Liver-derived IGF-I regulates kidney size, sodium reabsorption, and renal IGF-II expression

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Abstract

The GH/-IGF-I axis is important for kidney size and function and may also be involved in the development of renal failure. In this study, the role of liver-derived endocrine IGF-I for kidney size and function was investigated in mice with adult liver-specific IGF-I inactivation (LI-IGF-I$^{-/-}$ mice). These mice have an 80–85% reduction of serum IGF-I level and compensatory increased GH secretion. Seven-month-old as well as 24-month-old LI-IGF-I$^{-/-}$ mice had decreased kidney weight. Glomerular filtration rate, assessed using creatinine clearance as well as creatinine clearance corrected for body weight, was unchanged. The 24-h urine excretion of sodium and potassium was increased in the LI-IGF-I$^{-/-}$ mice. In the 24-month-old mice, there was no between-group difference in kidney morphology. Microarray and real-time PCR (RT-PCR) analyses showed a high renal expression of IGF-II in the control mice, whereas in the LI-IGF-I$^{-/-}$ mice, there was a tissue-specific decrease in the renal IGF-II mRNA levels (−79%, $P<0{\cdot}001$ vs controls using RT-PCR). In conclusion, deficiency of circulating liver-derived IGF-I in mice results, despite an increase in GH secretion, in a global symmetrical decrease in kidney size, increased urinary sodium and potassium excretion, and a clear downregulation of renal IGF-II expression. However, the LI-IGF-I$^{-/-}$ mice did not develop kidney failure or nephrosclerosis. One may speculate that liver-derived endocrine IGF-I induces renal IGF-II expression, resulting in symmetrical renal growth.

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Introduction

The growth hormone (GH)/insulin-like growth factor-I (IGF-I) system is important for kidney size and function. Treatment with GH or IGF-I increases kidney size and glomerular filtration rate (GFR; Guler *et al.* 1988). In diabetic as well as nondiabetic end-stage renal failure, there are marked abnormalities in the GH/IGF-I system (Jain *et al.* 1998, Rabkin & Schaefer 2004). In humans with diabetes mellitus (DM) type I, circulating IGF-I is low and circulating GH levels are compensatorily high (Carroll *et al.* 1998). At least in rodents, there is increased expression of IGF-I in the kidney of diabetic animals as well as multiple other changes in local renal growth factor levels (Cingel-Ristic *et al.* 2004, Rabkin & Schaefer 2004). These changes could be involved in the development of the pathological glomerular changes observed in DM type I (Cingel-Ristic *et al.* 2004, Rabkin & Schaefer 2004). Finally, GH as well as IGF-I treatment induces fluid retention due to increased renal sodium reabsorption (Feld *et al.* 1995, Hirschberg & Adler 1998, Cingel-Ristic *et al.* 2004, Rabkin & Schaefer 2004).

The expression pattern of GH and IGF-I receptors in the kidney is complex. This is partly due to the complex structure of the kidney itself, which includes a vascular network, tubules, and an interstitial component (Rabkin & Schaefer 2004). In the rat kidney, GH and IGF-I receptors colocalize in the thick ascending loop of Henle, whereas GH and IGF-I receptors otherwise are expressed separately (Rabkin & Schaefer 2004). The effect of GH/IGF-I on kidney function may mainly be an IGF-I-mediated effect since the effect after GH administration is slower than that after IGF-I administration and not seen until the circulating IGF-I has been increased (Feld *et al.* 1995, Hirschberg & Adler 1998). However, it has been suggested that GH can increase renal sodium reabsorption by a direct effect on the proximal tubuli (Rabkin & Schaefer 2004).

The major part of serum IGF-I is liver derived (Sjögren *et al.* 1999, Yakar *et al.* 1999). In addition to being regulated by GH (Bengtsson *et al.* 1993), serum IGF-I levels are also affected by factors, such as food intake, exercise, and age

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(Landin-Wilhelmsen *et al.* 1994, Kaklamani *et al.* 1999, Ehrnborg *et al.* 2003). A mouse model with liver-specific inducible inactivation of the *IGF-I* gene, using the Cre–LoxP conditional knockout system, has been developed (LI-IGF-$I^{-/-}$ mice; Sjögren *et al.* 1999, 2001, 2002, Wallenius *et al.* 2001, Tivesten *et al.* 2002). The selective inactivation of the *IGF-I* gene in the liver results in an 80–85% reduction in serum IGF-I, whereas the renal expression of IGF-I is unaffected (Sjögren *et al.* 1999). These mice have increased GH secretion secondary to the decrease in serum IGF-I (geometric mean plasma GH 3·1 times higher in LI-IGF-$I^{-/-}$ than in control mice (Sjögren *et al.* 1999, Wallenius *et al.* 2001)). In addition, as previously shown using western ligand blotting (Sjögren *et al.* 2002), the LI-IGF-$I^{-/-}$ mice have unchanged serum insulin-like growth factor-binding protein-1 (IGFBP-I) level, whereas IGFBP-2 and -3 in serum were decreased by 74 and 86% respectively, when compared with control mice.

