mouse insulin-like growth factor binding proteins. *Molecular and Cellular Endocrinology* **104** 57–66.

Segev Y, Landau D, Rasch R, Flyvbjerg A & Phillip M 1999 Growth hormone receptor antagonism prevents early renal changes in nonobese diabetic mice. *Journal of the American Society of Nephrology* **10** 2374–2381.

Wanke R, Hermanns W, Folger S, Wolf E & Brem G 1991 Accelerated growth and visceral lesions in transgenic mice expressing foreign genes of the growth hormone family: an overview. *Pediatric Nephrology* **5** 513–521.

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