

Growth Hormone, the Insulin-Like Growth Factor System, and the Kidney*

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- I. Introduction
 - A. GH
 - B. IGF-I and IGF-II
 - C. IGF-I receptor
 - D. IGF-II/Man-6-P receptor
 - E. IGF-binding proteins
- II. Expression of GH Receptors and the IGF System in the Kidney
 - A. Expression in the rat kidney
 - B. Expression in human kidney
 - C. Functional implications of anatomic data
 - D. Regulation of the renal expression of IGF-I
- III. Processing of GH, IGFs, and IGF-BPs by the Kidney
- IV. GH, IGF-I, and Glomerular Hemodynamics
 - A. GH
 - B. IGF-I
 - C. Effects of rhIGF-I on renal hemodynamics in normal humans
 - D. Physiological mechanisms of the IGF-I-induced rise in renal hemodynamics
 - E. Biochemical mediators of the IGF-I-induced effects on renal hemodynamics
 - F. Hemodynamic effects of IGF-I in nonrenal vascular beds
- V. Effects of GH and IGFs on Tubular Function
 - A. Tubular phosphate (Pi) handling
 - B. Tubular calcium (Ca) handling
 - C. Tubular sodium (Na) and water handling
 - D. Renal regulation of acid-base homeostasis
- VI. GH and the IGF-System in Renal Development
- VII. GH and IGFs in Renal (Compensatory) Growth
 - A. Renal growth caused by systemically elevated GH and IGF-I
 - B. Unilateral nephrectomy
 - C. Subtotal nephrectomy
 - D. Streptozotocin diabetes
 - E. Diet and renal growth
 - F. Other experimental models

- G. Summary of data on renal growth
 - H. Compensatory renal growth and progressive glomerular sclerosis and interstitial fibrosis: is there a role for GH and IGF-I?
- VIII. GH and the IGF System in Selected Renal Diseases
- A. Chronic renal failure
 - B. Acute renal failure
 - C. The IGF-system in the nephrotic syndrome
- IX. Summary

I. Introduction

GROWTH hormone and the insulin-like growth factors (IGFs) compile a complex, interrelated system of three peptide hormones, GH, IGF-I, and IGF-II, their specific receptors, and a series of binding proteins. The authors intend to review all aspects of GH, IGFs, and their binding proteins that relate to the normal anatomy and physiology of the kidney and their relationship to pathophysiology and pathobiology of the kidney and to selected kidney diseases. This manuscript will update and expand on previous review articles that were published by different authors (1–4). The reader's attention may also be drawn to an outstanding recent review by Jones and Clemmons (5) on the general biology of IGF-I and its binding proteins.

In this introduction a basic review of GH, IGFs, their receptors, and binding proteins will be provided as a basis for the subsequent discussion on their association with the kidney.

A. GH

GH is the strongest secretagogue for IGF-I, and some (but not all) effects of GH on peripheral tissues are physiologically mediated by IGF-I.

Two human GH genes have been identified and are located on chromosome 17 (6). The hGH-N gene, which consists of five exons, encodes pituitary GH (6, 7). The primary gene product is a prohormone that undergoes posttranslational proteolytic cleavage and, hence, is modified into the two active pituitary GHs with molecular masses of 21.5 kDa (191 amino acids) and 20 kDa (176 amino acids). The 21.5-kDa GH is the predominant circulating form and accounts for about 90% of serum GH (8). The hGH-V genes are expressed only in syncytiotrophoblasts of the placenta, and the two gene products (22 and 26 kDa) are variants of GH that appear to have specific functions during gestation (6, 9). The discussion of GH in the context of renal physiology, pathophysiology,

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and kidney diseases in this review will be limited to pituitary GH.

GH synthesis and release from pituitary somatotrophs is pulsatile, and serum GH levels fluctuate accordingly (10–12). In normal subjects, about 13 surges in serum GH concentrations occur in a 24-h period with a periodicity of roughly 2 h (12). Thus, an estimation of serum GH levels can only be made if profiles over prolonged periods of time are obtained. GH bioactivity may relate more closely to the number and peaks of surges rather than basal levels (11). For example, in normal subjects, the serum IGF-I levels do not correlate with the amount of GH secreted (11).

GH gene transcription and serum levels are controlled by many different neuronal, metabolic, and hormonal events. Among others, hypothalamic somatostatin as well as peripheral IGF-I, inhibit GH release whereas hypothalamic GH releasing hormone induces gene expression and release of pituitary GH (13).

GH acts through specific receptors. In man, the gene that encodes for the GH receptor protein is located on chromosome 5 (14). The receptor that consists of 620 amino acids is a glycoprotein with several glycosylation sites (14). The receptor protein spans the cell membrane. The extracellular ligand-binding domain occurs in a slightly modified form in plasma. These 'truncated' solubilized GH receptors are referred to as the "growth hormone binding protein" (GHBP) (8, 14–24). The serum half-life of free GH is rather short, about 20 min, but is prolonged to several hours by binding to GHBP. Another unrelated GHBP that appears to bind much of the 20-kDa variant of pituitary GH has also been found in human serum (25).

B. IGF-I and IGF-II

Insulin-like growth factor I (IGF-I) is synthesized and released from multiple tissues. However, hepatic biosynthesis appears to account for the vast majority of circulating IGF-I (26–29). IGF-I (molecular mass 7650 Da) is a single chain peptide growth factor that shares 50% sequence identity with Pro-insulin (30–32) and about 70% identity as well as structural similarities with IGF-II (33).

IGF-I genes from rat, mouse, and man have been identified, and their structure is known (34–38). These are relatively large genes (up to ~100 kb) with unusually complex regulation due to two leader exons, multiple transcription start sites, and alternate splicing, which all give rise to multiple mRNA species that can be found in different tissues by Northern analysis (39, 40).

IGF-I binds with high affinity to IGF-I receptors and with lesser affinity to IGF-II/mannose-6-phosphate receptors and insulin receptors (41). The lower affinity of IGF-I to insulin receptors (41) may explain why the "insulin-like effects" of IGF-I occur only at greater levels than other effects of the peptide.

The serum levels of IGF-I (~25–40 nM) are much greater than those of any other growth factor or peptide hormone. For example, serum IGF-I levels are 2 to 3 orders of magnitude greater than those of insulin in the fasting state. The biological effects of such large concentrations of IGF-I could be incompatible with life. However, only small amounts of

the circulating IGF-I are present in the free form (42–44). Greater than 99% of the serum IGF-I is bound to specific binding proteins, mainly in a ~150-kDa complex and to a lesser extent in a ~45-kDa complex (*vide infra*).

An analog of IGF-I, namely des(1–3)-IGF-I, may be naturally present in brain but has not been demonstrated in the kidney or any other tissue. This peptide differs functionally from IGF-I because of its reduced affinity to IGF-binding proteins. A number of IGF-I analogs have been synthesized that differ from the natural peptide by their reduced affinity to the IGF-I receptor and binding proteins (45–48). None of these or other analogs appear to occur naturally. Analogs of IGF-I that have reduced binding to binding proteins but similar receptor affinity, such as des(1–3)IGF-I, have been used as laboratory tools.

GH is the strongest secretagogue for IGF-I and activates IGF-I gene transcription. In the liver, exon 2 transcription of the IGF-I gene is sensitive to GH (49, 50). GH promotes IGF-I synthesis in most tissues (26, 49, 51) including the kidneys (52–55).

Insulin-like growth factor II (IGF-II) is present at even greater concentrations in human serum than IGF-I (56–58). Like IGF-I, ≥99% of the circulating IGF-II is bound to IGF-binding proteins (44). In contrast, rodent serum IGF-II levels are greatest before birth but decline rapidly thereafter. This results from a switch in promoter activity of the IGF-II gene at the time of birth (59–62).

IGF-II has high affinity for IGF-II/Man-6-P receptors but binds also to IGF-I receptors to which it has lesser affinity than IGF-I (41, 63, 64). However, all IGF-II-induced metabolic or mitogenic effects that have been examined primarily in cell culture studies thus far appear to be transduced by IGF-I receptors.

C. IGF-I receptor

The IGF-I receptor is the product of a single gene in rodents as well as in humans (65–67). Its structure is determined by posttranslational modification. Posttranslational cleavage generates a ~140-kDa α -subunit and a ~95 kDa β -subunit, which are linked by disulfide bonds. Two $\alpha\beta$ -dimers, in turn, are linked to form the mature, tetrameric $\alpha_2\beta_2$ -holoreceptor (5).

The receptor α -subunit is localized extracellularly and is the ligand-binding site of the receptor. IGF-I receptors preferentially bind IGF-I with $K_D \leq 1$ nM and have a several-fold lesser affinity for IGF-II (41, 63, 64). However, most studies found a difference in affinity of both IGFs to the IGF-I receptor to be within the same order of magnitude. Since IGF-II levels in human (but not rodent) serum are about 2 to 3-fold greater than those of IGF-I, the difference in receptor affinity may be neutralized by the difference in serum levels.

The IGF-I receptor β -subunit spans the cell membrane and extends into the intracellular compartment. The β -subunit contains a cytoplasmic tyrosine kinase domain that phosphorylates tyrosine residues in the respective contralateral β -subunit within the same receptor upon ligand binding to the α -subunit (5, 68–71). This autophosphorylation reaction initiates a series of cytoplasmic kinase activations that, like a cascade, activate nuclear transcription factors and, hence,

transmit the mitogenic and metabolic signals to the cell nucleus (5). The catalytic domain of the IGF-I receptor contains an ATP-binding site and a cluster of three tyrosine residues. Autophosphorylation of this tyrosine cluster in position 1131, 1135, and 1136 upon ligand binding plays a major role in the full activation of kinase activity and biological action of the receptor, although there are additional tyrosine residues in the receptor β -subunit that are autophosphorylated (72–75). Stannard *et al.* (76) elegantly demonstrated that a single mutation of tyrosine 1131 reduces IGF-I receptor β -subunit autophosphorylation significantly. This reduction in phosphorylation of the receptor does not reduce the IRS-1 phosphorylation. Furthermore, the hypertrophic/hyperplastic response in fibroblasts that were transfected with this mutant receptor was not reduced. This experimental finding suggests that differential tyrosine autophosphorylation of the IGF-I receptor may have different cellular effects. Furthermore, the growth effect of the stimulated IGF-I receptor may be transmitted through IRS-1 phosphorylation (76).

D. IGF-II/Man-6-P receptor

The rat IGF-II receptor amino acid sequence deduced from rat cDNA closely resembles those of the bovine cation-independent mannose-6-phosphate receptor (77). In fact, both receptors were found to be identical and are usually referred to as the IGF-II/Man-6-P receptor (78–80). The human IGF-II/Man-6-P receptor gene is located on chromosome 12 (79). Within the receptor there are different binding sites for IGF-II and Man-6-P or Man-6-P-containing glycoproteins (77). The IGF-II receptor consists of a single peptide chain (77, 81, 82) that lacks detectable kinase activity but is phosphorylated by extrareceptor kinase activity (83, 84). IGF-II/Man-6-P receptors have great affinity for IGF-II ($K_D = 0.1\text{--}1.0$ nM), but the affinity for IGF-I is at least 2 orders of magnitude less (5). Binding of Man-6-P to the receptor increases the affinity for IGF-II about 2-fold (77). In addition to IGF-II and with lesser affinity than IGF-I, IGF-II receptors may bind other ligands, such as proliferin, TGF β precursor, and β -galactosidase, all of which induce activation of phospholipase C and increase inositol-3-phosphate (85).

A function of cytoplasmic IGF-II/Man-6-P receptors is the intracellular targeting and sorting of lysosomal enzymes (86). The receptor recycles rapidly between intracellular compartments with an acidic endosome being the primary intracellular destination (87–89). Thus, cell surface IGF-II/Man-6-P receptors may function as scavenger receptors. However, there is also evidence, albeit limited, for a signaling function of the receptor. IGF-II receptors are coupled to and activate G proteins, and further details of this apparent IGF-II/IGF-II-receptor signal transduction pathway have recently been unfolded (90–97). IGF-II effects that are mediated through IGF-II/Man-6-P receptors may include the modulation of cellular Ca^{++} -fluxes (91, 93, 98–100). However, many IGF-II effects can be blocked by neutralizing IGF-I receptors with IR3 monoclonal antibody, suggesting that they are transmitted through IGF-I receptors (101–103). The possible role of IGF-II receptors in the transduction of IGF-II signals in the kidney are unknown and warrant further

study. IGF-II receptors may also play an important role during embryo- and organogenesis (*vide infra*; Ref. 104).

IGF-II/Man-6-P receptors are also present in a soluble form in serum (105–108). In immunoblots, soluble IGF-II receptors are about 10 kDa smaller compared to their tissue-residential counterparts (105). Soluble IGF-II receptors are released from tissues that also express residential IGF-II receptors, including heart, skeletal muscle, liver, and kidney. In fetal life, most of the soluble IGF-II receptors originate from the heart and muscle, but in the adult rat, the liver is the major source (107). The function of the soluble IGF-II/Man-6-P receptors in serum is unknown. They may serve as circulating IGF-II-specific binding proteins, although such a physiological role is yet unproven.

E. IGF-binding proteins (Table 1)

Six IGF-binding proteins (IGFBPs) and a IGFBP-3 supporting protein, the acid-labile subunit (ALS), have been identified and cloned thus far (109–140). All IGFBPs have sequence homologies (119). Both IGFs have similar affinity to IGFBP-1, -3, and -4, but IGFBP-2, -5, and -6 preferably bind IGF-II (119, 141–145). IGFBPs do not bind insulin or other known ligands. As a rule, the affinity of IGFBPs for the IGFs are in the same order of magnitude as for the IGF-receptors, suggesting that *in vivo* some competition occurs between the receptors and the IGFBPs for ligand binding.

IGFBP-1 and -2 contain an arginine-glycine-asparagine (RGD) triplet that allows for (nonspecific) binding to $\alpha_5\beta_1$ -integrins resulting in cell surface association (5, 113, 146–148). IGFBP-3 and -5 can also be found in association with cell surfaces, although these IGFBPs do not contain a RGD-sequence.

IGFBP-2, -3, -4, and -5 are glycosylated. In human serum, IGFBP-3 forms two bands in Western ligand blots, and in rat serum three bands are found due to different glycosylation stages of IGFBP-3. IGFBP-2 and -4 usually form only a single band in rat, as well as in human, serum.

IGFBP-1, -3, and -5 undergo intracellular phosphorylation on serine residues (5, 149–151). Phosphorylation of IGFBPs increases their affinity for the ligand (149). About 99% of the serum IGF-I and II is bound to IGFBPs (44). Most of the bound serum IGFs are present in a ~ 150 -kDa complex (142, 152–156) that contains IGFBP-3, ALS, and the ligands (152, 153, 157–159). The association of ALS with IGFBP-3 increases the affinity for IGFs to IGFBP-3. This ~ 150 -kDa complex is largely limited to the intravascular space and may be viewed as a circulating IGF reservoir (160, 161). About 30% of the serum IGF-I is found in small (~ 45 kDa) protein complexes that contain IGFBP-1, -2, -3, or -4 but no ALS (142). The small serum-binding complexes can leave the intravascular space and may deliver IGFs to tissue-binding sites (160, 161).

Most IGFBPs have a putative glycosaminoglycan-binding site, and IGFBP-3 has three such sequences that facilitate binding to extracellular matrix (162).

IGFBPs can be examined in serum, tissue, or cell lysates and in concentrated conditioned cell culture medium by Western ligand blotting (163–166) or Western immunoblotting. Antibodies are commercially available for the detection of all human IGFBPs but, unfortunately, do not cross-react

TABLE 1. Summary on IGF-binding proteins

	cDNA Reference	EMBL/GB Acc No	Theoretical molecular mass (kDa)	App Mwt SDS-PAGE	Glycosylation	RGD	Phosphorylation	↑	↓	Preferred binding
IGFBP-1	M:(136)	X81570								
	R:(112)	J04486	29.5	29	-	+	+	Fasting	Insulin	Similar
	H:(110)	Y00856	28.1						GH	
M:(136)	X81580									
IGFBP-2	R:(123)	M31672	29.5	31-33	-	+	-	IGF-I		IGF-II
	H:(109)	X16302	31.3	(1 band)						
IGFBP-3	M:(136)	X81581						IGF-I		Similar
	R:(129)	M31837	29.4	38-48	+	-	+		Fasting	
	H:(140)	X64875	28.7	R: 3 bands H: 2 bands				GH		
IGFBP-4	M:(136)	X81582								Similar
	R:		25.8	24 + 29	+	-	-			
	H:(121)	M62402	26.3							
IGFBP-5	M:(136)	X81583								IGF-II
	R:(134)	M62781	28.4		-	-	+			
	H:(121)	M62403	28.5							
IGFBP-6	M:(136)	X81584								IGF-II
	R:(133)	M69055	21.4			-				
ALS	M:									
	R:(118)	S46785								
	H:									

M, mouse; R, rat; H, human; EMBL/GB Acc No, European Molecular Biology Laboratory/GenBank accession number.

well with rodent IGFBPs. Anti-bovine IGFBP-2 polyclonal antisera cross-react well with human, mouse, and rat IGFBP-2 (167). Immunoassays have been developed for the measurements of human IGFBPs (168) and assay kits are commercially available for some of them.

In many instances, particularly in the measurement of rodent or rodent-cell culture-derived IGFBPs, Western ligand blotting is the only available method. However, investigators should be careful with quantitative interpretations of this qualitative, or at best, semiquantitative method. In Western ligand blots and subsequent densitometry of autoradiograms, curves derived from serially diluted sera do not necessarily run parallel with curves derived from titrated recombinant human IGFBPs (R. Hirschberg, own observation). This may be caused by incomplete dissociation of IGFs from the binding proteins in nonreducing SDS-PAGE procedures (169).

1. IGFBP-1. IGFBP-1 is expressed primarily in liver but also in kidney in both rats and humans (110, 119, 170-172). IGFBP-1 expression is suppressed by insulin and increased by insulin deficiency (172) and fasting (125, 173). GH as well as IGF-I reduce the (renal) expression of IGFBP-1 (174-176). In contrast, fasting increases the IGFBP-1 mRNA levels in kidney and other tissues (176). In adult serum, IGFBP-1 is a less abundant IGF carrier. IGFBP-1 is serine-phosphorylated, and its dephosphorylation decreases the affinity for IGF-I (174). IGFBP-1 interacts through its RGD-sequence with $\alpha_5\beta_1$ -integrins. *In vitro* cell culture experiments have demonstrated that IGFBP-1 has direct mitogenic actions via $\alpha_5\beta_1$ -integrins that are independent of its interaction with IGF-I (148). It is possible, although unproven, that IGFBP-1 has IGF-I-independent actions *in vivo* in kidney.