The LI-IGF-$I^{-/-}$ mice do not have any major disturbance in postnatal longitudinal bone growth, whereas they have increased blood pressure (BP) due to increased peripheral resistance without any detectable effect on the renin–aldosterone system (Tivesten *et al.* 2002). Blood glucose is normal, but circulating insulin levels are increased (Sjögren *et al.* 2001). Finally, kidney size is decreased (Sjögren *et al.* 1999). Here, we assess the role of adult expression of liver-derived IGF-I for renal function, morphology, and sodium handling and investigate to what extent changes in these factors are associated with alterations in renal global gene expression.

Materials and Methods

Animals and serum analyses of IGF-I and -II

The LI-IGF-$I^{-/-}$ mice were generated as previously described by Sjögren *et al.* (1999). Mice homozygous for LoxP (Liu *et al.* 1998), and heterozygous for Mx-Cre (Kuhn *et al.* 1995), were given polyinosinic–polycytidylic acid (PiPc; 6·25 μg/g body weight; Sigma–Aldrich Corp.) in three i.p. injections at 4 weeks of age to induce expression of the Cre protein in the liver (Kuhn *et al.* 1995). PiPc-treated littermates, homozygous for LoxP but lacking Mx-Cre, were used as controls. Seven days after the PiPc injections, blood was collected, and serum was assayed for IGF-I by a double-antibody IGF-binding protein-blocked RIA using a commercial kit (Mediagnost, Tubingen, Germany). In addition, in the 24-month-old mice, serum IGF-I (Mediagnost) was measured from serum collected when the mice were killed. In mice aged 9 months, serum IGF-II was measured by RIA after separation of IGFs from IGFBPs as described previously (Mohan & Baylink 1995, Miyakoshi *et al.* 2001). The animals had free access to fresh water and food pellets (B&K Universal AB, Sollentuna, Sweden). The study was approved by the ethical committee at the University of Göteborg.

Study design

In 6-month-old control ($n=7$) and LI-IGF-I ($n=7$) mice, serum creatinine and 24-h urine excretion of creatinine, sodium, and potassium were determined when the animals were placed in metabolic cages. Creatinine clearance was then calculated. One month later, at 7 months of age, basal tail-cuff systolic BP was assessed in these mice. Other 7-month-old mice (seven control and nine LI-IGF-$I^{-/-}$ mice) were anesthetized with a combination of fentanyl and fluanisone (0·55 and 17·5 mg/kg; Hypnorm, Janssen Pharmaceuticals, Beerse, Belgium) and midazolam (8·75 mg/kg; Dormicum, Hoffman-La-Roche Inc., Basel, Switzerland) and were killed by rapid excision of the heart. Kidney weights were assessed and microarray analyses (kidney, liver, muscle, heart, fat, and bone) were performed. In additional experiments, 24-month-old mice (eight control and five LI-IGF-$I^{-/-}$ mice) were killed in a similar way as the 7-month-old mice, kidney weights were determined and then examinations of kidney morphology were performed. Finally, serum IGF-II levels were determined in 9-month-old mice (nine control and seven LI-IGF-$I^{-/-}$ mice).

Systolic BP measurements

Systolic BP was measured using a computerized noninvasive tail-cuff system (RTBP Monitor; Harvard Apparatus, Inc., South Natick, MA, USA). Unanesthetized animals were kept in a restrainer, with a standardized acclimatization time of 10 min and gentle heating of the tail before the recordings. Basal systolic BP measurements were performed on three separate days, with at least three recordings for each time point. Final systolic BP was obtained by averaging the mean values from the different time points.

Measurements of sodium, potassium, creatinine, and creatinine clearance

Urine was collected from LI-IGF-$I^{-/-}$ and control mice placed in metabolic cages during 24 h, with free access to tap water and food pellets. Concentrations of sodium and potassium in urine were analyzed using flame photometry (FLM3, Radiometer, Copenhagen, Denmark). Creatinine in serum and urine was determined by a colorimetric method (Sigma–Aldrich Corp.). Creatinine clearance was then calculated and used as a measure of GFR (creatinine clearance = (urinary creatinine)/(serum creatinine) × (urinary flow)). GFR values are given as absolute values or values corrected for body weight.

Kidney morphology

Specimens were processed from formaldehyde-fixed paraffin-embedded tissue and sectioned at 3–4 μm. The sections were stained with either hematoxylin and eosin or periodic acid-Schiff and light microscopic examination was performed.

All determinations of kidney morphology were performed in a blinded fashion by the same pathologist.

DNA microarray analysis

Total RNA from kidneys was extracted by Tri Reagent (Sigma) and further purified using spin columns from Rneasy Total RNA Isolation Kit (Qiagen) according to the manufacturer's instructions. RNA from 7-month-old mice (six control and seven LI-IGF-I$^{-/-}$ mice) was prepared. For microarray analysis, the RNA samples were pooled into two or three, resulting in three pools per group. The pooled RNA was reverse transcribed into cDNA, labeled, and analyzed by DNA microarray (MG-U74Av2 Array; Affymetrix, Santa Clara, CA, USA). The array represents 7000 mouse genes and 5000 uncharacterized expressed sequence tag clones. Preparation of labeled cRNA and hybridization were done according to the Affymetrix Gene Chip Expression Analysis manual.

The gene expression of IGF-II was also determined in microarray analyses of liver, muscle, heart, fat (retroperitoneal fat), and bone (vertebrae). These microarray analyses were performed using similar methodology as described above.

Bioinformatics

Scanned output files were analyzed using Affymetrix Micro Array Suite version 4.0.1 software (Affymetrix). To allow comparison of gene expression, the gene chips were globally scaled to an average intensity of 500. Each of the three LI-IGF-I$^{-/-}$ chips was compared with the three control chips, generating nine comparison files in total. Only the genes that were regarded as 'changed,' according to the Affymetrix algorithm, in five to nine of the comparisons were selected for further analysis. An average-fold change for the nine comparisons of the selected genes was then calculated. For a gene to be regarded as regulated in the LI-IGF-I$^{-/-}$ mice, the average-fold increase or decrease of the nine comparisons was set to be at least threefold. These relatively strict criteria ensured that only genes that were strongly regulated by the circulating IGF-I were detected.

Real-time PCR (RT-PCR) analysis

In order to confirm the microarray findings, RT-PCR analyses were performed (ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Stockholm, Sweden)). The RT-PCR analyses were performed on renal tissue samples from individual mice (no pooling; control mice ($n=6$) and LI-IGF-I$^{-/-}$ mice ($n=7$)). An FAM-labeled probe specific for IGF-II was used (Accession no. NM_010514, forward primer: CCGTACTTCCGGACGACTTC, reverse primer: CGTCCCGCGGACTGTCT, probe: CGTGGGCAAGTTCTTCCAATATGACACC; PE Applied Biosystems). Predesigned primers (PE Applied Biosystems) and a VIC-labeled probe for 18S rRNA were included in the reactions as an internal standard. The cDNA was amplified at the following conditions: one cycle at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The mRNA amount of each gene was calculated using the standard curve method (multiplex reaction, following the instructions in User Bulletin no. 2, PE Applied Biosystems) and adjusted for the expression of 18S rRNA.

Statistical analyses

All the descriptive statistical results are presented as the mean ± S.E.M. Between-group differences were calculated using unpaired *t*-tests. Furthermore, for body weight, kidney weight, and kidney weight/body weight, a two-way ANOVA with age and study group as the independent variables was performed followed by Student–Neuman-Keul's multiple range test. A two-tailed $P<0{\cdot}05$ was considered significant.

Results

Serum IGF-I, body weight, and kidney size

Liver-specific inactivation of the *IGF-I* gene was induced at 4 weeks of age, resulting in a reduction of serum IGF-I level by 80–85% in the LI-IGF-I$^{-/-}$ mice when compared with the control mice (data not shown). This reduction in serum IGF-I level was sustained until 24 months of age (24-month-old LI-IGF-I$^{-/-}$ mice, 24 ± 7 ng/ml; 24-month-old controls, 149 ± 21 ng/ml; $P<0{\cdot}001$).

Body weight increased with increasing age, but there was no significant difference in body weight between the LI-IGF-I$^{-/-}$ and the control mice (Table 1). Kidney weight and kidney weight per body weight were not affected by age, but both absolute and relative kidney weight were reduced in the LI-IGF-I$^{-/-}$ mice (Table 1).