2. IGFBP-2. IGFBP-2 is expressed in liver as well as in kidney (174) and is second in line as a serum IGF carrier. It contains an RGD-sequence. IGFBP-2 has a (several fold) greater af-

finity for IGF-II than for IGF-I and, hence, may carry primarily IGF-II (177). Levels of this binding protein are increased by IGF-I (178) and appear to be up-regulated by IGF-II (179). IGFBP-2 is also elevated in the nephrotic syndrome in the rat, despite reduced serum IGF-I levels (167). GH or fasting have little effect on the renal expression of IGFBP-2 (175, 176). In the kidney, IGF-II and IGFBP-2 are coexpressed in the vasculature, suggesting that IGFBP-2 has a particular role in modifying IGF-II actions (180) but may also modify IGF-I effects, as has been shown in other tissues (181).

3. IGFBP-3. IGFBP-3 is the primary IGFBP in adult human and rat serum. IGFBP-3 serum levels are increased by IGF-I as well as GH (probably through IGF-I) (178). IGFBP-3 can undergo *in vivo* proteolysis, which leads to release of the ligand and is believed to be a measure of increasing IGF-I activity. Increased IGFBP-3 protease activity has been demonstrated in a number of physiological and abnormal conditions such as pregnancy (182, 183) and the nephrotic syndrome (167). Proteolytic fragments of IGFBP-3 bind IGF-I with a (50-fold) reduced affinity (167, 184).

4. IGFBP-4. IGFBP-4 is primarily expressed in adult rats (and humans?) in liver. Lesser levels of IGFBP-4 mRNA are also found in total RNA extracted from normal adult rat kidney (131). In human and rat serum, IGFBP-4 contributes to IGF binding to a lesser extent (5, 142).

5. IGFBP-5. IGFBP-5 is primarily expressed in kidney (132, 134, 185-187). In contrast to other IGFBPs, liver mRNA levels encoding IGFBP-5 are relatively low (134). In the kidney IGFBP-5 resides in the renal interstitium where it appears to be bound to extracellular matrix proteins (162, 186, 188-190), and may provide a "intermediate IGF-I reservoir" to facilitate IGF-I actions. Such a facilitating function of IGFBP-5 has been demonstrated in *in vitro* experiments (188).

6. *IGFBP-6*. *IGFBP-6* is widely expressed in adult rat tissues particularly in lung. Lesser levels of *IGFBP-6* mRNA are found in liver and kidney (120, 133, 134). *IGFBP-6* has a 70-fold greater affinity for IGF-II compared to IGF-I. In some *in vitro* cell culture experiments *IGFBP-6* was found to inhibit the actions of IGF-II (191, 192). Thus, this binding protein's primary function may be inhibition of IGF-II actions. In the rat kidney the expression of *IGFBP-6* mRNA is lower than the mRNA of the other five binding proteins and is strictly localized to fibroblasts adjacent to the vasculature (189). Since the renal vasculature expresses IGF-II, the role of *IGFBP-6* in the kidney may also be the regulation (inhibition) of IGF-II effects on the vasculature (which are not yet well known).

II. Expression of GH Receptors and the IGF System in the Kidney

GH receptors, IGF-I, IGF-II, IGF-I and -II receptors, and *IGFBPs* are normally expressed in the adult (rat) kidney suggesting a role for GH and the IGFs in some aspects of physiological nephron function. The expression of these proteins is highly organized and differs among the different anatomical and functional segments of the nephron. Studies examining the spatial distribution of the GH/IGF-I system in the nephron were predominantly performed in rats using *ex vivo* peptide or RNA tissue extraction techniques with subsequent RIA or Northern analysis of the extracts or *in situ* hybridization or immunohistochemical techniques. Less information is available about the expression of these proteins in normal human nephrons. Available data from human kidneys suggest that there are some species differences (193). Notably, neither IGF-II nor IGF-II mRNA have been demonstrated in the normal rat nephron in most studies (except in intrarenal blood vessels), and IGF-I and IGF-I mRNA have not been found in the human kidney (194). However, IGF-II receptor mRNA and IGF-II were found in the rat nephron by Evan and associates (see Fig. 2) (180). In humans, the expression of IGF-II is apparently limited to the vasculature (193–195). Furthermore, in the rat, the expression of members of the IGF-system is known to be altered by physiological maneuvers and in a number of experimental disease states (*vide infra*). The expression of the renal GH-IGF-I system changes during development of the kidney, which suggests a role for this system during nephrogenesis and renal development (*vide infra*).

A. Expression in the rat kidney (Fig 1)

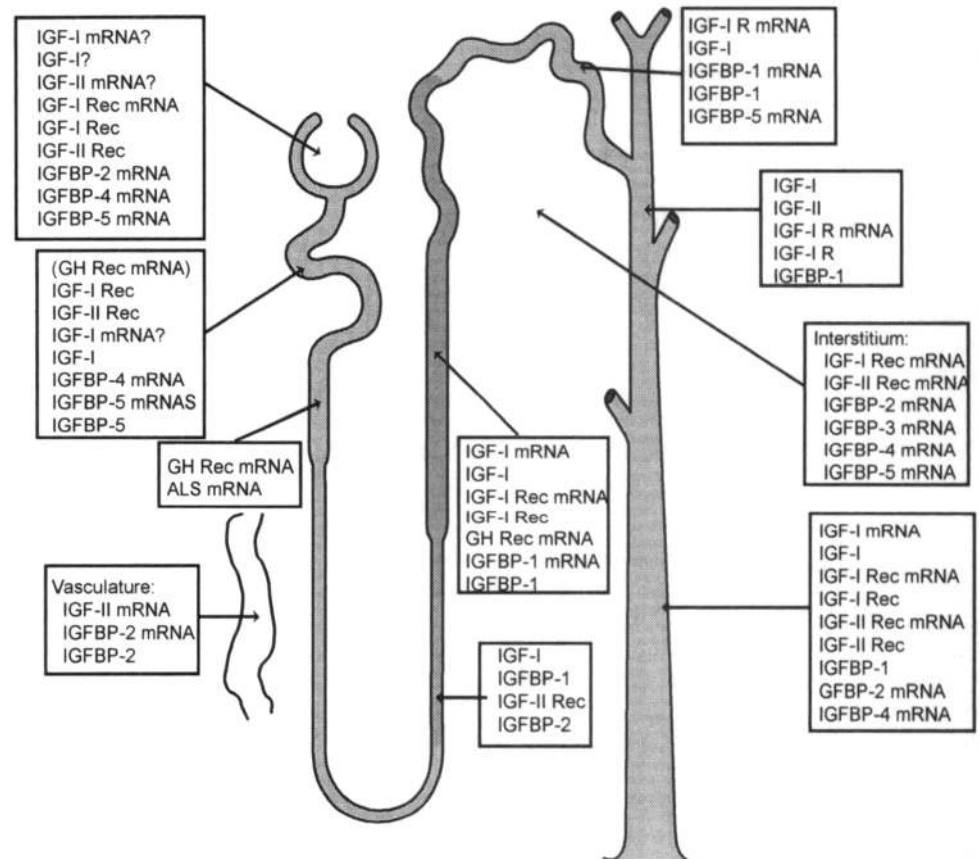
Both IGF-I and IGF-II have been measured in rat kidneys by tissue extraction and RIA or extraction of separated rat renal cortex and medulla (27–29, 196). The peptides measured in these studies may be trapped by receptor or surface binding or may have been synthesized *in situ*. Since the IGF-I levels are greater in renal venous blood compared with the concentration in arterial blood, the kidney synthesizes IGF-I (55). More recent studies employing *in situ* hybridization of RNA and *in situ* immunohistochemistry have indicated that surface binding as well as *in situ* synthesis occur in different parts of the nephron. A major proportion of IGF-I peptide that can be extracted from kidney may have been trapped

from the circulation and bound to cell surfaces or the interstitium rather than being synthesized *in situ*. This is supported by a comparison of the levels of extracted IGF-I peptide with the levels of IGF-I mRNA that can be extracted from rodent tissues. Comparable amounts of IGF-I can be extracted from (rat) liver and kidney (27), whereas the IGF-I mRNA is at least 1 order of magnitude greater in (mouse) liver compared to kidney (51). Trapping of IGF-I rather than *in situ* synthesis is also suggested by the distribution of immunoreactive IGF-I in a pericellular pattern and by the dissociation of IGF-I and IGF-I mRNA in some segments of the nephron, as will be examined in the following paragraphs.

1. *Glomeruli*. Normal adult rat glomeruli possibly do not express IGF-I, but express IGF-I receptors, IGF-II receptors, and *IGFBPs-2*, -4, and -5 (186, 197–200). The expression of IGF-I receptors in rat glomeruli has been demonstrated by the presence of IGF-I receptor mRNA with *in situ* hybridization (198) as well as with RNase protection assay of RNA that was extracted from isolated rat glomeruli (52). In rat glomeruli IGF-I receptors are expressed at higher density than in other parts of the nephron (199) and bind radiolabeled IGF-I with high affinity (195, 199).

The expression of high-affinity IGF-I receptors provides the basis for the effects of IGF-I on glomerular function and structure that are discussed later in this review. These effects of IGF-I on the glomerulus could either follow endocrine modes or result from autocrine/paracrine activity of the peptide. Studies on the expression of IGF-I in rat glomeruli suggest an endocrine mode of action, because IGF-I may not be expressed in the glomerulus. Several investigators did not find IGF-I peptide or mRNA with *in situ* immune histology or hybridization techniques (180, 186, 197, 198, 200–204). However, there has been one exception. Matejka and co-workers examined the expression of IGF-I mRNA by *in situ* hybridization using a digoxigenin-labeled probe (205). In this study, glomerular IGF-I mRNA was confined to some cells in the glomerular tuft. Furthermore, Bortz and associates (52) found low levels of IGF-I mRNA by *ex vivo* solution-hybridization/RNase protection assay in glomeruli that were isolated from rat kidney. These investigators failed to demonstrate the presence of IGF-I peptide in glomeruli by *in situ* immunohistochemistry (52). The discrepancy between findings from different laboratories may have several explanations. First, the studies by Matejka *et al.* also demonstrated IGF-I mRNA at low levels in proximal tubules in normal rats, which was not found by any other investigator. Thus, the *in situ* hybridization technique used may have resulted in "false positives." Second, Bortz *et al.* isolated glomeruli by sequential sieving. This preparation is usually not completely pure but contains fragments from other tubular segments. Small amounts of fragments of collecting ducts that express relatively large amounts of IGF-I mRNA may have contaminated the preparation. Second, IGF-I is indeed expressed in rat glomeruli at low levels and may have escaped detection in studies by several other investigators. This is possible but probably less likely. Thus, the experimental evidence suggests that IGF-I is not expressed by normal rat glomeruli and IGF-I-induced glomerular dynamic effects result from circulating peptide. Whether IGF-I peptide is bound to recep-

FIG. 1. Expression of GH receptors and the IGF system in the rat nephron. The cartoon is compiled from several original data sources (180, 194, 197, 198, 201–205, 217, 481, 557). Rec, Receptor; ?, *in vitro* finding, but not confirmed in *in vivo* study. The figure differentiates between proteins and mRNAs. The presence of the latter suggests *in situ* synthesis.



tors was not delineated in immunohistochemical studies, probably because the levels are rather low.

(Primary) cell cultures of glomerular mesangial, endothelial, and epithelial cells express IGF-I, IGF-BPs, and IGF-I receptors *in vitro* (206–210). In cultured mesangial cells, IGF-I is a progression factor, suggesting a mitogenic role in the mesangium *in vivo*, possibly by an autocrine or paracrine mode (206–211). The fact that residential rat glomerular cells express IGF-I in culture but apparently not *in vivo* may be explained with dedifferentiation of the cells in the cell culture environment. The expression of IGF-I in cultured cells certainly does not allow for the assumption that these cells express IGF-I also *in vivo*.

Endothelial cells from large arteries such as aorta and possibly the renal arteries secrete IGF-I (212–214). Renal glomeruli and peritubular capillaries are strategically located downstream from these large vessels, and IGF-I that is secreted into the renal circulation may act in glomeruli and tubules.

Rat glomeruli also express IGF-II receptors as indicated by the presence of IGF-II receptor mRNA by *in situ* hybridization (180) and in extracted RNA from isolated glomeruli (52), and the receptor protein can be demonstrated by immunohistochemistry in glomeruli *in situ* (215).

IGF-II or IGF-II mRNA are not found in the rat glomerulus (180), but IGF-II mRNA is expressed in association with IGFBP-2 mRNA and protein in the wall of interlobular arteries and afferent arterioles (180), giving rise to the notion that arteriolar IGF-II may act downstream through glomerular IGF-I (and IGF-II?) receptors. At present, no particular

function has been associated with glomerular IGF-II receptors. Not all studies have confirmed that IGF-II mRNA is found in these vessel in rat kidney (193). The reasons for this discrepancy among findings by different investigators are unclear.

mRNAs encoding for IGFBP-2, -4, and -5 have also been found in rat glomeruli by *in situ* hybridization (180, 189, 200). Neither IGFBP-1 nor IGFBP-1 mRNA could be demonstrated in the rat glomerulus (198, 201). IGFBP-3 mRNA is also not found in glomeruli in normal rats (216).

2. Proximal tubules. Under physiological conditions, proximal tubules do not, or only minimally, express IGF-I mRNA (205, 217), but IGF-I peptide is clearly found in proximal tubules by immunohistology (201). In contrast to other authors, Bortz *et al.* (52) also found IGF-I mRNA by RNase protection assay of RNA that was extracted from isolated rat proximal tubules and failed to demonstrate the presence of IGF-I by *in situ* immunostaining. Taken together, these studies suggest that IGF-I is probably not expressed in greater amounts in normal rat tubules. However, IGF-I mRNA and peptide become transiently expressed in regenerating proximal tubules after acute injury. In this setting, new epithelial cells that are not yet fully differentiated and macrophages that participate in the repair process express IGF-I mRNA and peptide (203, 217). The lack of synthesis of IGF-I in normal rat proximal tubules is further suggested by the pattern of immunostaining that was described by Kobayashi *et al.* These investigators found IGF-I peptide along the brush border and the basolateral membrane but not in cytoplasm (201). This distribu-

tion is best compatible with (receptor) binding of serum IGF-I or IGF-I that has undergone glomerular ultrafiltration.

Proximal tubules express IGF-I- and IGF-II receptors abundantly (195, 197, 198, 200, 205, 217, 218). IGF-II receptors appear to be distributed evenly between the apical (luminal) and basolateral tubule membranes (218–220). In contrast, IGF-I receptors may be more abundant in the basolateral membrane compared to the brush-border membrane (218, 221). However, functional studies by Quigley and Baum (222) in isolated-perfused rabbit proximal tubules indicate greater efficacy of IGF-I-induced effects through the apical as compared to the basolateral proximal tubule membrane. These latter studies strongly suggest that apical IGF-I receptors are functionally important. The location of IGF-I- and IGF-II receptors in both cell membranes and the (likely) lack of expression of IGF-I in proximal tubules suggests physiological actions of circulating as well as ultrafiltered IGF-I. Possible physiological and pathological effects that may be mediated by these modes of action include alteration of transport processes, growth (hypertrophy and hyperplasia), and possibly increased secretion of extracellular matrix proteins, as is discussed in later parts of this review.

Rat proximal tubules also express GH receptors (197, 216). Since IGF-I is probably not expressed in proximal tubules in normal rats (202, 203, 216, 217), GH may function independently of IGF-I in proximal tubules. Studies by Rogers and associates (223) demonstrated that GH directly stimulates gluconeogenesis in canine proximal tubules without increasing IGF-I. *In vivo*, cells in the S₃-segment of proximal tubules also express ALS and GH receptors (216). The proximal tubule ALS mRNA levels are under GH control and are reduced in hypophysectomized rats and increase during treatment with GH (216). In this segment of the nephron, IGFBP-3 is not expressed (216). IGFBP-3 and (most likely) IGF-I are not synthesized in proximal tubules, and the 150-kDa complex could only be assembled in proximal tubules if these latter two proteins are "imported." This anatomic distribution of ALS gives rise to the possibility that ALS has local intrinsic biological functions that are independent of its association with the large IGF-binding complex. However, at present, this is speculative and warrants further experimental investigation. Proximal tubules also express IGFBP-5 as was shown by Hise and associates (190) on the mRNA as well as the protein level.

3. Loop of Henle. The presence of IGF-I peptide in the thin segment of the loop of Henle in normal rat renal medulla has been shown by immunostaining (180, 201, 202, 204). This segment also contains IGFBP-1, IGFBP-2, and IGF-II receptors (180, 201). The thick ascending limb of the loop of Henle expresses IGF-I mRNA (197, 200, 201), and *in situ* hybridization depicts the expression of mRNA encoding IGFBP-1, IGF-receptors, and GH receptors (197). Immunostaining indicates also the presence of IGFBP-1 protein (201). Thus, the thick ascending limb of the loop of Henle is the only segment within the nephron that colocalizes GH receptors, IGF-I, and IGF-I receptors, suggesting that GH may act via local induction of IGF-I which, in turn, acts through the IGF-I receptor by autocrine or paracrine modes (197).

4. Distal tubules and collecting ducts. Rat distal convoluted tubules do not express IGF-I mRNA (198) but may (201) or may not (202, 204) contain small amounts of IGF-I peptide, probably reflecting circulating and/or ultrafiltered IGF-I that is bound to receptors. Distal tubules express IGF-I receptor mRNA (197, 198) as well as IGFBP-1 mRNA and protein and IGFBP-5 mRNA (197, 198, 200, 201).

Cortical collecting ducts contain IGF-I receptor mRNA (197, 200). In only one study (180) IGF-II receptors were also found by immunohistology. This may require confirmation before major conclusions can be made. Cortical collecting ducts do not express IGF-I- or IGF-II mRNA, but both peptides as well as IGFBP-1 have been found, by immunohistology, and are probably of ultrafiltered or circulating origin (180, 201).

Several investigators found IGF-I mRNA and/or peptide in medullary collecting ducts, and the IGF-I expression was found in some studies to be greater than in other parts of the nephron (52, 53, 180, 198, 201, 202, 204, 205, 224, 225). However, other investigators have not confirmed the presence of IGF-I mRNA in cortical or medullary collecting ducts (197, 200). Thus, there are discrepancies that are not readily explained. IGF-I mRNA was also found by assaying extracted RNA from microdissected collecting ducts as well as by *in situ* hybridization (52, 53, 180). Furthermore, the peptide is diffusely distributed in the cytoplasm in collecting ducts as was shown by immunohistology. This finding suggests local synthesis of IGF-I in medullary and probably cortical collecting ducts (204). Most likely, IGF-I is indeed synthesized in rat renal collecting ducts.