GFR, BP, excretion of sodium and potassium, and kidney morphology

In mice aged 6 months, serum and 24-h urine concentrations of creatinine were similar in the two study groups (Table 2). There was no between-group difference in GFR, as assessed using creatinine clearance or creatinine clearance corrected for body weight (Table 2). At 7 months of age, systolic BP was increased in the LI-IGF-I$^{-/-}$ mice (LI-IGF-I$^{-/-}$ mice ($n=7$), 141 ± 5 mm Hg; control mice ($n=7$), 126 ± 3 mm Hg; $P<0{\cdot}05$).

The 24-h urine volume (Fig. 1A) was unchanged, whereas the 24-h urinary excretion of sodium (Fig. 1B) and potassium (Fig. 1C), determined when the mice were placed in metabolic cages, were both increased in the LI-IGF-I$^{-/-}$ mice when compared with the control mice.

In 24-month-old mice, there was no change in kidney morphology in the LI-IGF-I$^{-/-}$ mice when compared with

Table 1 Body and kidney weights in 7- and 24-month-old controls and LI-IGF-I$^{-/-}$ mice. Kidney weights are given as the combined weight of right and left kidney. Values are given as the mean (S.E.M.). A two-way ANOVA with age and study group as the independent variables was performed followed by Student–Neuman-Keul's multiple range test

	7-month-old mice		24-month-old mice		Two-way ANOVA	
	Control	LI-IGF-I$^{-/-}$	Control	LI-IGF-I$^{-/-}$	*P*-value for age	*P*-value for group
n	7	9	8	5		
Body weight (g)	32·4 (2·4)	32·0 (1·8)	43·6 (4·4)	36·7 (3·9)	<0·05	N.S.
Kidney weight (g)	0·30 (0·01)	0·26 (0·02)	0·43 (0·02)	0·30 (0·03)*	N.S.	<0·01
Kidney weight/body weight (%)	0·94 (0·03)	0·83 (0·02)*	1·04 (0·09)	0·84 (0·04)	N.S.	<0·01

N.S., non significant. *$P<0·01$ vs age-matched control mice using unpaired *t*-tests.

age-matched control mice (Fig. 2). There was no glomerulosclerosis or changes in the number of glomeruli, blood vessels, or tubules. No difference in mesangial cell proliferation was seen (Fig. 2). Focal inflammation but no fibrosis was seen in both LI-IGF-I$^{-/-}$ and control mice.

Microarray and RT-PCR analyses

DNA microarray analyses were performed to investigate if there were alterations in the global gene expression pattern in kidneys from LI-IGF-I$^{-/-}$ mice. The analyses revealed that only three probe sets, representing FK506-binding protein 5 (FKBP51; GB U16959), Mouse A12 mRNA (GB L22977), and IGF-II (X71922), were strongly regulated (all downregulated) in LI-IGF-I$^{-/-}$ mice when compared with control mice (Table 3). IGF-II was, however, the only regulated gene that had a high renal expression using the Affymetrix arbitrary scale (Table 3). Furthermore, the microarray analyses showed no change in any of the members of the IGF-I family except for IGF-II (Table 3).

The expression of the *IGF-II* gene, as determined using microarray, was similar in the LI-IGF-I$^{-/-}$ and the control mice in muscle, heart, and bone (vertebra). As shown in Fig. 3A, the mRNA levels of IGF-II in control mice were considerably higher in the kidney than in the muscle, heart, and bone (Fig. 3A). In liver and fat (retroperitoneal fat), no IGF-II expression was detected using microarray (data not shown).

IGF-II was the only regulated gene that had a high renal expression using the Affymetrix arbitrary scale. Furthermore, FK506-binding protein 5 (FKBP51; GB U16959) and Mouse A12 mRNA (GB L22977) had no obvious known relation to kidney function or morphology. Therefore, only the renal expression of IGF-II was verified using RT-PCR. The RT-PCR analyses were performed on tissue samples from individual mice (no pooling) and confirmed a clear downregulation of renal IGF-II mRNA levels in the LI-IGF-I$^{-/-}$ mice (−79%, $P<0·001$ vs controls; Fig. 3B). Furthermore, the renal IGF-II mRNA level, as determined using RT-PCR, correlated positively with relative kidney weight (kidney weight/body weight) in both the LI-IGF-I$^{-/-}$ mice ($n=7$; $r=0·80$, $P=0·01$, Fig. 3C) and the total number of mice ($n=13$; $r=0·57$, $P<0·05$; data not shown).