Medullary collecting ducts possibly contain IGF-II mRNA (180), although this has also not been confirmed in other studies (194). One study also found IGF-II receptors in medullary collecting ducts in rats (180), but this finding may also need confirmation before major conclusions are drawn. This segment also expresses IGFBP-2 and -4 mRNAs, and the presence of IGFBP-2 protein has been demonstrated (180, 200).

In rats, both IGF-I- and II- receptors have been localized to the renal interstitium, probably on interstitial fibroblasts. Interstitial cells also express IGFBP-3 and -5 mRNAs (216, 226). In one study IGFBP-2 and -4 were also found in the cortical interstitium (186). Northern analysis of whole kidney RNA demonstrates that the amounts of IGFBP-5 mRNA in kidney are greater than in other organs (134). IGFBP-5 protein may be bound to extracellular matrix proteins in the renal interstitium and may provide a "intermediary reservoir" for IGF-I and enhance the local action of the peptide (188, 227).

B. Expression in human kidney (Fig 2)

Less information is available about the expression of GH receptors and the IGF system in human kidneys. Data on the GH-dependent regulation are not available. In adult humans, IGF-II (but not IGF-I) has been localized to the glomerular and peritubular vasculature as well as the interstitium (193–195). Chin and associates (195) did not find IGF-I mRNA expression in any location of the human nephron by *in situ* hybridization. Both IGF-I and -II receptors are present in

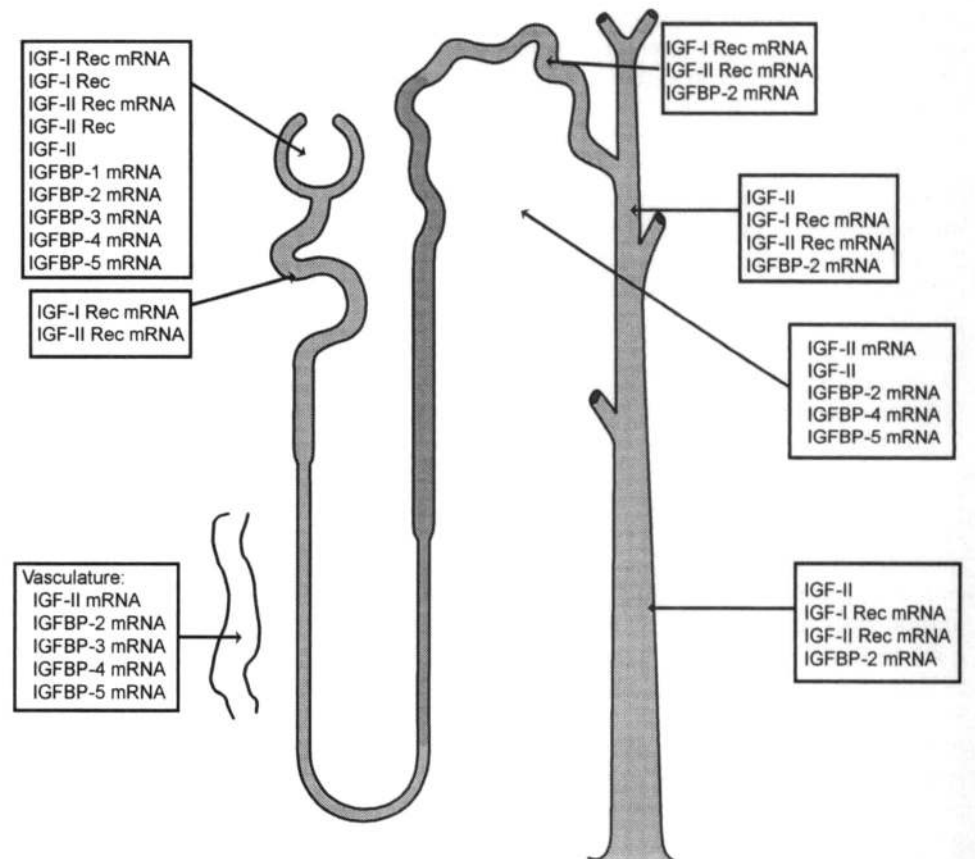


FIG. 2. Expression of IGF-I and -II, IGF-I and -II receptors, and IGFBPs in the normal human kidney (193–195). The figure differentiates between proteins and mRNAs. The presence of the latter suggests *in situ* synthesis.

various segments. mRNAs encoding for IGFBPs are found by *in situ* hybridization in the glomerulus and in tubules (193, 195). A composite of the location of IGFs, their receptors, and binding proteins and/or their mRNAs in the human nephron is provided in Fig. 2. The current picture of the localization and presence of members of the IGF system in human kidney is, most likely, incomplete and warrants further examination.

C. Functional implications of anatomic data

The distribution of the GH-IGF-system in the (rat) kidney raises several questions as to how GH and IGF-I interrelate with regard to their effects on renal function and metabolism. Both GH and IGF-I increase glomerular hemodynamics, as will be reviewed in detail in a later section. Yet, (rat) glomeruli do not express GH receptors and may not synthesize IGF-I *in vivo*, although the expression of the peptide has been found by some investigators (*vide supra*). Thus, GH-induced effects on glomerular function could only be explained by an indirect action by which GH induces an increase in systemic IGF-I, which then acts through glomerular IGF-I receptors. Indeed, as will be discussed in further detail, the hemodynamic effects of GH occur only with a time delay and after the serum IGF-I levels have risen. Thus, all GH effects on the glomerulus appear to be systemically mediated by circulating IGF-I.

On the other hand, mice transgenic for GH, but not those transgenic for IGF-I, develop premature glomerular sclerosis, although in both models, animals have elevated circulating levels of IGF-I of similar magnitudes (207). This would

suggest that GH may act directly on glomerular metabolism independently of IGF-I. Possibly, these mice express glomerular GH receptors, in contrast to rats, but this has not been examined.

All members of the GH-IGF-I loop, namely GH receptors, IGF-I mRNA, and IGF-I receptors, are expressed in only one location in the nephron, namely the thick ascending limb of the loop of Henle. This finding suggests that GH-induced IGF-I functions in an autocrine/paracrine mode in the loop of Henle. There may also be actions of GH that are independent of IGF-I. Since the major function of this nephron segment is the extraction of chloride from tubular fluid and the maintenance of an increased interstitial osmolal tonicity, GH and/or IGF-I may participate in the regulation of the primary transporter of this segment, the Na/K/2 Cl transporter. However, such a role has not been directly demonstrated.

Although anatomic studies have not demonstrated that GH receptors are invariably expressed in the cortical and/or medullary collecting duct (197), there is the suggestion that functioning GH receptors are present in collecting ducts. This evidence is derived from experiments that demonstrate a GH-induced rise in the collecting duct IGF-I mRNA levels (52, 53, 224). In conclusion, GH receptors appear to be expressed in cortical and/or medullary collecting ducts.

Proximal tubules express transport proteins for many (absorptive and secretory) transport processes, and some of these, such as the phosphate and possibly sodium absorption, are partially regulated by IGF-I. Both GH and IGF-I may

have direct actions on proximal tubules independent from each other and increase tubular cell gluconeogenesis (223, 228). The evidence from several sets of anatomic studies indicates a lack of IGF-I mRNA in proximal tubules in normal rats but the presence of IGF-I that is bound to apical and basolateral membranes but not in the cytoplasm. In concert, these findings suggest that circulating and ultrafiltered IGF-I affect the function of proximal tubules. This is a rather intriguing hypothesis since it suggests that the proximal tubule operates under "endocrine-like" regulation by IGF-I.

D. Regulation of the renal expression of IGF-I

Our current understanding of different mechanisms that contribute to the regulation of the renal expression of IGF-I is largely obtained from animal studies, and it remains unclear whether the findings also apply to humans. However, it is likely that most mechanistic principals apply to both humans and rodents. In this section we will review what is known about the effects of GH, nutrition, and epidermal growth factor (EGF) as physiological regulators of renal IGF-I.

1. *GH.* The kidney contributes to the circulating IGF-I pool as inferred by greater somatomedin activity in renal venous compared to arterial blood (55). However, these early studies did not indicate that the renal expression of IGF-I is increased by GH (54). It is now known that GH regulates IGF-I synthesis in liver, kidney, and brain but not in several other tissues such as lung, spleen, and heart (51). In GH-deficient and control mice exogenous GH raises kidney IGF-I mRNA levels significantly and quickly, within <2 h. After administration of GH peak IGF-I mRNA levels in kidney are observed at about 12 h. GH increases IGF-I gene expression by activating transcription (51). A similar time course for a rise in extractable and immunoreactive IGF-I has been found in hypophysectomized rats that were injected with a single dose of GH (27). In GH-deficient mice and probably also in normal mice the GH-induced rise in liver IGF-I mRNA is greater than in kidney (51). This may result from a greater GH receptor density in liver compared to kidney.

Renal IGF-I mRNA levels are reduced in GH-deficient dwarf rats compared to normal controls. In these animals the IGF-I mRNA levels increase upon administration of GH (175). Implantation of GH-producing tumor tissue or pituitary cells into normal rats also increases the renal IGF-I mRNA levels (53, 226).

The GH-induced rise in renal IGF-I mRNA levels is caused by focal stimulation of the IGF-I gene in collecting ducts and the medullary thick ascending limb of the loop of Henle (53, 224). Hypophysectomy reduces the IGF-I expression in these segments of the nephron. This focal action of GH on IGF-I mRNA in the kidney may explain the lesser increase in extractable IGF-I mRNA in kidney compared to liver on a per cell basis (51).

IGF-I mRNA is detected in glomeruli from normal rats neither at baseline nor after stimulation with GH (198). Thus, all glomerular effects of GH are most likely transmitted by systemic elevation of IGF-I that may primarily result from increased transcription of the hepatic IGF-I genes (51).

Hypophysectomy reduces and exogenous GH raises the renal expression of GH receptors in straight proximal tubules and thick ascending limbs of the loop of Henle (197). Since IGF-I mRNA is not found in normal rat proximal tubules, GH apparently acts directly through its own receptor in this segment of the nephron. However, effects of GH are most likely also transduced indirectly through systemic and/or ultrafiltered IGF-I and its interaction with IGF-I receptors in proximal tubules. GH may modestly down-regulate the renal expression of IGF-I receptors (226).

GH also regulates the expression of IGFBPs in rat kidney. In response to GH, renal IGFBP-1 and -4 mRNAs decrease moderately (175, 226). In GH-deficient rats IGFBP-1 and -4 mRNAs are increased and administration of GH normalizes the levels (175, 176). The effect of GH on IGFBP-4 mRNA seems to be less compared to effects on IGFBP-1 mRNA (175, 226). Renal IGFBP-3 mRNA increases modestly with administration of GH to normal rats, and the effect appears to be quantitatively less important (175, 216, 226). Some authors found in hypophysectomized or in dwarf rats that GH does not increase the renal expression of IGFBP-3 significantly (175, 176, 216). This is in contrast to hepatic IGFBP-3 mRNA, which is GH dependent (216). The ALS mRNA that is found in straight proximal tubules increases with GH treatment in hypophysectomized rats (216). Hypophysectomy also increases the expression of IGFBP-5 in proximal tubules and glomeruli in the rat (227).

2. *Nutrition.* The renal IGF-system is also regulated by nutrition, particularly calorie and protein nutrition. Short-term fasting decreases the renal IGF-I mRNA and raises renal IGFBP-1 levels in GH-deficient and normal rats but does not affect IGFBP-2 to -5 mRNAs in kidney (175). Since these fasting-induced changes occur similarly in normal and GH-deficient rats, they are apparently independent of GH (i.e. not mediated by GH).

High protein diets fed to normal rats increase the renal IGF-I mRNA levels and reduce IGFBP-1 mRNA (229). Low protein diets reduce IGF-I and increase IGFBP-1 mRNA in the kidney (229, 230). Since IGFBP-1 may reduce IGF-I activity by binding the peptide and preventing receptor interaction, these changes may augment the effects of low and high protein diets to further up- and down-regulate renal IGF-I activity beyond a direct effect on IGF-I mRNA. Since IGF-I and IGFBP-1 are coexpressed in medullary thick ascending limb of the loop of Henle, this dietary protein-dependent regulation of IGF-I activity may refer particularly to this segment of the nephron. Indeed, the thick ascending limb of the loop of Henle is particularly sensitive to high dietary protein-induced hypertrophy (231, 232). However, dietary protein also regulates systemic, circulating IGF-I activity. A low protein diet reduces liver synthesis of IGF-I and increases hepatic IGFBP-1 mRNA.

IGF-I receptors and IGFBP-5 in proximal tubules are also regulated by dietary protein in the rat. Both increase with reduced dietary protein intake (190). Low protein diets also decrease the renal mRNA levels for IGFBP-2 and do not affect the IGFBP-3, -4, and -6 mRNAs in the kidney (230).

Low protein diets decrease the levels of extractable and immunoassayable IGF-I in isolated rat glomeruli, and the

levels are increased if the rats are fed a high protein diet (233). Since IGF-I is probably not synthesized in rat glomeruli, the extracted IGF-I probably represents circulating peptide that was bound/trapped in glomeruli.

3. *Epidermal growth factor (EGF)*. EGF is also expressed in thick ascending limbs of the loop of Henle and in distal convoluted tubules (234). EGF is obviously synthesized in these two locations in the nephron since both mRNA and peptide are colocalized (234). There is evidence that GH regulates renal EGF expression. Renal EGF mRNA is reduced in hypophysectomized rats and increases with GH administration in these animals. However, in normal rats GH does not increase the renal EGF levels above normal (235). Furthermore, EGF increases the expression of IGF-I in isolated rat collecting ducts *in vitro* (235, 236), but it is unclear whether this occurs also *in vivo* and whether EGF also stimulates IGF-I synthesis in the loop of Henle. It is possible, that EGF that is synthesized and released in the loop of Henle or distal tubules increases the expression of IGF-I in the thick ascending limb and/or in downstream segments of the nephron. Thus, there is the possibility that a "GH- EGF- IGF-I" axis exists in the kidney. Interactions between EGF and IGF-I may have important implications in mechanisms that regulate renal compensatory growth and in the accelerated healing after ischemic renal injury. Both of these aspects are discussed below in separate sections of this review.

III. Processing of GH, IGFs, and IGF-BPs by the Kidney

The normal glomerular capillary wall prevents the ultrafiltration of great amounts of serum albumin by its charge selectivity (due to anionic charges in the basement membrane) and of large molecular mass serum proteins by size selectivity of the pore structure. However, small amounts of albumin (molecular mass ~68 kDa) and even IgG (molecular mass ~158 kDa) are ultrafiltered physiologically (237). Low molecular mass proteins, such as β_2 -microglobulin, are normally ultrafiltered in the glomerulus at greater rates. Tubule absorption and degradation are the primary means of their metabolism. In this respect, the kidney plays a major role in the degradation of small molecular mass proteins and peptides, including peptide hormones such as insulin (238–242).

GH also undergoes glomerular ultrafiltration and subsequent tubular absorption and degradation. Bilateral nephrectomy in experimental animals results in increased plasma levels and half-life of GH (243, 244). Similarly, GH serum levels are increased in patients with chronic renal failure, and the half-life is prolonged (245–249). Under normal conditions small amounts of GH are excreted with urine (250–252).

Several years ago, Johnson and Maack (253) examined the renal extraction and ultrafiltration of radiolabeled rat GH in intact rats and in isolated-perfused rat kidneys. These studies demonstrated a high sieving coefficient¹ for GH of about 0.6, indicating that the ultrafiltration of GH is as great as 60% of

the filtration rate of water (assuming that there is no substantial tubular secretion of GH). However, the fractional excretion² of GH in the urine was <0.01. This large difference between the sieving coefficient and the fractional excretion indicates that most of the ultrafiltered GH is processed by the tubules (253). In fact, studies have indicated that GH undergoes endocytotic absorption and subsequent proteolysis in tubule cells (241, 242, 253, 254). The absorption of GH into tubule cells occurs via the luminal (apical) cell membrane, not through the basolateral membrane (253). Thus, the glomerular ultrafiltration of GH is the rate-limiting step in the metabolism of the protein and, hence, explains why serum GH hormone levels are elevated in subjects with renal failure and that the kidney is the major organ causing most of the plasma GH turnover (253, 255). Intense renal uptake of GH in the rat was also demonstrated by Krogsgaard Thomsen *et al.* (256). These investigators provided evidence that [¹²⁵I] GH may also be extracted from plasma by peritubular renal uptake, a finding that is incompatible with those of other investigators (253).

Haffner and associates (255) examined the steady state metabolic clearance rate (MCR) of recombinant human GH in normal subjects and in patients with chronic renal failure. The MCR is decreased and the serum half-life is increased with increased serum levels of GH. In patients with chronic renal failure, the MCR is decreased and the serum half-life is increased. This fact confirms the importance of the kidneys for GH metabolism (255). These investigators estimated that about 50% of the GH catabolism is normally accomplished by renal extraction and degradation; this value is similar in magnitude to earlier experimental estimates (253). The normal kidney handles GH similarly to other microproteins, namely by glomerular ultrafiltration, tubular reabsorption via endocytosis, lysosomal degradation in tubule cells, and some urinary excretion (253, 255).

Due to their low molecular mass (~7.6 kDa), the glomerular ultrafiltration of IGF-I and IGF-II should occur at great rates, with a sieving coefficient that approaches 1.0. However, since most of the circulating IGFs are present in molecular forms of ~150 and ~45 kDa, ultrafiltration of these protein complexes occurs certainly at much lesser rates. Indeed, in normal rats, the glomerular ultrafiltration of IGF-I is extremely low (257–259).

Several investigators demonstrated the presence of IGF-I in bladder urine of normal subjects and patients with renal disease. Hizuka and associates (260) found IGF-I levels in urine of normal subjects of 78 ± 13 pg/ml (or 72 ± 7 pg/mg creatinine). Although the sieving coefficient for IGF-I cannot be estimated from these data, one may estimate the fractional excretion of IGF-I to be about 0.00001 (or 0.0000001%) based on data by Hizuka *et al.* (260). The urinary excretion of IGF-I is even slightly lower in patients with hypopituitarism and about 3-fold greater in acromegalics (260). Other authors reported similar rates of urinary IGF-I excretion (250, 251,

¹ The glomerular sieving coefficient of GH is the ratio of the GFR of GH divided by the GFR of water and small solutes, which is usually measured as the clearance of inulin or creatinine.

² The fractional excretion of GH is the ratio of the (whole kidney) renal clearance of GH, divided by the GFR or creatinine clearance. The difference between the sieving coefficient and the fractional excretion of a compound indicates the amount of tubular binding, absorption and/or degradation, or tubular secretion.

261–264). Several investigators used gel filtration chromatography to estimate the molecular size of IGF-I in urine. The data are somewhat dissimilar. This results most likely from elution of the columns with acidic or neutral buffer systems. Hizuka *et al.* found that about 70% of urinary IGF-I is present in a ~43 kDa molecular form, and ~30% is excreted as free IGF-I (260). However, Yokoya and associates (263) found the vast majority of urinary IGF-I to be present in the free form. As expected, ~150 kDa complexes are not found in urine from subjects without renal glomerular diseases. The urinary excretion of IGF-I is greater in young children but tends to decline with age (250, 263) and correlates with the urinary excretion of GH (251).

The contribution of the kidney to the metabolism of IGF-I is exemplified by comparison of pharmacokinetic data obtained from normal subjects compared to patients with advanced renal failure or end-stage renal disease. Rabkin and associates (265) found a greater maximal serum concentration and reduced volume of distribution after administration of recombinant human IGF-I (rhIGF-I) in patients with chronic renal failure compared to controls (265). The plasma clearance of IGF-I was similar in both groups, despite low renal clearance rates in chronic renal failure patients, suggesting that normally the kidney plays only a minor role in the metabolism of IGF-I. Even the serum half-life was reduced in renal failure patients (265). Fouque and associates (266–268) also examined the pharmacokinetics of rhIGF-I in some detail in normals and in patients with end-stage renal disease. With administration of rhIGF-I, 50 $\mu\text{g}/\text{kg}$, the pharmacokinetic parameters were similar in both groups of subjects. The half-life was decreased in renal failure patients with administration of a larger dose, 100 $\mu\text{g}/\text{kg}$ (268). Thus, the kidney plays only a modest role in the removal of IGF-I from the circulation. This is much different in patients with the nephrotic syndrome (*vide infra*).

The fate of IGFs that have been ultrafiltered by glomeruli may include binding to specific IGF receptors and induction of metabolic effects in renal tubules (257, 258), and/or the peptides may be absorbed and degraded (269). Flyvbjerg and associates (221) studied the uptake and processing of IGF-I in isolated-perfused rabbit proximal tubules. These investigators found that some uptake of IGF-I occurs upon perfusion of the apical as well as the basolateral membrane with IGF-I. The uptake of [^{125}I]IGF-I was inhibited by excess cold IGF-I, suggesting involvement of IGF-I (and/or IGF-II-) receptors (221). A minor fraction (~20%) of bound IGF-I undergoes lysosomal degradation. Thus, renal extraction and degradation of IGF-I may occur by glomerular ultrafiltration and luminal uptake but also by uptake from peritubular capillaries through tubule cell basolateral membranes (221).

Fawcett and associates (270) confirmed some of these findings in an *in vitro* model of cultured proximal tubule-like epithelial cells. These authors demonstrated that the cellular uptake of IGF-I was inhibited by IGFBP-3, but enhanced by IGFBP-5 (271). The mechanisms by which IGFBP-5 actually facilitate cellular uptake of IGF-I remain unclear.

In humans, IGF-II is also excreted with urine (Fig. 3). The rate of urinary excretion tends to decline with age and is greatest in prepubertal children in some studies (261) but not in others (272). The urinary excretion of IGF-II is several fold

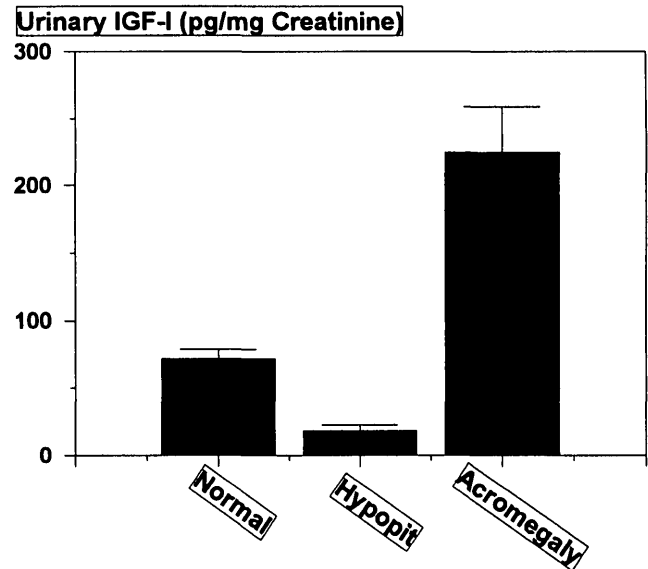


FIG. 3. Urinary excretion of IGF-I in eight normal subject, nine patients with hypopituitarism (Hypopit), and 10 patients with acromegaly. [Data derived from Hizuka *et al.* (260).]

greater than that of IGF-I. This may result from greater serum levels compared to the IGF-I levels in human as compared to rat serum, and/or a greater free, unbound fraction of IGF-II in serum (272). In normal subjects the absolute concentration of free IGF-II in serum is at least 50% greater than the free IGF-I (44).

Excretion of IGFBPs in urine has also been demonstrated. Zunkeller and Hall (272) demonstrated the presence of IGFBP-1 in urine from normal healthy adults. Furthermore, urine from normal subjects contains IGFBP-2 and -3 (261, 273). IGF-binding in dialyzed urine from normal subjects is found in Western ligand blots at 30–40 kDa and 31 kDa corresponding to IGFBP-3 and -2, respectively (273). These investigators also demonstrated that the 150-kDa complex is absent from normal urine (273). This is due to the size-selective ultrafiltration barrier of the normal glomerulus. Furthermore, the urinary IGFBP-3/IGFBP-2 ratio is reversed compared to serum (273). There may be several explanations for this finding. First, most IGFBP-3 in serum is present in the 150-kDa binding complex and thereby virtually excluded from glomerular ultrafiltration. Second, some of the urinary IGFBP-3 may result from tubular secretion, although this has not been demonstrated in appropriate experiments. Third, there may be differential absorption or binding of IGFBPs to tubule cells during the tubular downstream passage.

Arany and associates (274) injected [^{125}I]IGFBP-3 into normal rats. Within 1 h, the majority of IGFBP-3 had become associated with ALS, and this complex was excluded from urinary excretion, most likely because it was excluded from glomerular ultrafiltration. Only a small amount (8%) of the administered IGFBP-3 was recovered in urine. Some of this fraction may have been fragmented IGFBP-3 (274). During the initial few minutes, the serum half-life of [^{125}I]IGFBP-3 was short (~25 min). Subsequently, the serum IGFBP-3 levels remained fairly constant (274). Thus, before complexing with ALS, free IGFBP-3 could disappear from the intravascular space. The ALS-IGFBP-3 complex could not (274).

In patients with glomerular diseases and proteinuria, the rate of urinary excretion of IGF/IGFBP-complexes is increased (261, 273), particularly when associated with the nephrotic syndrome (167, 257, 258, 275). However, the urinary excretion of intact IGFBP-3 is relatively low (167) or absent (276). This may have pathophysiological importance (*vide infra*).

IV. GH, IGF-I, and Glomerular Hemodynamics

A. GH

Circumstantial evidence linking GH to increased renal hemodynamics was suggested several decades ago. Ikkos and associates (277) found elevated glomerular filtration rates (GFR) and renal plasma flow (RPF) rates in patients with acromegaly. Thus, the hypersomatotrophic state of acromegaly is associated with both increased renal function and greater renal mass. Corvilain and co-workers (278) were the first to demonstrate that administration of GH-containing pituitary extracts increased GFR in normal subjects (278). Falkheden (279) and Falkheden and Wickbom (280) examined the kidney function in patients with acromegaly or breast carcinomas before and after hypophysectomy. Falkheden found that the GFR decreased after the procedure (279). This investigator posed the important question of whether GH raises GFR secondary to increasing renal mass or owing to a direct effect on renal hemodynamics independent of the effect of the hormone to increase kidney size. Falkheden could separate the effects on hemodynamics and kidney size in patients with serial measurements of kidney function at different times after hypophysectomy. The decrease in renal function occurred before a (noticeable) reduction in renal mass (280).

Ingestion of protein-rich meals or administration of amino acid solutions are known to induce a rise in serum GH levels as well as GFR. Several investigators hypothesized that the protein- or amino acid-induced rise in GFR may be mediated by GH. In one study from this laboratory, normal subjects and patients with GH deficiency underwent short-term serial measurements of renal hemodynamics [inulin and p-aminohippuric acid (PAH) clearances] before and after a 30-min infusion of L-arginine (281). The GFR at baseline was significantly lower in the GH-deficient subjects compared to normal controls. However, after the arginine infusion, the GFR [and renal plasma flow (RPF)] rose in both groups (281). If expressed as percent of baseline, the rise in GFR tended to be greater in the GH-deficient patients compared to the healthy subjects. Furthermore, in the normal subjects, the rise and peak in GFR preceded the peak in plasma GH levels (281), suggesting that the effect of intravenous amino acids on GFR is GH-independent.

These findings were confirmed by Ruilope and associates (282). These investigators administered a mixed amino acid infusion to patients with panhypopituitarism that increased GFR (282). In contrast, a challenge with a protein-rich meal failed to cause a rise in GFR in a group of GH-deficient patients (283). However, in the latter study, some of the normal control subjects also had no change in GFR in response to the same protein meal. This finding renders the

study somewhat indeterminant (283). In concert, these studies indicate that GH does not mediate the rise in GFR and RPF that occurs with acute infusions of amino acids.

Since the original study by Corvilain and his associates (278), several studies have been performed that indicate that administration of GH (or recombinant human GH) increases GFR. Haffner and co-workers (284, 285) administered daily GH to normal subjects for 3 days. In one study, GFR was increased on day 3 by 11% above baseline ($P < 0.02$) (284). In a separate but similar study, GFR rose significantly after 3 days of GH treatment by 18% above baseline values (285). Parving and associates (286) acutely infused GH into normal subjects and found that this short-term GH administration had no effect on GFR. Similar findings had been reported by Westby and associates (287) who infused bovine GH acutely into normal or hypophysectomized dogs and did not find an increase in GFR or RPF within several hours. Christiansen and collaborators administered GH to normal subjects for 1 week. GFR and sonographic renal size measurements were obtained before and after 1 week of GH treatment (288). In contrast to the acute study, GFR was significantly increased (288). Furthermore, the rise in GFR occurred without a concomitant increase in renal size (288).

This series of studies indicated that GH would not raise GFR very acutely, but would do so chronically. To demonstrate this circumstantial finding more directly, we measured GFR and RPF in a single GH-deficient subject before and during the initial week of treatment with GH (289). The findings are summarized in Fig. 4 and demonstrate that the GFR did not rise during the initial pharmacological rise in serum GH hormone levels that occurred shortly after the first GH injection (289). However, 24 h after the first dose and before the administration of the second dose of GH, GFR and RPF were increased above baseline. At this time serum IGF-I levels were also increased in response to GH (Fig. 4) (289). Both renal function and serum IGF-I levels remained elevated for the remainder of the 1 week study (Fig. 4).

Similar findings were obtained in a group of seven normal subjects in whom serial measurements of GFR and RPF were performed for 3 consecutive days (290). After baseline measurements, a single intramuscular injection of recombinant human GH was given. Despite the pharmacological rise in serum GH levels shortly after the injection (Fig. 5), GFR, RPF, and serum IGF-I levels remained at baseline during the first day of the study (290). However, about 20 h after the injection of GH, serum IGF-I levels as well as GFR and RPF were increased significantly and remained elevated on the third day (Fig. 5) (290).

The latter two studies clearly explain the apparent discrepancies between the previous results on the effects of GH on GFR. GH increases GFR with a delay of many hours up to 1 day. Furthermore, these findings gave rise to the hypothesis that GH would not directly affect the renal vasculature. Rather, it induced IGF-I, which in turn, acts on renal hemodynamics. This hypothesis was subsequently confirmed (*vide infra*).

GH receptors are apparently not expressed in the glomerular microvasculature. Since IGF-I mRNA has also not been found *in situ* in glomeruli in most studies, it appears that the GH-induced and IGF-I mediated increase in glomerular he-

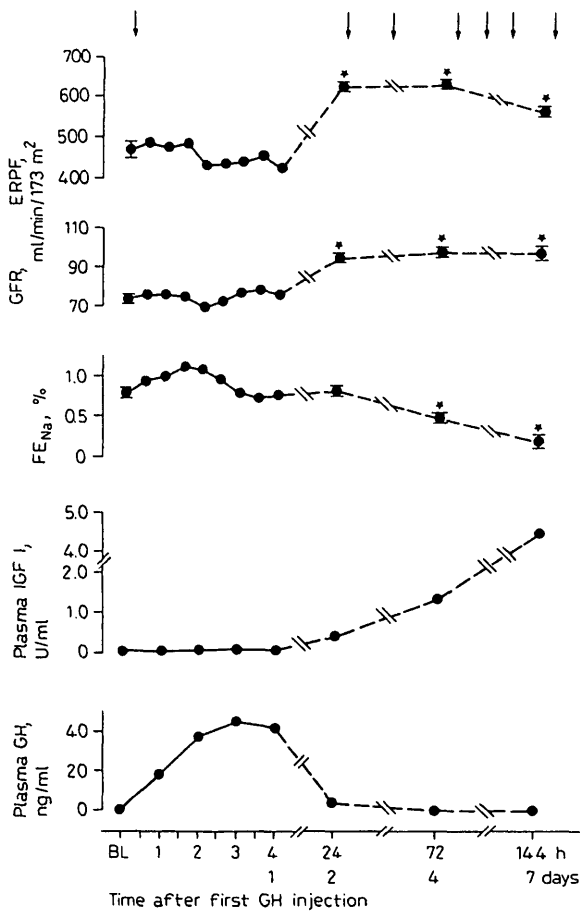


FIG. 4. Effective renal plasma flow (ERPF), GFR, fractional urinary excretion of sodium (FE_{Na}), and plasma IGF-I and GH levels at baseline (BL) and during the first 4 h and at 24, 72, and 144 h after initiation of treatment with recombinant human GH 0.125 mg/kg per day (arrows) in a GH-deficient adult. Note that neither ERPF nor GFR increase during the initial 4 h after the first dose of GH, when serum GH levels peaked. ERPF and GFR were elevated at 24, 72, and 144 h, and this rise in renal function occurred concomitantly with an increase in serum IGF-I levels. There was a decrease in the fractional excretion of Na, but the patient did not develop frank edema. [Reprinted with permission R.R. Hirschberg and J.D. Kopple: from *Am J Nephrol* 8:249–254, 1988 (289).]

modynamics is not directly regulated in glomeruli. It is more likely that GH induces a rise in IGF-I synthesis and release from other tissues such as liver (51), which causes a rise in the circulating IGF-I levels, which in turn act through glomerular IGF-I receptors. This hypothesis might be definitively proven in experiments using direct infusions of GH into renal arteries or perfusion of isolated-perfused kidneys with GH. To the authors' knowledge such experiments have not yet been performed.

Renal hemodynamic function covaries with endogenous GH status. In hypersomatotropic states (*i.e.* acromegaly), GFR and RPF are elevated and in GH deficiency both are decreased below normal (277, 281, 291–297). Hoogenberg and associates (298) measured the creatinine clearance in 14 acromegalics before and during chronic treatment with the somatostatin analog octreotide. The creatinine clearance of the acromegalic patients was greater compared to normal subjects. Treatment with octreotide lowered GH levels by

63% and resulted in a decline in the creatinine clearance (298). A similar study was reported by Dullaart and associates (291). These investigators measured the GFR and RPF as [¹²⁵I]iothalamate and [¹³¹I]hippuran clearances, respectively, before and after 3 months of treatment of acromegalic patients with octreotide (291). The somatostatin analog reduced serum GH levels by about two-thirds and lowered GFR and RPF to normal (291). Continuous administration of a peptidic antagonist to GHRF reduced endogenous GH synthesis and serum levels in normal rats (299–301). Concomitantly, the GFR was decreased below normal values (299).

B. IGF-I

Observations suggesting that exogenous or endogenous GH excess would increase GFR and that reduced GH activity would reduce renal function, but only after similar changes in serum IGF-I had occurred, led to the hypothesis that IGF-I mediates the effects of GH on renal function. Furthermore, IGF-I may affect renal hemodynamics acutely or chronically independent of the GH status.

Series of studies were performed in this and other laboratories to prove this hypothesis. We performed experiments in fasted anesthetized rats that underwent repetitive renal clearance measurements (302). After baseline measurements, animals received an injection and a 20-min infusion of rhIGF-I (Fig. 6). Within ≤ 20 min after onset of the IGF-I infusion, RPF and GFR began to rise and renal vascular resistance fell; renal hemodynamics remained elevated for about 2 h after cessation of the IGF-I infusion (Fig. 6). This was the first indication that a growth factor, namely IGF-I, has vasoactive properties and raises GFR and RPF. This supports the hypothesis that IGF-I mediates the effects of GH on renal hemodynamics (302).

More recently, Baumann and associates (303) confirmed the above findings. In addition, these investigators raised the important question as to whether the IGF-I-induced rise in renal hemodynamics is a IGF-I receptor-mediated effect or whether it is an insulin-like effect of IGF-I and possibly mediated through insulin receptors. Theoretically, IGF-I may increase renal function by lowering blood glucose concentrations and inducing hyperglucagonemia. An acute increase in serum glucagon levels is known to increase GFR (304, 305). Baumann *et al.* (303) infused rhIGF-I at different rates into anesthetized rats while maintaining an euglycemic clamp. Despite clamping the glucose levels at baseline values, IGF-I induced a dose-dependent rise in GFR by up to 35%, and half-maximal stimulation of GFR was achieved at a total serum IGF-I concentration of 24 nM.