IGF-II in serum

The microarray and RT-PCR analyses showed a strong downregulation of the renal IGF-II mRNA levels. We therefore determined IGF-II levels in serum of 9-month-old mice. Serum IGF-II concentration was found to be similar in the two study groups as measured using RIA (mean serum IGF-II was $14·4\pm1·2$ ng/ml in the LI-IGF-I$^{-/-}$ mice ($n=7$) and $13·9\pm2·3$ ng/ml in the control mice ($n=9$); $P=0·6$).

Discussion

This study shows that selective deficiency of hepatic IGF-I causes a global symmetrical decrease in kidney size. Kidney function, as assessed using creatinine clearance, was not changed and there was no sign of glomerulopathy in old

Table 2 Serum and urine creatinine levels as well as creatinine clearance in 6-month-old control and LI-IGF-I$^{-/-}$ mice. Values are given as the mean (S.E.M.)

	Control mice	LI-IGF-I$^{-/-}$ mice	*P*-value
n	7	7	
Body weight (g)	29·5 (2·5)	26·2 (1·3)	0·28
Serum creatinine (mg/dl)	1·62 (0·20)	1·63 (0·23)	0·98
24-h urine creatinine (mg/24 h)	0·43 (0·08)	0·38 (0·08)	0·65
Creatinine clearance (ml/24 h)	31·1 (8·0)	29·2 (8·5)	0·87
Creatinine clearance/body weight (ml/g per 24 h)	1·08 (0·31)	1·08 (0·28)	1·00

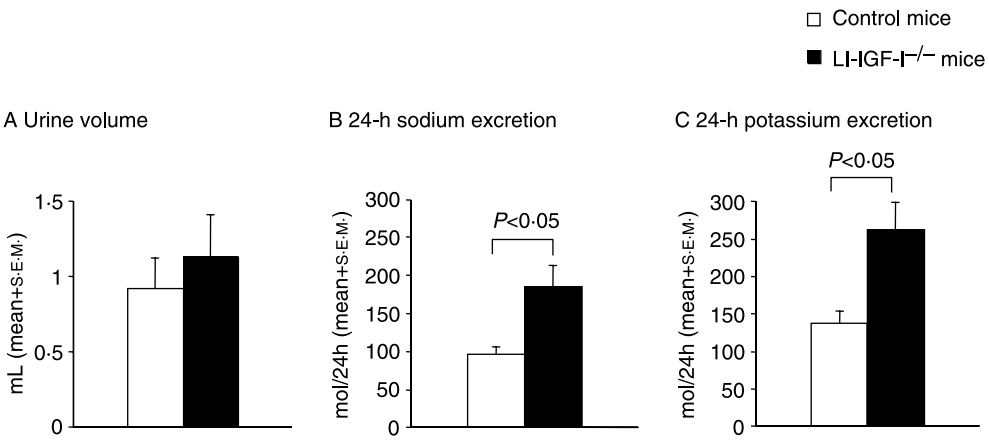

Figure 1 The 24-h (A) urinary volume and (B) urine excretion of sodium, and (C) potassium, determined when the mice were placed in metabolic cages, in control ($n=7$) and LI-IGF-I$^{-/-}$ ($n=7$) mice. Values are given as means ± S.E.M.

LI-IGF-I$^{-/-}$ mice. However, the LI-IGF-I$^{-/-}$ mice had increased 24-h urinary loss of sodium and potassium, suggesting decreased renal reabsorption of sodium and potassium. Microarray analyses, confirmed by RT-PCR analyses, demonstrated that the observed abnormalities in the LI-IGF-I$^{-/-}$ mice were associated with a marked downregulation of IGF-II mRNA levels in the kidney. This decline in renal *IGF-II* gene expression preceded the decline in kidney growth in 7-month-old LI-IGF-I$^{-/-}$ mice.

Transgenic mice with global overexpression of GH and/or IGF-I have selective renal enlargement and glomerular hypertrophy, whereas only the mice with global overexpression of GH have mesangial proliferation followed by progressive glomerulosclerosis (Doi *et al.* 1988, 1990, Mathews *et al.* 1988, Cingel-Ristic *et al.* 2004). Mice with global inactivation of the *IGF-I* gene that survive the postnatal period have reduced body weight, proportionally reduced kidney size, reduced glomerular size, and decreased number of nephrons (Rogers *et al.* 1999, Cingel-Ristic *et al.* 2004). However, these studies cannot evaluate the role of IGF-I in adult or aging animals, as they are confounded by affected IGF-I activity during development. The LI-IGF-I$^{-/-}$ mice do not have decreased IGF-I expression in the kidney at 3 (Sjögren *et al.* 1999) and 7 months of age (the present study). The inactivation of liver-derived IGF-I at 4 weeks of age resulted in a marked reduction in serum IGF-I that was maintained at 24 months of age. Therefore, although renal IGF-I expression were not determined at 24 months of age, the observed alterations were likely not, or only to a relatively small extent, due to the developmental changes in the kidney.