Several lines of evidence suggest that IGF-I raises GFR, not only acutely and when administered exogenously in pharmacological dosages, but also that endogenous IGF-I may chronically contribute to the regulation of glomerular filtration. Circulating and/or renal tissue IGF-I levels covary with the GFR in a number of physiological and pathophysiological conditions. Both are elevated in acromegalics as outlined above (291, 294, 306), in pregnancy (29, 42, 307–318), and during high protein diets (10, 190, 231, 233, 319–330). GFR as well as serum IGF-I levels are chronically reduced in isolated GH deficiency, panhypopituitarism (279, 280, 282, 283, 294,

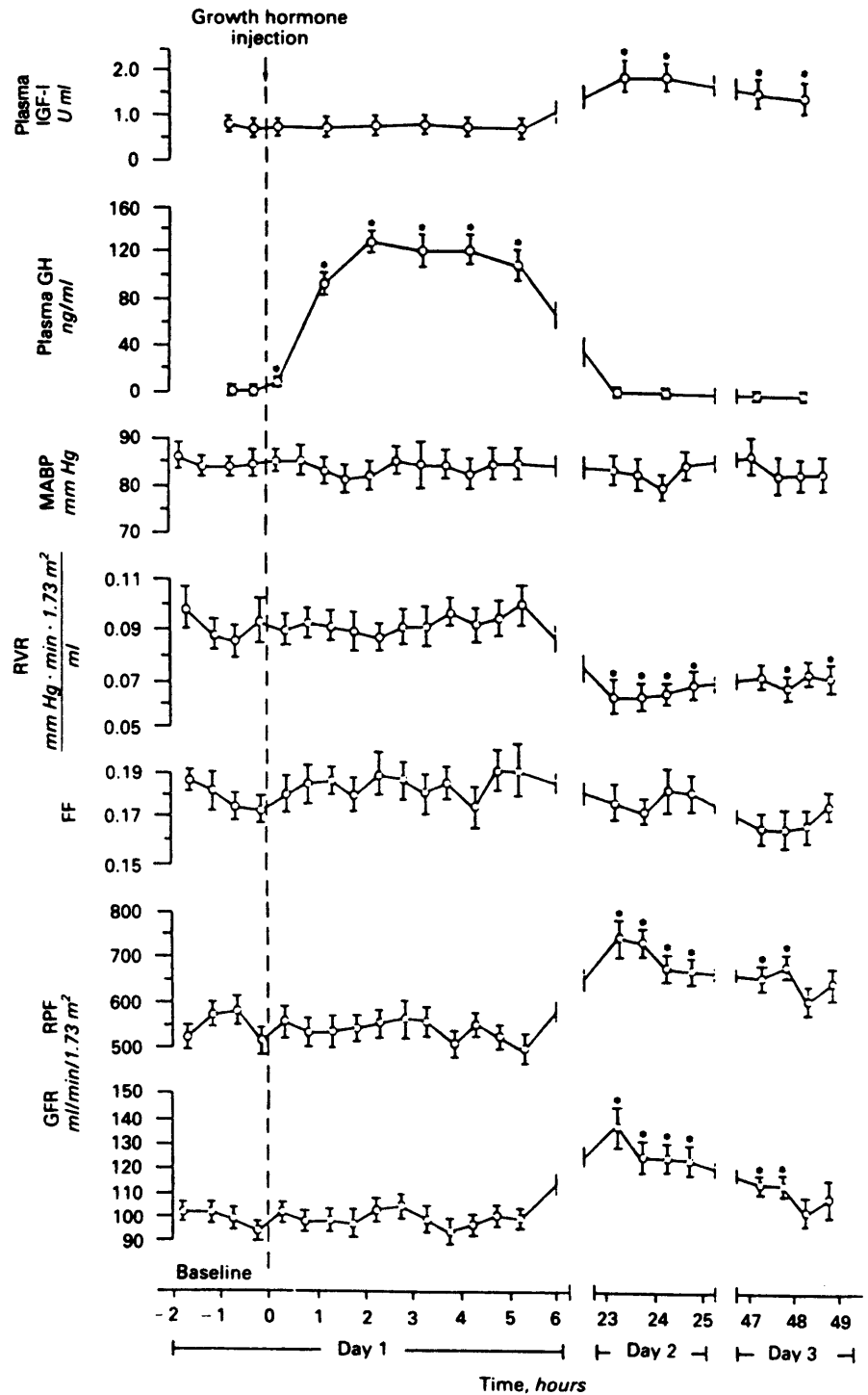


FIG. 5. Effects of a single injection of GH (0.15 mg/kg) on plasma hormone levels, mean arterial blood pressure (MABP), renal vascular resistance (RVR), filtration fraction (FF), renal plasma flow (RPF), and GFR in normal subjects. The GH injection was given as indicated by the arrow and the dotted vertical line. Note that the increase in renal function occurred with a delay and was paralleled by the rise in plasma IGF-I. [Reprinted with permission from R. Hirschberg *et al.*: *Kidney Int* 35: 865-870, 1989 (290).]

323, 329, 330), during treatment of actomegalics or type I diabetics with somatostatin analog (291, 294, 331-335), low protein intake (233, 323, 324, 326, 336-338), starvation, and malnutrition (23, 336, 339-345). In concert, these observations suggest a role for IGF-I in the maintenance of an adequate GFR.

Feeding high-protein diets to laboratory rats for 2 weeks increases GFR as well as serum and glomerular IGF-I levels compared to rats fed a low-protein diet (233). The GFR cor-

relates with both glomerular and serum IGF-I levels in these animals ($r = 0.67$ and 0.82 , respectively) (233). In 5%-nephrectomized rats in which hyperfiltration in the remnant kidney is well documented (337), high-protein diets also raise the renal tissue levels of IGF-I (320, 346). These findings are consistent with, but do not prove, that the dietary protein-induced rise in endogenous IGF-I may contribute to the up-regulation of GFR.

Long-term continuous subcutaneous administration of

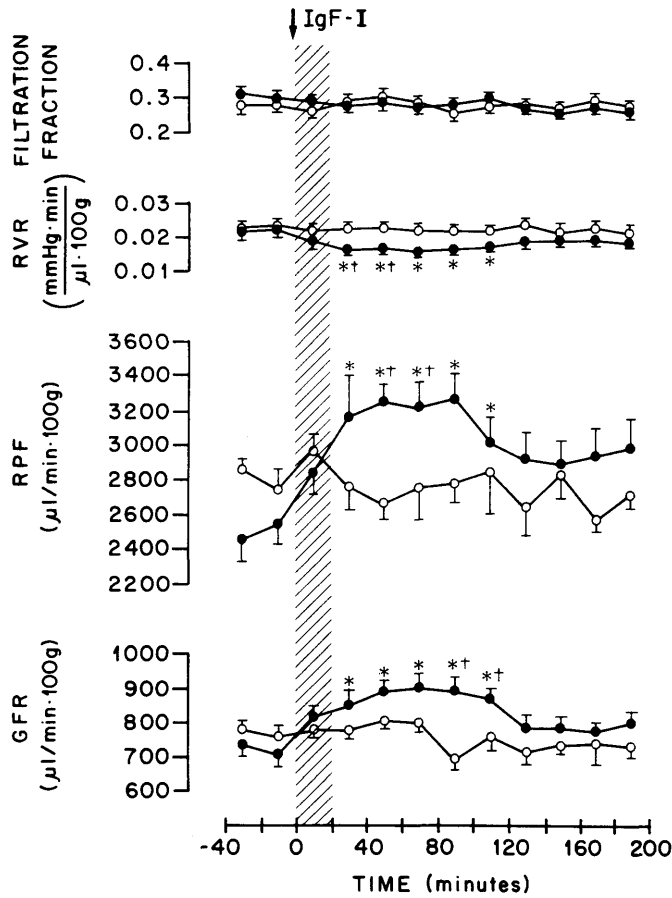


FIG. 6. Effect of an injection (arrow) and 20-min infusion (vertical shaded bar) of rhIGF-I (closed circles) or vehicle (open circles) in normal rats. RVR, Renal vascular resistance; RPF, renal plasma flow; GFR, glomerular filtration rate. [Reprinted with permission from R. Hirschberg and J.D. Kopple: *J Clin Invest* 83:326-330, 1989 (302) by copyright permission of The American Society for Clinical Investigation.]

rhIGF-I to normal rats for 1 week increases serum IGF-I levels by about 90%. Concomitantly, GFR rises significantly, on average by 20% (Fig. 7) (347). In this study, a second group of rats received subcutaneous infusions of a peptidic GHRF receptor inhibitor, N-Ac-Tyr, D-Arg-GHRH-(1-29)-NH₂ (347). This peptide reduces the endogenous pulsatile GH release as well as the serum IGF-I levels (299-301, 347). In this latter group of rats, a decrease in GFR occurred concurrent with a decrease in serum IGF-I levels (Fig. 7). A similar observation was reported by Mulrone *et al.* (299). These studies indicate that manipulations of endogenous IGF-I activity result in expected responses in GFR.

Similar findings were derived from *in vivo* experiments in rats with inherited GH deficiency (314, 346, 348-353). In contrast to appropriate controls with normal GH status, the GH-deficient rats have reduced serum and renal tissue IGF-I levels (314, 346, 348-353). Concomitantly, these animals have significantly reduced GFR (Fig. 8). Chronic low-dose administration of a IGF-I derivative, des(1-3)-IGF-I, restores GFR to normal (353). This study also supports the hypothesis that the reduced GFR in GH deficiency states results, in fact, from reduced serum and/or renal IGF-I activity. These data are

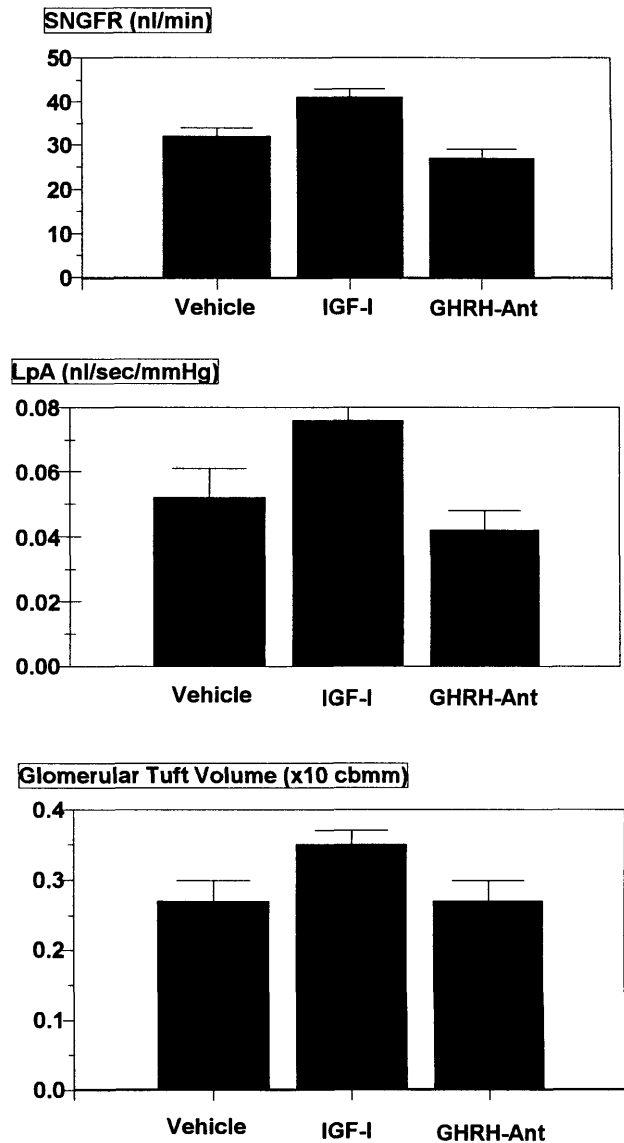


FIG. 7. Single nephron GFR (SNGFR), glomerular ultrafiltration coefficient (LpA) and glomerular tuft volume in normal rats treated with vehicle, IGF-I, or a peptidic GH releasing hormone antagonist by subcutaneous infusion for one week. [Data derived from (347).]

further confirmed by an “experiment of nature”; namely, observations in Laron-type dwarfs, in whom GH receptors are defective and serum IGF-I levels are low. Their creatinine clearance is reduced but is increased toward normal during treatment with exogenous rhIGF-I (354).

C. Effects of rhIGF-I on renal hemodynamics in normal humans

In vivo experiments in rats are useful to demonstrate biological principals or answer mechanistic questions, but proof must be provided that similar effects occur in man. Guler and associates (355, 356), in two small studies, were the first to demonstrate that rhIGF-I raises GFR in normal subjects. These investigators infused rhIGF-I subcutaneously for 3 days into two normal volunteers and measured the 24-h

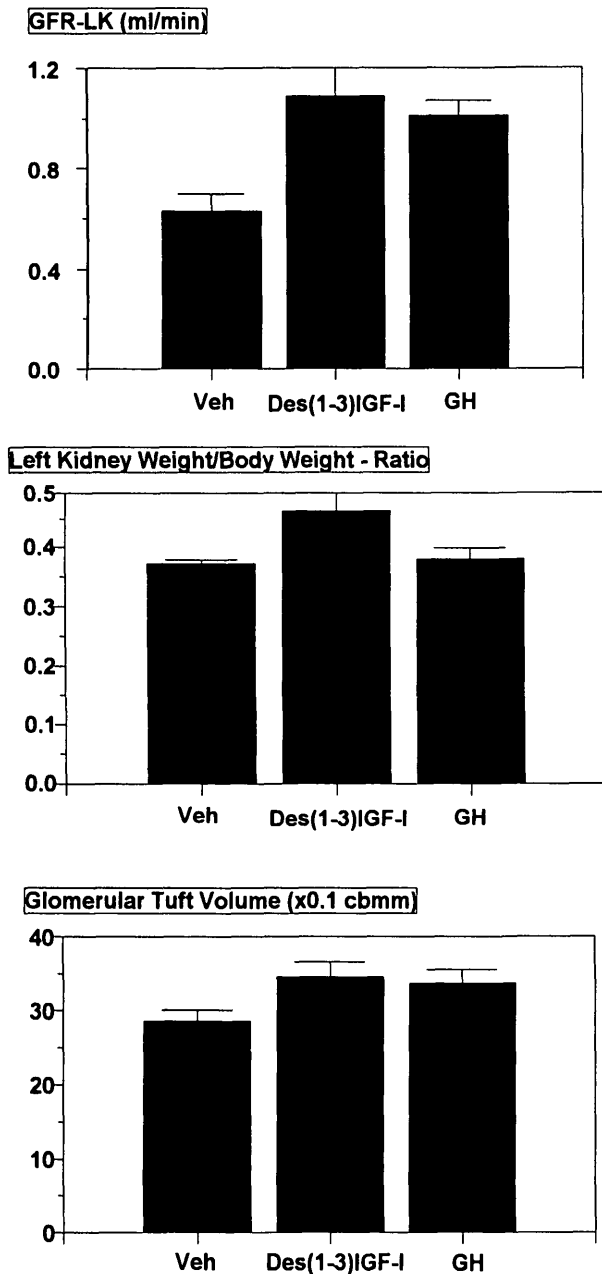


FIG. 8. GFR of the left kidney (GFR-LK), the ratio of left kidney weight/body weight, and the glomerular tuft volume in GH-deficient *dw/dw* dwarf rats after treatment with vehicle, subcutaneous infusions of des(1-3)IGF-I, or GH for 1 week. [Data derived from (353).]

creatinine clearance (355). The creatinine clearance increased during the period of IGF-I infusion and returned to baseline after cessation of treatment with IGF-I (355). In a separate study, Guler and associates (356) measured the GFR and RPF with iothalamate and iodohippurate clearances before, during, and after administration of rhIGF-I for 5 days. In this latter study, the authors also found an increase in renal perfusion and glomerular filtration.

In studies from this laboratory, eight normal subjects underwent repetitive inulin- and PAH-clearance measurements to determine RPF, GFR, and the renal vascular resistance for 5 consecutive days (237) (Fig. 9). Clearances

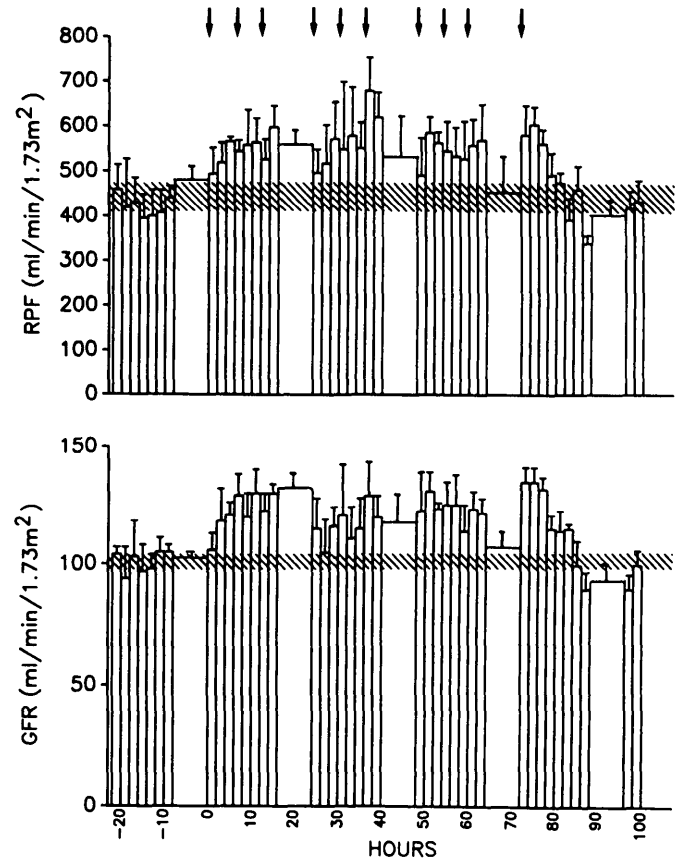


FIG. 9. Effect of subcutaneous injections of IGF-I ($60 \mu\text{g}/\text{kg}$ three times daily) in four normal subjects for 3.5 days on renal plasma flow (RPF) and GFR. Subjects received a constant infusion of saline ($50 \text{ ml}/\text{h}$) throughout the study. Each bar represents the mean (\pm SEM) of a 2-h clearance period. Wide vertical bars represent 8-h clearance periods collected overnight. The shaded horizontal bar indicates the grand mean ± 1 SD of baseline values. Arrows indicate each IGF-I injection. [Reproduced with permission from R. Hirschberg *et al.*: *Kidney Int* 43:387-397, 1993 (237).]

were measured during eight 2-h periods and one 8-h period each day. After baseline measurements, subjects were injected with rhIGF-I, $60 \mu\text{g}/\text{kg}$, three times daily for 3 consecutive days. By 6 h after onset of the IGF-I treatment, RPF and GFR had increased and remained elevated throughout the 3 days of treatment with rhIGF-I (Fig. 9) (237). Over the subsequent 24 h following cessation of the treatment, RPF and GFR, as well as the serum IGF-I levels, decreased toward baseline. A rapid rise in GFR and RPF during a short-term (3-h) infusion of IGF-I to triple the serum IGF-I levels was also demonstrated in eight normal subjects by Giordano and DeFronzo (357). These findings clearly indicate that the effects of exogenous IGF-I on renal function that were previously demonstrated in rats occur similarly in humans. It is reasonable to assume that the mechanisms through which IGF-I raises renal hemodynamics in man are similar to those observed in rats.