The histological analyses in the 24-month-old LI-IGF-I$^{-/-}$ mice showed a global and symmetrical decrease in kidney size. There were no relative changes in the size, number, or distribution of renal structures, and there was no between-group difference in inflammation or fibrosis. Somewhat surprisingly, since the LI-IGF-I$^{-/-}$ mice have increased BP

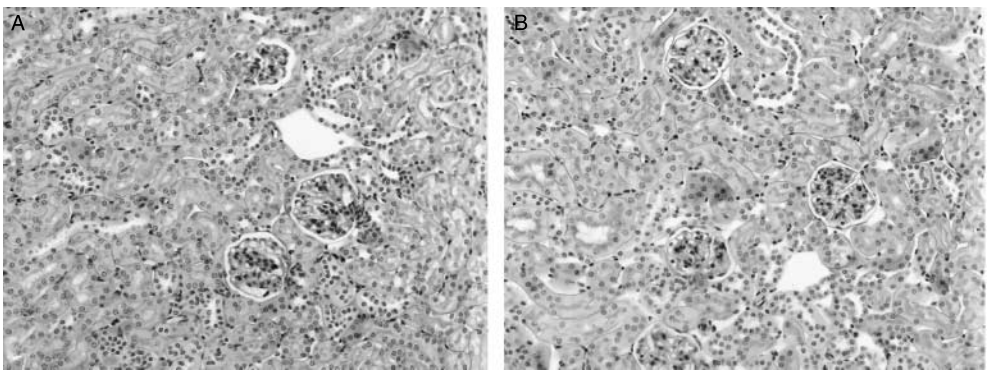

Figure 2 Representative light micrographs (PAS stain) of (A) control mice and (B) LI-IGF-I$^{-/-}$ mice. The micrographs show normal cortex containing glomeruli and tubules. No glomerulosclerosis, mesangial sclerosis, or cortical fibrosis is seen.

Table 3 Genes regulated in the kidney in 7-month-old control and LI-IGF-I$^{-/-}$ mice as determined by DNA microarray analyses as well as expression and regulation of the IGF family members as assessed using DNA microarray. The regulation of mRNA levels of different genes is indicated by the average-fold change (FC) according to the rules given in the Materials and Methods

Gene	Genebank accession	Expression	Regulation FC
Regulated genes			
FKBP51	U16959	†	−11·4
Mouse *A12* mRNA	L22977	*	−3·94
IGF-II	X71922	§	−3·31
Genes from the IGF family			
IGFBP-1	X81579	†	NC
IGFBP-2	X81580	*	NC
IGFBP-3	X81581	ND	NC
IGFBP-4	X76066	†	NC
IGFBP-5	L12447	‡	NC
IGFBP-6	X81584	ND	NC
IGF-I	X04480	†	NC
IGF-IR	AF056187	‡	NC
IGF-IIR	U04710	*	NC
ALS	U66900	*	NC
GHR	U15012	‡	NC

NC indicates no change according to Affymetrix. ND indicates not detectable. *, 0–500; †, 501–2000; ‡, 2001–8000; §, >8000 (Arbitrary units by Affymetrix).

(Tivesten *et al.* 2002) and compensated hyperinsulinemia (Sjögren *et al.* 2001), we did not find any increase in glomerulopathy or nephrosclerosis in the 24-month-old LI-IGF-I$^{-/-}$ mice. However, it has previously been shown that the LI-IGF-I$^{-/-}$ mice have decreased total body fat (Sjögren *et al.* 2001), which may possibly counteract the impact of the increased BP and the hyperinsulinemia.

Creatinine clearance and creatinine clearance corrected for body weight was similar in the LI-IGF-I$^{-/-}$ and the control mice. Previous studies have shown that both systemic GH and IGF-I treatment can increase GFR (Feld *et al.* 1995, Hirschberg & Adler 1998, Cingel-Ristic *et al.* 2004, Rabkin & Schaefer 2004). In the present study, it was not possible to correct GFR for kidney weight since it was not determined at the time when GFR was measured. However, in the LI-IGF-I$^{-/-}$ mice, unchanged GFR combined with reduced kidney weight suggest that the smaller kidneys were functioning at similar or even increased rate when compared with that in the control mice.

GH treatment increases both serum IGF-I concentration and sodium reabsorption, resulting in fluid retention (Feld *et al.* 1995, Hirschberg & Adler 1998, Cingel-Ristic *et al.* 2004, Rabkin & Schaefer 2004). The LI-IGF-I$^{-/-}$ mice have high circulating GH secondary to their low circulating IGF-I (Sjögren *et al.* 1999, Wallenius *et al.* 2001). Therefore, the present results, with increased urinary loss of sodium over 24 h in the LI-IGF-I$^{-/-}$ mice, indicate that GH cannot increase the renal reabsorption of sodium without a concomitant increase in serum IGF-I level, and that the combination of high circulating GH and low IGF-I even result in increased urinary loss of sodium. It is not clear to what extent the increased BP in the LI-IGF-I$^{-/-}$ mice ((Tivesten *et al.* 2002) and the present study) affected renal sodium handling. The increased BP in the LI-IGF-I$^{-/-}$ mice is due to increased peripheral vascular resistance without

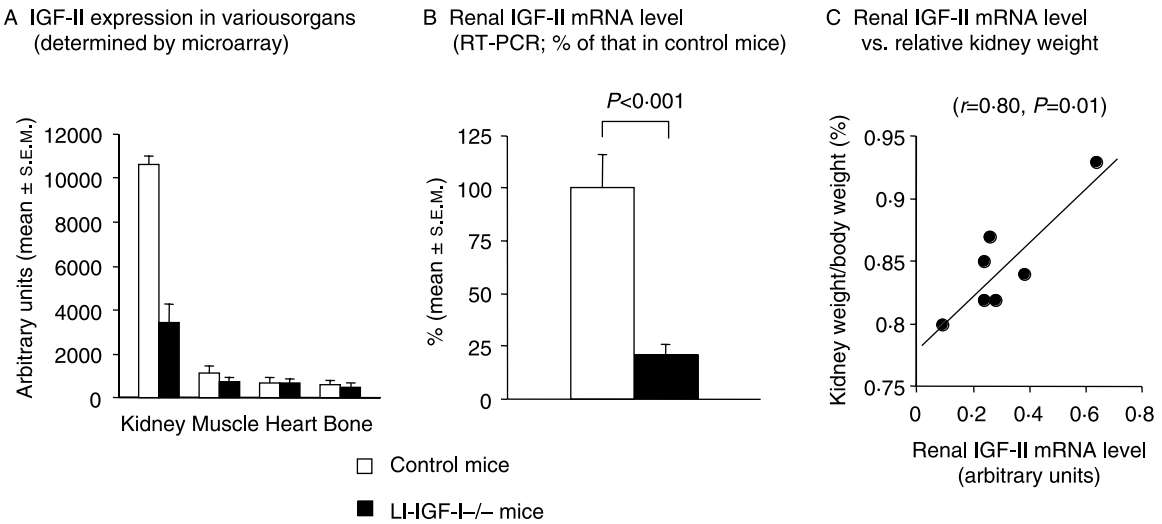

Figure 3 (A) The expression of IGF-II, as determined using microarray, in kidney, muscle, heart, and bone (vertebrae) in control ($n=6$) and LI-IGF-I$^{-/-}$ ($n=7$) mice. No detectable expression of IGF-II mRNA was found in the liver and retroperitoneal fat (data not shown). (B) The renal IGF-II mRNA levels, as determined by RT-PCR, in the LI-IGF-I$^{-/-}$ mice ($n=7$) expressed as percentage of that in control mice ($n=6$). The RT-PCR analyses were performed on tissue samples from individual mice (no pooling). In Fig. 3A and B, the vertical bars indicate the S.E.M. for the mean values shown. (C) The positive correlation in the LI-IGF-I$^{-/-}$ mice ($n=7$) between renal IGF-II mRNA levels, as determined using RT-PCR, and relative kidney weight ($r=0·80$, $P=0·01$).

any detectable effect on the renin–aldosterone system (Tivesten *et al.* 2002). It cannot be fully excluded that the increased sodium excretion in the LI-IGF-I$^{-/-}$ mice is a compensatory mechanism in order to counteract the BP elevation via a pressure–natriuretic action (Guyton 1991). However, GH treatment in humans generally increased serum IGF-I level, sodium reabsorption, and fluid retention without affecting BP (Svensson *et al.* 2005), thus suggesting that the effect of GH/IGF-I on renal sodium handling is independent of the BP changes. Therefore, we propose that the increased sodium reabsorption seen during GH treatment is mediated by the concomitant increase in circulating IGF-I.