D. Physiological mechanisms of the IGF-I-induced rise in renal hemodynamics

To examine the physiological determinants of nephron ultrafiltration through which IGF-I affects glomerular he-

modynamics, studies were performed in rats using micropuncture techniques. The nephron micropuncture studies were conducted in normal fasted and nonfasted rats that received intravenously an acute injection and short-term infusion of rhIGF-I or vehicle. In both the fasted and nonfasted animals, IGF-I induced a fall in the efferent arteriolar resistance, and there was a trend toward reduced afferent arteriolar resistance (Fig. 10). Since the systemic blood pressure remained unchanged during the IGF-I infusion, the decrease in arteriolar and renal vascular resistance resulted in an increase in the single nephron plasma flow rate of 22% and 16% in the fasted and nonfasted rats, respectively (358). The rise in nephron plasma (and blood) flow rates contributed to the increase in single nephron (and whole kidney) GFR (Fig. 10). However, the magnitude of the rise in nephron ultrafiltration exceeded the increase in plasma flow (358). This latter finding strongly suggests, that in addition to the effect on arteriolar resistance and the rise in single nephron plasma flow, IGF-I must have affected another determinant of nephron ultrafiltration. The glomerular transcapillary hydraulic pressure difference, however, did not change from control values in the animals receiving IGF-I (358). However, IGF-I consistently increased the glomerular ultrafiltration coefficient, LpA, about 2-fold (Fig. 10) (358). The LpA is the product of the glomerular capillary permeability for water and small solutes and the surface area that is available for ultrafiltration. It is not known which of the two parameters was altered by IGF-I, since it is experimentally impossible to separate the two. Most likely, IGF-I relaxed the glomerular mesangium, which resulted in expansion of the capillary surface area. In general, smooth muscle cell-like glomerular

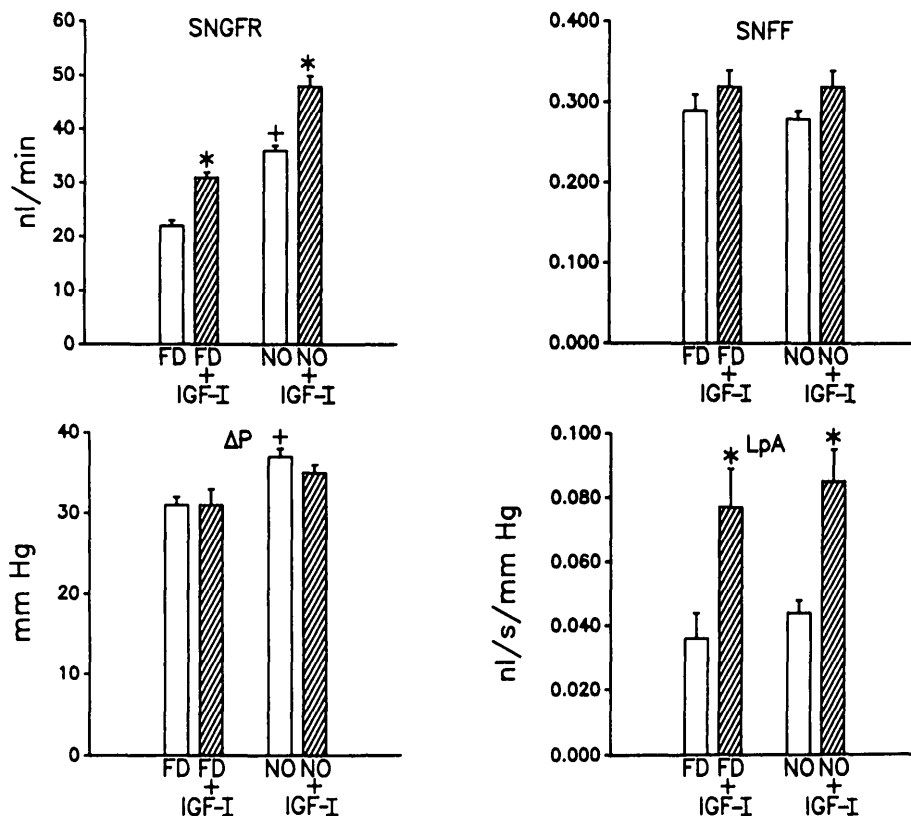
mesangial cells tend to react similarly as vascular smooth muscle cells.

E. Biochemical mediators of the IGF-I-induced effects on renal hemodynamics

There is circumstantial, experimental evidence that suggests that the IGF-I-induced renal arteriolar vasodilation and the rise in LpA are mediated by other autacoids that are quickly induced by IGF-I. Studies in rats receiving IGF-I indicated that the acute rise in GFR may be mediated by cyclooxygenase metabolites such as vasodilating prostaglandins. This was indicated by the finding that concomitant administration of a cyclooxygenase inhibitor blocked the IGF-I-induced rise in GFR (302) as well as in renal blood flow (359). However, these indirect studies do not prove that prostaglandins mediate the IGF-I effects on the renal microvasculature. It is possible that the reduction of vasodilating prostanoids results in a milieu of unbalanced vasoconstrictors that would not allow IGF-I to induce vasodilation.

Angiotensin II, which is synthesized and released in the nephron, acts as a mesangial constrictor, increases the (efferent) arteriolar resistance, and increases the transcapillary hydraulic pressure difference in the glomerulus resulting in glomerular hyperfiltration (360). However, circumstantial evidence suggests that angiotensin II does not mediate the IGF-I-induced effects that cause an increase in glomerular filtration. First, with IGF-I the efferent arteriole vasodilates (358) whereas angiotensin II constricts it (360). Second, in contrast to angiotensin II, IGF-I does not increase the glomerular capillary ultrafiltration pressure. This reasoning is

FIG. 10. Effect of an acute infusion of IGF-I on single nephron hemodynamics in food-deprived or normal rats receiving either vehicle or IGF-I. SNGFR, Single nephron GFR; SNFF, single nephron filtration fraction; ΔP , glomerular transcapillary pressure gradient; LpA, glomerular ultrafiltration coefficient; FD, food deprived; NO, normal controls; open bars, vehicle infusion; shaded bars, IGF-I infusion; *, $P < 0.05$ compared to respective vehicle control; +, $P < 0.05$ compared to food-deprived vehicle control. Note that IGF-I increases SNGFR and LpA, but does not increase ΔP . [Reproduced with permission from R. Hirschberg *et al.*: *J Clin Invest* 87:1200-1206, 1991 (358) by copyright permission of The American Society for Clinical Investigation.]



further corroborated in studies by Haffner and associates (285). These investigators coadministered an angiotensin I-converting enzyme inhibitor and GH for 3 days. GH increased GFR, most likely through IGF-I (*vide supra*), and this effect was not ameliorated by the ACE-inhibitor (285).

Several lines of evidence strongly suggest that nitric oxide (NO), formerly called endothelium-derived relaxation factor, mediates some or all of the effects of IGF-I on glomerular hemodynamics. NO is a powerful, short-lived vasodilator that is released from the vascular endothelium upon stimulation with several vasoactive compounds, such as acetylcholine, bradykinin, histamine, thrombin, substance P, hydralazine, and others (361–366). After release from endothelial cells, nitric oxide appears to act in a paracrine fashion on adjacent and downstream vascular smooth muscle cells in resistance-regulating micro vessels, such as renal arterioles (363–366). Cyclic GMP (cGMP) mediates the intracellular effects of NO on smooth muscle cells (367). Interestingly, urinary excretion of cGMP increases during and shortly after administration of IGF-I in rats, which possibly results from IGF-I-induced NO activity (368).

Further evidence that NO mediates the IGF-I-induced acute hemodynamic response in the kidney was provided by Haylor and associates (359). These investigators infused rhIGF-I acutely into rats that were instrumented with an electromagnetic flow probe to continuously measure renal blood flow (RBF) and renal vascular resistance (359). Even with low infusion rates of IGF-I (2.5 ng/100 g/min), RBF increased and renal vascular resistance decreased significantly. Furthermore, coadministration of the NO synthase inhibitor, *N*^G-nitro-L-arginine methyl ester, completely abolished the IGF-I-induced rise in RBF (359).

Tsukahara *et al.* (369) recently confirmed that IGF-I induces NO synthesis and release by cultured vascular endothelial cells (369). Provision of an IGF-I receptor-neutralizing antibody abolished the NO release upon incubation of the cells with IGF-I, indicating that this action of the peptide is mediated through IGF-I receptors (369). Furthermore, inhibition of receptor autophosphorylation by antiphosphotyrosine antibodies also inhibited the NO release when the cells were stimulated with IGF-I (369).

These sets of experimental data provide significant evidence to postulate an important role of NO to mediate the renal vasodilatory response upon administration of IGF-I. In addition to its effect on the renal microvasculature, NO has been shown to relax cultured mesangial cells *in vitro* (366). Inishi and associates (370) demonstrated *in vitro* that IGF-I, in part, inhibits the cell contraction that occurs upon incubation with angiotensin II in cultured mesangial cells. This experimental finding may provide a basis for the increase in LpA that is observed *in vivo* in rats receiving IGF-I. The lesser state of contraction of mesangial cells would increase the surface area that is available for glomerular ultrafiltration and, hence, would result in a greater LpA. Indeed, as described above, a rise in LpA contributes to the increase in nephron ultrafiltration that occurs during acute administration of IGF-I in the rat (358). In cultured glomerular mesangial cells, insulin also tends to decrease the degree of cell contraction that occurs with angiotensin II (370). *In vivo* in the rat, large doses of insulin during euglycemic clamp increase

nephron hemodynamics (371). Although this is unknown at present, insulin may increase nephron perfusion and filtration through IGF-I receptors to which it has (weaker) affinity.

Glomerular hyperfiltration and, more specifically, chronically increased single nephron GFR in remnant functioning nephrons, were found to contribute to the progression of renal failure in experimental models of chronic renal disease, such as streptozotocin-induced diabetic glomerular sclerosis in the rat, as well as in unilateral or subtotal nephrectomized rats (337, 372–375). Recently, Haylor and co-workers (376) demonstrated in the isolated-perfused remnant kidney from unilateral nephrectomized rats that postnephrectomy renal hyperfiltration can be inhibited acutely when anti-IGF-I-antibody is added to the perfusate. In these studies, a reduction in GFR was also achieved with addition of genistein (a tyrosine kinase inhibitor) or a NO synthase inhibitor to the perfusate (376). This finding would suggest that the renal expression of IGF-I, which is known to be increased in the remnant kidney after contralateral nephrectomy, would mediate the increased GFR. However, caution must be used in the judgement of the validity of these conclusions. First, in the renal disease models in rats that are cited above, the increased nephron filtration rate is largely caused by increased glomerular transcapillary hydraulic pressure. In contrast, IGF-I does not increase the glomerular filtration pressure (358) (Fig. 10). Furthermore, due to the absence of renal nerve innervation and the lack of many hormonal regulators in the isolated-perfused kidney, the renal vascular resistance of this preparation is extremely low, and physiological filtration pressures can only be maintained with very high perfusion rates. Additional physiological experiments may be needed in order to establish a role for IGF-I in nephron hyperfiltration after partial nephrectomy or in experimental diabetic glomerular sclerosis.

In summary, exogenous and endogenous IGF-I raises RPF and GFR by reducing renal arteriolar resistance and increasing the glomerular ultrafiltration coefficient, possibly by relaxing the mesangium. Experimental evidence suggests that these effects of IGF-I are mediated through IGF-I receptors and by induction and release of NO.

F. Hemodynamic effects of IGF-I in nonrenal vascular beds

There is evidence that IGF-I may induce vasodilation with a resulting increase in blood flow systemically and in other vascular beds. However, these effects may occur only with very high dosages of IGF-I and are most likely pharmacological rather than physiological. Elahi and associates (377) infused rhIGF-I intravenously for 4 h in normal subjects while clamping plasma glucose at euglycemic levels. This maneuver resulted in an increase in cardiac output and a reduction in systemic vascular resistance.

Local infusion of rhIGF-I into the brachial artery of normal subjects resulted in a significant increase in blood flow rate through the dependent arm (378). The increase in arm blood flow was 2-fold above baseline after 3 h of intraarterial infusion of IGF-I, 100 ng/kg/min, which is certainly a pharmacological dose. The mechanisms leading to reduction of the vascular resistance in the arm during IGF-I infusion were not delineated in this study. Interestingly, intraarterial in-

sulin infusion also raised local blood flow to a similar extent (378). Thus, at large dosages, IGF-I reduces vascular resistance not only in the kidney but also in other vascular beds.

V. Effects of GH and IGFs on Tubular Function

Many *in vivo* and *in vitro* studies have been performed to examine the effects of GH and IGF-I on tubule cell functions, but very little information is available on the effects of IGF-II on tubules. In this section we will review data on the effects of the three peptides on tubule transport. Effects on tubule hypertrophy will be reviewed separately. Most published data describe changes in the phosphate (Pi) and sodium (Na) and water absorption, and much less information is available on the tubular absorption of other minerals. As described above, IGF-I and IGF-II receptors are expressed in proximal, distal, and collecting tubules in both the apical and basolateral membrane (Fig. 1). GH receptors are expressed in the proximal tubule and in the loop of Henle (*vide supra*).

A. Tubular phosphate (Pi) handling

About three decades ago, Corvilain and associates (278, 379, 380) demonstrated that treatment with GH raises the renal tubular phosphate absorption in normal subjects. Patients with Laron-type (GH receptor defect) dwarfism have reduced serum Pi levels due to renal phosphate losses (354). Treatment of rats with a GH releasing hormone antagonist reduces pulsatile GH activity as well as serum IGF-I levels, which results in reduced renal Pi absorption (299). Several clinical trials that have been performed more recently have found that recombinant human GH administration reduces the urinary phosphate excretion and thus, have confirmed Corvilain's original finding (381–384). GH clearly induces a positive phosphate balance due to enhanced renal phosphate absorption.

IGF-I also reduces renal phosphate excretion when administered to normal subjects. An acute decrease in the fractional urinary excretion of phosphate by about 49% was observed in a group of normal subjects who received a short-term intravenous infusion or rhIGF-I (357). IGF-I was also shown to decrease urinary Pi during a 3-day treatment with rhIGF-I (237). Since not only the absolute excretion but also the fractional renal excretion of phosphate fell in this study to about half the pretreatment values, IGF-I must have increased the tubular absorption of Pi (237) (Fig. 11). The Pi excretion in normal subjects follows a circadian pattern in which the phosphate excretion is reduced during the morning hours and somewhat greater excretion rates of Pi are observed in the afternoon (385, 386). This pattern of pulsatile renal phosphate excretion is believed to result from pulsatile excretion of PTH (385, 387), but not all observations are compatible with this hypothesis (388, 389). This rhythmicity is maintained during treatment with IGF-I except that the circadian undulations of the renal Pi excretion occur around a lesser average (237) (Fig. 11). This finding suggests, but does not prove, that IGF-I increases the renal Pi absorption acutely through a mechanism that is independent of the PTH effects on renal phosphate absorption.

The regulation of the transcellular Pi flux involves alter-

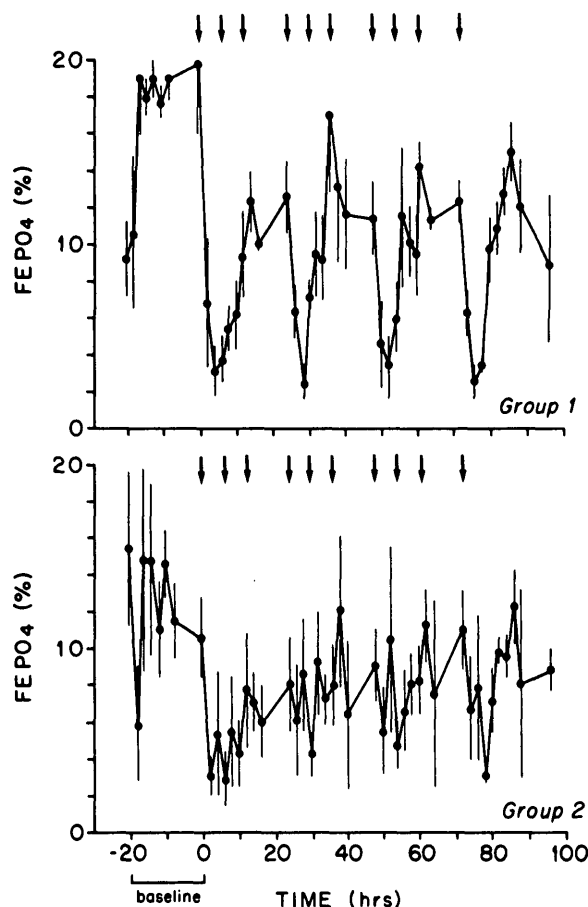


FIG. 11. Effect of treatment with IGF-I (60 $\mu\text{g}/\text{kg}$ three times daily, arrows) on the fractional excretion of phosphate in normal subjects. Subjects ($n = 4$) in group 1 (upper panel) were continuously infused with saline, 50 ml/h, and subjects in group 2 ($n = 4$) received saline, 150 ml/h. Note that the normal circadian rhythm of the phosphate excretion is preserved during treatment with IGF-I. [Data derived from (353).]

ations in the tubule cell's apical Na-Pi cotransport. At least three different cellular control systems that appear to drive this regulation include: inhibition by PTH, increased activity by phosphate depletion, and a IGF-I-dependent increase in the Na-Pi cotransport (390–392). Experimental results obtained in cultured OK cells (opossum kidney cells; a culture model of proximal tubule cells) strongly suggest that distinct mechanisms regulate the IGF-I- and phosphate depletion-induced rise in tubule cell Pi absorption (393).

Many, but not all, effects of GH on target tissues are mediated through IGF-I. For example, GH reduces glucose tolerance whereas IGF-I improves glucose metabolism (382, 394). Several investigators have contributed different *in vivo* and *in vitro* experiments and have provided a rather complete mechanistic understanding of how GH and IGF-I affect renal Pi absorption.

There is now experimental proof that indicates that the GH-induced increase in renal Pi retention is mediated entirely by IGF-I (222, 395–397), and the physiological increase in renal Pi absorption that occurs during growth in children or weanling laboratory rats appears to be mediated by IGF-I, not directly by GH (299, 384, 398–402).

Quigley and Baum (222) perfused isolated rabbit renal proximal tubules with either GH or IGF-I. GH had no effect on phosphate absorption, but IGF-I increased phosphate transport by up to 46%. This effect of IGF-I could be obtained by exposure of the basolateral membrane of the tubule to the peptide but was greater when IGF-I was presented to the apical membrane (222). This latter finding exemplifies the fact that apical IGF-I receptors, although apparently less densely distributed compared to their number in the basolateral membrane (218), are very active in transmitting the increase in tubular phosphate uptake (222). Although IGF-I is present in only minute amounts in tubular fluid under physiological conditions, it may still contribute to the regulation of Pi absorption (222). Moreover, the increased levels of tubular fluid IGF-I that are found in the nephrotic syndrome may further increase the renal phosphate retention under this pathological condition (257).

Further experimental findings also demonstrate that GH does not directly increase tubular Pi transport. In hypophysectomized rats, as compared to controls, renal phosphate absorption is decreased (401). Selective suppression of endogenous pulsatile GH secretion also increases renal phosphate losses (299, 300, 402). Conversely, stimulation of the fractional Pi absorption by the kidney is observed during chronic GH treatment in humans, laboratory animals (278, 379, 381–384, 398), or acromegalic patients (380). Brush border membrane vesicles that were isolated from proximal tubules of young, growing rats display greater Pi uptake than those obtained from adult rats (396, 397), suggesting that the (Na-dependent Pi-) cotransport system is expressed at greater levels in the growing state. Administration of GH acutely affects renal phosphate transport only after a substantial lag time (287). In contrast, administration of IGF-I to rats quickly increases renal tubular Pi absorption (395, 403, 404). The Na-Pi cotransport in isolated brush border membrane vesicles obtained from renal cortex of hypophysectomized rats is selectively activated by IGF-I (395), but other sodium-dependent transport systems such as the glucose or alanine transport are not affected (395).

Although both phosphate depletion and IGF-I (or GH via IGF-I) increase renal phosphate retention through activation of the apical proximal tubule Na-Pi cotransporter, the mechanisms appear to be different (390–392). The increase in Pi uptake in phosphate-deprived rats occurs more quickly than the effect of IGF-I (395). Furthermore, Pi repletion and administration of IGF-I increase the Pi absorption about 5-fold, which is a more than additive effect (395) (Fig. 12).

During Pi deprivation of cultured MDCK cells (a proximal tubule-like cell line), IGF-II mRNA was increased, but this increase occurred several hours after the onset of the increase in Pi absorption, suggesting that IGF-II does not mediate this effect of Pi depletion; rather, both events appear to be epiphenomena (405). In these cells, Pi depletion raises the IGF-II mRNA levels by 77% (405). Furthermore, IGF-I (10^{-8} M) and IGF-II (10^{-7} M) stimulated the Na-dependent Pi uptake. Whether IGF-II plays a physiological role in the increase in renal Pi retention during Pi deprivation during growth is questionable and will require further (*in vivo*) study.

IGF-I is most likely not a key mediator in the increased renal Pi absorption that occurs with Pi depletion or phos-

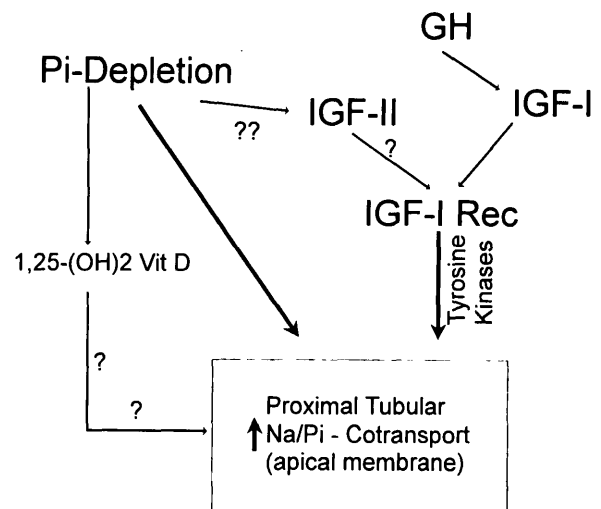


FIG. 12. Proposed algorithm summarizing the mechanisms of renal phosphate homeostasis. ? indicates potential mechanisms that have not clearly been demonstrated in appropriate experiments.

phate deprivation *in vivo* and in tubule epithelial cell cultures *in vitro*. During phosphate restriction, serum, liver, or kidney IGF-I levels are not increased in normal or in hypophysectomized rats (403, 406, 407). Although maintenance of phosphate homeostasis during Pi deprivation does not require the GH-IGF-I system, this system mediates the adaptation to increased phosphate requirements during growth (403). The adaptive response to Pi depletion may, however, require calcitriol, since mice with x-linked hypophosphatemia lost both the adaptive response to Pi depletion as well as the rise in calcitriol levels, but have normal serum, kidney, and liver IGF-I expression (403, 408, 409).

The IGF-I-induced increase in tubular phosphate transport activity is apparently transmitted through IGF-I receptors (101, 396, 405). This can be inferred from experiments from this laboratory that demonstrate that the IGF-I-induced effect on Pi transcellular transport in cultured proximal tubule cells can be blocked with monoclonal anti-IGF-I receptor antibodies (101). Vanadate, a phosphatase inhibitor, simulates tyrosine phosphorylation activity, and the Pi transport that is increased by IGF-I in OK cells is mimicked by vanadate with regard to time course and selectivity (VO_4 also does not raise the Na-alanine cotransport system) (396). In contrast, genistein, a tyrosine kinase inhibitor, blocks the IGF-I-induced rise in Pi uptake in these cells (396).

1. *Calcitriol*. Chronic administration of IGF-I to hypophysectomized rats increased not only the renal Pi absorption but also plasma calcitriol levels (395). Similarly, GH increases serum calcitriol levels (406). However, calcitriol may stimulate or decrease renal Pi absorption (410). Apparently, IGF-I activates the proximal tubular 25-hydroxyvitamin D-1 α -hydroxylase and directly stimulates 1,25(OH) $_2$ -vitamin D synthesis (411, 412). The rise in renal Pi absorption that occurs as an adaptation to phosphate depletion is also associated with a rise in serum 1,25(OH) $_2$ -vitamin D levels (408), but this adaptive response is found in both hypophysectomized and normal rats (401).

In acromegaly, serum 1,25(OH) $_2$ -vitamin D levels and the

levels of (liver derived) 24,25(OH)₂-vitamin D are elevated (413). As pointed out above, the increased 1,25(OH)₂-vitamin D levels result from an IGF-I-induced rise in the renal tubular activity of the respective hydroxylase enzyme (413). Whether the 24-hydroxylation of vitamin D in liver is also increased by IGF-I or is a direct GH effect is currently unknown. Treatment of acromegalics with bromocriptine reduces serum GH and the serum 1,25- and 24,25(OH)₂-vitamin D levels (413). GH treatment in man also increases serum 1,25(OH)₂-vitamin D levels (414). Activated vitamin D may increase the intestinal absorption of calcium (414). However, not all investigators were able to demonstrate this latter effect of GH (415). Although attractive as a hypothesis, it is not yet established whether IGF-I raises renal Pi absorption through calcitriol.

In summary, the increased Pi balance that is observed during juvenile body growth is mediated by IGF-I and GH via IGF-I. IGF-I and possibly IGF-II increase renal tubular phosphate absorption by increasing the expression or activating proximal tubular Na-Pi-cotransporter. These effects of IGF-I are mediated through IGF-I receptors. IGF-I also increases calcitriol production by activating the proximal tubular hydroxylase. The contribution of this latter mechanisms to the IGF-I-induced renal Pi retention is unclear. The increased Pi absorption that is observed during phosphate depletion appears to be independent of GH and IGF-I.

B. Tubular calcium (Ca) handling

Clinical studies and experiments in intact laboratory animals as well as in cultured osteoblast-like cells indicate an anabolic effect of GH and IGF-I on bone. IGF-I and -II and IGF-I receptors are expressed in bone (295, 400, 416-431). The renal handling of calcium is usually inversely correlated to phosphate; *i.e.* increased urinary phosphate excretion is associated with reduced calcium excretion. Thus, one might predict that IGF-I (or GH) may induce calciuresis. Indeed, some experimental hypersomatotrophic states induced by the implantation of GH and PRL-secreting anterior pituitary tissue or tumor cell lines into rats increase urinary calcium excretion (432). However, this effect may be caused by PRL, rather than GH (432).

Nutting and associates (433) examined eight pediatric patients with Duchenne's muscular dystrophy in a metabolic ward before and during treatment with octreotide. As one might have expected, serum GH and IGF-I were reduced during treatment with the somatostatin analog. In these subjects, octreotide markedly lowered the absolute and fractional urinary calcium excretion without a concomitant change in the serum levels of either Ca, Pi, or PTH and with only a minor change in fecal Ca excretion (433). The Ca balance became more positive during treatment with octreotide. These authors argued that octreotide may act directly on bone where somatostatin receptors are expressed (433). However, this treatment apparently acted also directly or indirectly on renal tubules, possibly due to the reduction in systemic GH or IGF-I levels or by reducing renal IGF-I activity, since the fractional excretion of Ca also changed (433). This latter finding could only be explained by a reduction in tubular secretion or, more likely, by an increase in

the tubular absorption of Ca. In both of the above studies, serum PTH levels and the urinary cAMP excretion did not change, suggesting that the effects on calciuresis were not mediated by changes in PTH activity (432, 433).

Treatment of acromegalic patients with bromocriptine reduces serum GH levels as well as urinary Ca excretion (413). However, this treatment may also reduce PRL levels, and PRL may increase the urinary Ca excretion (432). Burstein *et al.* examined a group of GH-deficient children before and during treatment with GH. Although neither serum PTH nor serum Ca levels changed during treatment, urinary cAMP excretion rose, possibly due to an increase in the sensitivity to circulating PTH (418). Thus, the effects of GH and IGF-I on renal calcium excretion are not mediated through changes in PTH, but they may up-regulate renal PTH-receptors.

Possibly, GH and IGF-I affect calcium homeostasis through the IGF-I-induced rise in 1,25-dihydroxyvitamin D. The effect is most likely not acute but requires longer periods of time and would be overlooked in acute studies. Calcitriol may have several long-term effects, such as an increase in gastrointestinal Ca absorption and Ca deposition in bone, but may also reduce the release of PTH. At present, experimental or clinical study data are not available to suggest that such a mechanism is of major importance.

Administration of rhIGF-I, 150 µg/kg, three times daily for 3 consecutive days to normal subjects has only a modest effect on renal Ca excretion (237, 434). In this study the serum Ca levels did not change significantly. The absolute and fractional urinary Ca excretion also did not change dramatically. Since multiple urine collection periods were obtained each day, the circadian rhythms of the urinary Ca excretion could be shown and the rhythmicity was maintained (237, 434). In a subgroup of subjects who received only a small amount of saline maintenance infusion, the Ca excretion tended to decrease transiently during the first day of IGF-I treatment (237, 434). In these subjects, the grand mean of the renal Ca excretion on the first day of treatment with IGF-I was significantly reduced below baseline levels, but not during the subsequent 2 days despite continued IGF-I treatment (237, 434). Takano and associates (43) treated normal subjects with subcutaneous rhIGF-I for 7 days and found no change in the rate of urinary calcium excretion. In a case report by Rubin *et al.* (430), an increase in urinary calcium excretion was found in a patient with Werner syndrome. In this patient the urinary calcium excretion rose with rhIGF-I treatment. Thus, in contrast to the effects of GH and IGF-I on renal phosphate absorption, if any, the effects on the renal handling of calcium are probably less significant compared with other metabolic systems that regulate calcium homeostasis.

C. Tubular sodium (Na) and water handling

Clinical investigators recently became alerted to the possible effect of rhIGF-I on renal sodium and water absorption when they observed edema formation in insulin-resistant type II diabetics during treatment with rhIGF-I. Edema formation was severe enough to cause premature withdrawal of some of the study participants (435, 436). Although edema formation in subjects receiving rhIGF-I and/or GH was observed by other investigators, it was usually of only modest

severity (354, 382). For unknown reasons, type II diabetics with severe insulin resistance may be particularly sensitive to rhIGF-I with regard to edema formation. The dose of rhIGF-I or GH may also play a role. Furthermore, these effects may only be pharmacological and may not play a role in the physiological regulation of water and sodium balance. Recently, further insights into the mechanisms of tubular water and sodium retention in response to IGF-I have been obtained from experimental study.

Bergenstal *et al.* (438) as well as Beck and co-workers (437) reported edema formation in humans when treated with human or monkey GH. Some authors have hypothesized that GH may contribute to fluid retention by activation of the renin-angiotensin system (439) but this is rather speculative and could not be substantiated by animal studies (256). Marcus and colleagues (383, 440) demonstrated a decrease in the urinary Na excretion in elderly subjects receiving rhIGF-I, but this was not associated with edema formation or a significant rise in body weight. Bengtsson *et al.* (381) administered GH for 26 weeks to adults with GH deficiency and one of 10 subjects was prematurely withdrawn from the study because of edema formation. Transient retention of water and NaCl was also observed in GH-deficient subjects who were treated with GH and studied in a metabolic ward (441). The Na retention occurred only during the first 3 days of treatment and amounted to ≤ 61 mmol/day. The increase in sodium retention resolved spontaneously after 3 days despite continued treatment with GH (441).

Kaiser and associates (442) administered GH to a group of elderly malnourished subjects for 3 weeks. In the GH-treated patients, a significant weight gain of 2.2 kg occurred during the 3 weeks of treatment, but this was not associated with clinical edema and may have been caused by anabolism. In a previous case report from this laboratory we examined a GH-deficient adult before and repeatedly during the first week of treatment with GH (289). Although GH caused a significant decrease in the absolute and fractional urinary sodium excretion, this patient did not develop clinically manifest edema. Furthermore, a single pharmacological dose of GH did not cause sodium retention in a group of normal subjects (290). Although not all studies have confirmed that sodium and fluid retention causing edema formation occurs in all or most subjects receiving GH, the matter remains of some concern and should be addressed in further clinical and experimental studies.

As described above, treatment with rhIGF-I has also been associated with edema formation, particularly in a trial to reduce the insulin resistance in type II diabetics (435). Clemmons *et al.* (443) observed mild edema formation during rhIGF-I treatment of normal subjects undergoing controlled catabolism, but catabolism and malnutrition cause edema formation, obviously by IGF-I-independent mechanisms. Caverzasio *et al.* (395) demonstrated reduced urinary sodium excretion in hypophysectomized rats receiving rhIGF-I (395). Laron and Klinger (354) found only a transient rise in NaCl and water retention in patients with Laron-type dwarfism who were treated with rhIGF-I for 9 months.

Giordano and DeFronzo (357) found an approximately 50% decrease in the fractional urinary excretion of sodium in a group of normal subjects receiving intravenous IGF-I for 3 h

(357). In a short-term (3-day) study we treated normal subjects with rhIGF-I (237). Subjects were infused continuously with normal saline, either 50 (n = 4) or 150 ml/h (n = 4). In the subjects receiving the lesser amount of saline, urinary Na excretion was slightly but significantly decreased during the first day of treatment with rhIGF-I and tended (not significantly) to be slightly decreased on the second and third days of treatment (237, 434). These subjects experienced an insignificant body weight gain of 0.2 kg, and none of the subjects developed clinically detectable edema (Fig. 13).

In the saline-loaded subjects one might expect a more significant degree of Na and fluid retention as well as a gain in body water. Indeed, in these latter subjects, a gradual rise in body weight occurred during the 3 days of rhIGF-I administration (237, 434). At the end of the study, the body weight gain averaged 1.3 kg, but frank edema did not develop. In these subjects, the renal Na excretion increased significantly by about 50% during rhIGF-I treatment, and the fractional excretion of Na also tended to rise (237, 434). However, the rise in Na excretion may not have been adequate during IGF-I treatment, since some increase in body weight occurred (Fig. 13). These findings suggest that in normal subjects, IGF-I may reduce somewhat the renal capacity to excrete salt, which may induce frank edema in individuals who have greater salt or fluid intake. Diabetics, particularly those with subclinical or overt diabetic nephropathy, are

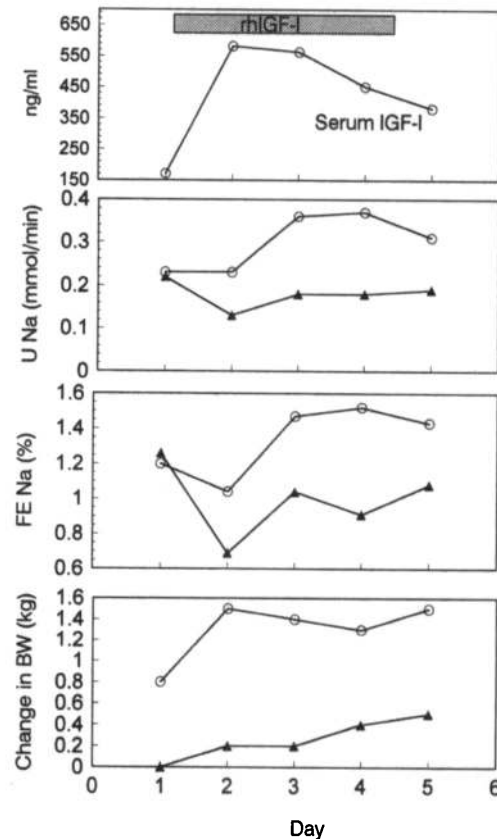


FIG. 13. Effect of exogenous IGF-I on serum IGF-I levels and the absolute (U Na) and fractional (FE Na) urinary excretion of sodium and change in body weight (presumably due to fluid retention) in normal subjects receiving continuous infusions of saline throughout the 5 days of study, either at a rate of 50 ml/h (n = 4, \blacktriangle) or 150 ml/h (n = 4, \circ). [Data derived from (353).]

known to handle Na and fluid challenges with difficulty and are prone to edema formation. Perhaps, this is why rhIGF-I induces sodium retention and edema formation more frequently in diabetic patients.

Although not proven, IGF-I may mediate the effects of GH to reduce urinary Na excretion. The physiological mechanisms are still somewhat unclear, although some experimental data are available to delineate the mediators at the level of the nephron or the tubular cell. The GH and/or IGF-I-induced increase in tubular Na and water reabsorption does not occur in the proximal tubule. This is quite plausibly inferred from studies by Quigley and Baum who perfused rabbit proximal tubules with GH or IGF-I (222). Neither physiological nor pharmacological concentrations of GH affect fluid absorption. This was independent of whether the peptide was presented to the apical or the basolateral membrane (222). As with GH, IGF-I also did not affect proximal tubular water absorption. Since proximal tubular Na absorption is isoosmolar, Na absorption was almost certainly not affected in these experiments, although it was not directly measured (222). IGF-I stimulates the Na-dependent Pi co-transport in the proximal tubule (*vide supra*), but this system does not account for sufficient Na absorption that would cause edema formation and the reduction in urinary Na excretion that was observed in some of the clinical studies.

Gesek and Schoolwerth (444) examined isolated tubules to assess whether IGF-I increases the Na⁺/H⁺ exchange activity. However, neither IGF-I nor IGF-II affected this system. In contrast, insulin stimulated Na uptake through this system, which is localized in the basolateral membrane. These studies suggest, if not convincingly prove, that neither GH nor IGF-I induce Na and water retention through a direct effect on proximal tubules. In contrast to IGF-I, IGF-II has been noted *in vitro* to raise the sodium uptake through a G protein-dependent mechanism in proximal tubule brush border membrane vesicles (445).