The LI-IGF-I$^{-/-}$ mice did not only have increased urinary loss of sodium but also increased urinary loss of potassium. Although the effect of GH/IGF-I on urinary potassium excretion is less well characterized than that on urinary sodium handling, decreased urinary excretion of potassium has previously been described during IGF-I treatment (Giordano & DeFronzo 1995), and GH treatment increases total body potassium content (Bengtsson *et al.* 1993). In addition, increased renal IGF-I expression has been observed in potassium-depleted rats (Flyvberg *et al.* 1992). Potassium excretion is, to a large extent, driven by urinary flow rates (Wright 1982). The similar flow rates in the experimental groups suggest that endocrine liver-derived IGF-I interferes with specific tubular mechanisms for potassium transport. Therefore, the present and other studies suggest that IGF-I not only regulates urinary sodium handling but also urinary potassium handling.

IGF-II is of major importance for body and tissue growth in fetal life. Also in adult rodents, IGF-II mRNA and protein are abundantly expressed in the kidney (Wolf *et al.* 1994, Hirschberg & Adler 1998), and transgenic mice over-expressing IGF-II have increased kidney weight (Wolf *et al.* 1994, Blackburn *et al.* 1997). In transgenic mice with global deficiency of IGF-I, global overexpression of IGF-II has no effect on body weight gain or the weight of organs except for an increase in absolute and relative kidney weight (Moerth *et al.* 2007). Furthermore, *in vitro* studies have indicated that IGF-II can increase sodium uptake in proximal tubule brush border membrane vesicles (Yanagawa *et al.* 1991, Feld & Hirschberg 1996, Hirschberg & Adler 1998). In line with these previous results, the control mice in the present study had a high renal expression of IGF-II, whereas IGF-II expression was much lower in the other organs studied (liver, muscle, heart, fat, and bone). The LI-IGF-I$^{-/-}$ mice had unchanged serum IGF-II level and the kidney was the only organ studied in which the *IGF-II* gene was downregulated. Thus, our results demonstrate that IGF-II is highly expressed in the adult kidney where it is regulated by endocrine liver-derived IGF-I in a tissue-specific manner. Therefore, one could speculate that affected IGF-II expression in the kidney could be important for the effects of circulating IGF-I on kidney size and sodium handling. This is supported by our observation of a positive correlation between renal IGF-II mRNA levels, as determined using RT-PCR, and relative kidney weight (kidney weight/body weight).

In the LI-IGF-I$^{-/-}$ mice (Sjögren *et al.* 1999, Wallenius *et al.* 2001), as well as in humans with DM type I (Carroll *et al.* 1998, Jain *et al.* 1998, Cingel-Ristic *et al.* 2004, Rabkin & Schaefer 2004), circulating IGF-I levels are low and circulating GH levels are high. Furthermore, in animal models of type 1 DM, the discordance between circulating GH and IGF-I levels is often due to elevated glucocorticoids (Unterman *et al.* 1993, Rodgers *et al.* 1995). In the LI-IGF-I$^{-/-}$ mice, serum corticosterone levels are increased at 1 month of age and still tend to be increased at 13 months of age (Sjögren *et al.* 2001). The absence of renal structural, vascular, and/or glomerular complications in the LI-IGF-I$^{-/-}$ mice might suggest that high circulating GH and low circulating IGF-I in itself do not cause renal failure. However, the present results do not exclude the possibility that high circulating GH levels combined with low circulating IGF-I levels can accelerate the renal disease in diabetic patients with already existing renal complications.

In conclusion, the LI-IGF$^{-/-}$ mice have a global and symmetrical decrease in kidney size and increased 24-h urinary sodium and potassium excretion. Since the LI-IGF-I$^{-/-}$ mice have compensatory high circulating GH levels, the present results suggest that the well-known stimulatory effects by GH treatment on kidney size and sodium reabsorption are mediated by circulating liver-derived IGF-I. There were no signs of glomerulopathy or nephrosclerosis in the old LI-IGF-I$^{-/-}$ mice, suggesting that the constellation of high circulating GH and low serum IGF-I is not a primary cause of diabetic or nondiabetic renal failure. The microarray analyses, confirmed by RT-PCR analyses, showed a marked decrease in the renal IGF-II mRNA levels, suggesting that the effects of circulating IGF-I on kidney size could be mediated by renal IGF-II.

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