The above experimental findings favor either an indirect, systemic mechanism that mediates GH and IGF-I-induced tubular absorption of Na and water and/or suggest the involvement of distal tubular mechanisms. Indeed, the sodium uptake in A6-cells, a distal tubule-derived cell line, has been shown to be stimulated by insulin through a tyrosine kinase activity-requiring mechanism (446). In the toad bladder epithelium, which shares characteristics of the distal tubules, both IGF-I and insulin increase Na uptake, apparently through activation of apical Na channels, rather than through basolateral Na/H antiporters (447). The toad bladder epithelium expresses IGF-I as well as insulin receptors, and the IGF-I effects may be transmitted through either receptor. Interestingly, the toad bladder reacts very sensitively to both IGF-I and insulin. The half-maximal stimulation of the Na uptake occurs at 100 pM of either peptide (447). In the toad bladder, the basolateral IGF-I-induced vectorial sodium transport occurs quickly (in minutes), is sustained for ≥5h, and is blocked by amiloride, which (semi-)specifically blocks the conductive apical Na channel activity. Na channel activation does not require protein synthesis (448).

Recently, Gallego *et al.* (449) examined the effects of IGF-I on the Na transport through amiloride-sensitive apical Na channels in cultured (distal tubular epithelium-like) A6-cells.

Within 5 min, IGF-I (10 nM) increased the Na channel activity about 3-fold. This immediate-early effect of IGF-I on cell Na uptake was associated with autophosphorylation of the IGF-I receptor β-subunit and phosphorylation of IRS-I (449), suggesting that IGF-I increases Na channel conductance through IGF-I receptor activation. However, receptor autophosphorylation and the increase in Na channel activity could certainly be epiphenomena. IGF-I does not activate sodium absorption in cortical collecting ducts (450). In both the studies in toad bladder as well as in A6 cells, IGF-I was presented to the basolateral membrane. Although very suggestive, these studies do not prove that IGF-I increases the Na uptake through apical distal tubular Na channels. Yet, these findings are very suggestive that this is a/the major mechanism through which IGF-I (and GH through IGF-I) increases renal Na and fluid retention, although confirmatory *in vivo* studies may be needed.³

Moller and associates administered GH systemically in a double-blind, placebo-controlled cross-over study to GH-deficient patients and to normal subjects for 2 weeks (456). In this study, the plasma volume, extracellular volume, and plasma vasopressin, renin, angiotensin II, and atrial natriuretic peptide (ANP) levels were measured. Similar to findings by other investigators, water retention with facial edema developed in some of the subjects within 2 to 3 days of treatment but diminished spontaneously thereafter. The extracellular volume increased but the plasma volume did not change (456). Plasma renin, angiotensin II, and vasopressin did not change, and aldosterone tended to decrease during GH treatment as compared to placebo (456). However, plasma atrial natriuretic peptide decreased significantly (although modestly) with administration of GH. These investigators suggested that the GH-induced fall in plasma atrial natriuretic peptide may cause or contribute to the reduced natriuresis that may result in sodium and fluid retention and edema formation. Whether these GH effects are mediated through IGF-I remains to be determined. Although this decrease in ANP levels may contribute to the Na retention during GH (or rhIGF-I) treatment, the direct tubule effects of IGF-I on Na reabsorption through increased sodium channel activity seems to be of greater importance.

In summary, clinical studies suggest that (pharmacological) administration of GH hormone and IGF-I causes edema formation, which is transient in most normal subjects, but may be more severe in some diabetics. Experimental studies

³ It may be of interest to note that epidermal growth factor (EGF), when administered systemically to sheep, causes polyuria due to natriuresis (451, 452). Atrial natriuretic peptide was not responsible for this change since plasma levels tended to be decreased, rather than increased. EGF receptors are expressed in collecting tubules and EGF synthesis and apical secretion occur in distal convoluted tubules in the rat (234, 453, 454). Possibly, EGF that is secreted into the tubule lumen in distal convoluted tubules acts further downstream. Pre-Pro-EGF has been demonstrated in urine (454, 455). Apparently, basolateral, but possibly not luminal, EGF decreases the luminal Na absorption through a quabain-sensitive mechanism (450). Moreover, EGF induces IGF-I synthesis in collecting ducts (85). Whether tubule fluid EGF has any physiological function on the apical membrane is unclear. A detailed discussion of EGF is not intended in this review. However, growth factors may be involved in the renal regulation of sodium homeostasis either increasing (EGF) or decreasing (IGF-I) urinary sodium excretion.

suggest that IGF-I may activate distal tubular Na channels, which could account for increased distal tubular Na and fluid absorption.

D. Renal regulation of acid-base homeostasis

Neither GH nor IGF-I affect proximal tubular bicarbonate reabsorption (222). However, GH increases the production of ammonia and glucose from L-glutamine substrate *in vitro* in proximal tubule segments or in cultured cells (457–459). Ammonia is secreted into the tubular lumen to bind protons and maintain an appropriate proton gradient that facilitates proton secretion. In cultured proximal tubule-like cells GH accelerates glycolysis, and the resulting lactate is converted to bicarbonate whereas the proton is secreted and undergoes urinary excretion, in part as NH_4^+ . Thus, GH may facilitate the renal response to acidosis, such as during exercise. Acidosis may contribute to an increase in serum GH levels (460), but the increase in serum GH and IGF-I levels during exercise may be independent of the exercise-induced lactic acidosis (460–463). However, during exercise, GH may contribute to the renal compensation of acidosis. GH may also contribute to renal acid base regulation through enhanced distal tubular Na absorption, which favors distal nephron proton secretion.

In chronic metabolic acidosis in the rat, serum IGF-I levels are reduced (464), and liver IGFBP-1 and -2 mRNAs are increased. However, in serum, only the IGFBP-1 levels are elevated whereas IGFBP-2 values are decreased (464). It is not known whether similar changes also occur in renal tubules and whether the IGF system is involved in the regulatory renal adaptation to acidosis.

VI. GH and the IGF System in Renal Development

During development of the embryo, three successive excretory organs appear, the pronephros, the mesonephros and the metanephros, all of which arise from the mesodermal nephrogenic cord. The first two organs are only transient, but the metanephros develops into the definitive kidney (465). Nephron formation in the metanephros begins at about the eighth week with small foci of condensed mesenchyme near the ureteric bud. This mass of cells elongates to form the S-shaped body, the lower portion of which develops into the glomerulus. The remaining parts of the S-shaped body give rise to the proximal, loop, and distal convoluted segments of the nephron (466, 467). Metanephric nephrons may begin functioning as early as the eleventh week of gestation. Postpartum, further development of the nephron occurs during the subsequent 3–4 weeks in rabbits (465). The GFR in newborn rats is low, about 0.045 ml/min/g kidney and rises steadily to reach the adult filtration rate of about 1.0 ml/min/g at age 40 days (465). Single nephron filtration rates of superficial nephrons were examined by Horster and Valtin in dogs (468). The single nephron GFR in puppies on day 21 after birth was 3.2 nl/min and rose 7-fold to 23 nl/min by day 77 (468).

The formation of the metanephric kidney is highly regulated through sequential and timed expression of growth factors such as IGF-I, IGF-II, EGF, $\text{TGF}\alpha$, $\text{TGF}\beta$, PDGF, EGF, and others in association with their respective receptors

(469–474). Mouse metanephroi in organ culture become enlarged if exposed *in vitro* to IGF-I (475), and the nephron population increases. In fact, IGF-I is necessary for normal metanephric development (236, 469), and IGF-I antisense oligonucleotide causes growth retardation (475). Similarly, the growth and development of cultured rat metanephroi are inhibited by neutralizing antibodies to IGF-I, IGF-II, and IGF-II receptors as well as by anti- $\text{TGF}\alpha$ (471, 472).

Avner and Seoney (476) examined the effects of EGF and $\text{TGF}\beta$ on organotypic renal growth in murine metanephric organ cultures. EGF induces overall growth and augments the differentiation of distal and collecting tubules but retards the differentiation of glomeruli and proximal tubules (476). $\text{TGF}\beta$ retards nephrogenesis (476). This may suggest a counter regulatory role of EGF and $\text{TGF}\beta$ to the effects of IGF-I in metanephric development. Indeed, $\text{TGF}\beta$ mRNA is expressed in kidneys from 13-day-old rat embryos, and metanephroi contain $\text{TGF}\beta$ protein (473). Exogenous $\text{TGF}\beta$ inhibits tubulogenesis in rat metanephric cultures but does not inhibit the synthesis of IGF-I and IGF-II (473), suggesting a direct effect of each of the growth factors rather than through induction or inhibition of each other. $\text{TGF}\beta$ inhibition with neutralizing antibodies improves tubulogenesis (473).

In the above experiments the respective investigators examined the effects of the IGF system on metanephros development after its initial induction. Weller and associates (477) investigated whether IGF-I or -II can induce embryonic kidney mesenchyme to differentiate into epithelium or interstitial cells, as occurs *in vivo* by the effects of inducer tissues. However, neither IGF-I nor IGF-II could mimic this effect of inducer tissues in the *in vitro* model (477). These findings indicate the dependence of the development of the metanephros but not its induction on the IGF-system (478).

IGF-I receptors also become expressed during metanephric development (478, 479). Liu and associates (479) examined the expression and role of the IGF-I receptor in the *in vitro* development of metanephroi obtained from mouse embryos at days 9–13 of gestation and maintained in organ culture. IGF-I receptors were expressed at greatest density on day 13, and their numbers decreased thereafter, but the receptors were still present until after birth (479). The functional importance of the IGF-I receptor was also demonstrated by these investigators in antisense oligodeoxynucleotide experiments. The inhibition of IGF-I receptor expression in murine metanephroi resulted in inhibition of kidney growth, reduction in nephron number, and disorganization of ureteric bud branching (66, 479). As with the inhibition of IGF-I receptor expression, the reduction in IGF-I gene transcription also alters metanephros development. Inhibition of IGF-I by the addition of antisense-oligodeoxynucleotide to the culture medium inhibits both metanephric development as well as the expression of extracellular matrix proteoglycans (475). Both can be reversed by addition of IGF-I to the culture medium (475). Since the sequential effects of IGF-I on metanephros size and nephron number correlate with increased extracellular matrix synthesis, the latter is possibly a prerequisite for accelerated metanephros development by IGF-I (475). These experiments, in concert, suggest that IGF-I acts through IGF-I receptors during metanephros development,

and the effects of IGF-I are regulated through transcriptional regulation of both the receptor and the peptide.

Both IGF-I and IGF-II are expressed by the embryonic kidney. Although the regulation of the IGF-gene expression is not well understood (236, 469), there is evidence for unique regulation of IGF-I gene transcription in the kidney (480). During kidney development, IGF-II is greatly expressed, in particular by the glomerular progenitor cells of the S-shaped body. IGF-II is also found in the medullary interstitium in association with IGFBP-2 and IGF-I as well as IGF-II receptors (185, 481). In the sheep, IGF-II mRNA is also expressed at high levels in the fetal kidney (482). Interestingly, the renal IGF-II expression does not decrease postpartum, whereas it decreases in most other organs (482). *In vitro* assay measurements of IGF-II levels after extraction of the peptide from fetal kidneys have confirmed the high renal levels, which are only surpassed by the IGF-II concentration in the pituitary (483). IGF-I has also been demonstrated in the fetal kidney, and renal as well as serum levels of IGF-I in fetal pigs are raised by maternal diabetes.

Latimer and associates (484) examined the IGF-I and -II levels in fetal pig tissues after hypophysectomy of the fetus with or without T_4 replacement. Hypophysectomy reduced IGF-I as well as IGF-II expression in the fetal pig kidney. In hypophysectomized fetal pigs, the serum T_4 levels, as well as the serum IGF-I, IGFBP-1 and IGFBP-2 levels, were decreased but IGFBP-4 was elevated. Administration of T_4 did not affect kidney IGF-levels but increased serum IGF-I (although not IGF-II), IGFBP-1, -2, and -4 levels (484). In human fetuses (weeks 14–18 of gestation) IGFBP-4, -5, and -6 mRNA is found in most tissues including the kidney by Northern blot analysis as well as by *in situ* hybridization. IGFBP-4 mRNA is particularly abundant in the developing human fetal kidney (485, 486).

The importance of the IGF-I receptor during metanephros development has been stressed above (66, 236, 470, 472, 479, 487). IGF-II receptors are also found in fetal rat tissues (day 19 postgestation) including the kidneys (104, 105). Similarly, serum levels of soluble IGF-II receptors are also high. Both tissue expression and serum levels of the IGF-II receptor decrease sharply in the rat after birth (105, 107).

GH receptors are more abundantly expressed in the fetal (rabbit) kidney compared to all other organs, suggesting an important role of GH during kidney development (24). The GH receptor expression in the kidney remains relatively high during the postnatal period. The expression of this protein in heart, liver, and muscle increases after birth during the following several months (24). In different fetal rabbit tissues the locations and levels of IGF-I mRNA do not covary well with the respective tissue GH receptor mRNA expression, suggesting that during fetal development, IGF-I may not be primarily under GH control (24).

Postnatally, several changes occur in the renal IGF-system. IGF-II receptor levels fall sharply at birth (105). IGF-II receptor levels were examined by Ballesteros *et al.* in fetal (day 21 of gestation) and newborn (day 2–42) rats (488). In the kidney as well as in other tissues, the IGF-II mRNA levels are greatest during gestation and decrease rapidly after birth. However, in adult humans, IGF-II serum levels are greater than IGF-I levels, suggesting that the finding in rats may not

be comparable to humans. Postnatal kidney growth appears to be modestly pituitary dependent (489). In hypophysectomized neonatal rats, IGF-I and IGF-II levels in serum fall quickly and liver IGF-II mRNA levels also decrease. IGFBP-2 increases in postnatal hypophysectomized rats compared to control counterparts with intact pituitaries (489). In the newborn rat, IGFBP-3 serum levels are very low at birth and increase subsequently. This rise is apparently (GH? and) pituitary-dependent, since postnatal hypophysectomy prevents much of this rise in serum IGFBP-2 (489).

In sheep, renal IGF-II and IGFBP-2 mRNA levels decline during late gestation (486). However, the expression of IGFBP-2 in liver as well as plasma IGFBP-2 levels increase during late gestation and after birth, suggesting that liver synthesis defines the serum IGFBP-2 levels (486). Funk *et al.* (490) examined the levels of IGFBPs by Western ligand blot of extracts from human fetal and newborn tissues from week 23 of gestation until 2 yr of age. In the kidney of a 3-month-old infant, ligand blotting demonstrated the presence of IGFBP-3 and a 30-kDa IGFBP, but IGFBP-2 was absent (490). In muscle, the 30-kDa IGFBP and small amounts of IGFBP-3 were present throughout gestation (20–41 weeks) and persisted after birth for at least 2 yr. In contrast, IGFBP-2 was present between 20 and 26 weeks of gestation, but not in the postnatal period (up to 2 yr of age). Unfortunately, serum IGFBP levels were not examined in this study (490).

In neonatal IGF-II transgenic mice, serum IGF-II levels are increased about 2- to 3-fold above nontransgenic control mice (179). In the transgenic mice, IGFBP-2 levels are also increased, but IGF-I in serum is reduced, possibly by competitive displacement from binding proteins. Overexpression of IGF-II had no significant effect on body growth or growth of most organs but increased kidney weight significantly (179).

In pigs, IGF-I and IGFBP-3 serum levels are also increased during fetal life (491) and increase even further after birth. However, kidney IGF-I and IGFBP-3 mRNA levels do not increase after birth, despite an increment in GH receptor expression. IGF-II levels in serum are greater than those of IGF-I before as well as after birth in the piglet (491). IGF-II mRNA levels in kidney decline after birth. In these animals, serum IGFBP-2 increases during late gestation and decline postpartally (491). IGF-II and IGFBP-2 serum levels also decrease in sheep after birth (486).

The importance of GH and IGFs for the renal development may be questioned. However, there is evidence that genetic deficiency in IGF-II, for example, reduces prenatal body and organ growth (492). To the authors' knowledge there has been no report of overt developmental renal abnormalities in GH- or IGF-deficient mice or rats, or in patients with Laron-type dwarfism. However, no study has examined in detail aspects of renal anatomy and function, such as nephron number and size, and concentrating and diluting capacities and others. Furthermore, a deficiency of one factor is often compensated for by other regulatory principles, and the pure lack of an obvious defect may not disprove that GH and IGFs are regulators during normal renal development.

VII. GH and IGFs in Renal (Compensatory) Growth (Tables 2 and 3)

In humans as well as in laboratory animals, nephron size and function adjust to chronic demand. After unilateral or subtotal nephrectomy, the remaining intact nephrons undergo growth that may be largely hypertrophic but also includes some hyperplasia. This compensatory renal growth may maintain greater levels of renal function, but there has been some concern, largely derived from studies in the rat, that nephron hypertrophy may induce progressive glomerular sclerosis, interstitial fibrosis, and tubule atrophy, and hence, accelerate the rate of progression of renal failure (493–497). In such states of loss of renal mass, which may be induced by experimental surgery or by renal disease, remnant functional nephrons also adapt their nephron ultrafiltration rate. In such kidneys, single nephron filtration rate increases, largely due to a rise in the glomerular capillary hydraulic pressure. Glomerular hypertension apparently contributes to the development of premature glomerular sclerosis and progressive renal failure (337, 372, 374, 375). For more than 10 yr, the mechanisms that induce these adaptive responses to loss of functional renal mass, namely compensatory renal growth and glomerular hyperfiltration, have undergone intense study. Some evidence has arisen indicating that GH and IGF-I may be involved in these adaptive mechanisms, particularly with regard to the induction of renal/nephron hypertrophy.

Most research examining the involvement and mechanisms of GH and the IGF system in renal compensatory growth has been performed in animal models such as exogenous administration of GH and IGF-I to rats, unilateral nephrectomy, subtotal nephrectomy, streptozotocin-induced diabetes in rats, feeding high-protein diets to rats, and

transgenic mice expressing GH or IGF-I transgenes. At present, there has been virtually no study to examine the relationship of the renal IGF-system with kidney growth in humans. In the following paragraphs we will review the most pertinent work that has been performed using different rat models. As will be seen, there are many similarities between different models, such as the transient increase in renal extractable IGF-I that lasts for 4 to 7 days and precedes the onset of increase in renal mass (498). However, some discrepancies in the findings from different laboratories will also be pointed out, such as the question of whether the increase in renal IGF-I reflects local synthesis or trapping of circulating IGF-I, and whether there are differences between immature and adult rats with regard to the involvement of GH and IGF-I in compensatory renal growth.

A. Renal growth caused by systemically elevated GH and IGF-I

Administration of rhIGF-I to rats does not cause major body growth (although weight gain due to anabolism is achieved) but induces selective growth (hypertrophy and/or hyperplasia) of certain organs, most notably the kidneys (499). There is ample evidence that elevated levels of circulating GH, whether caused by exogenous or endogenous means, also cause renal growth. GH administration was shown to induce a gain in renal mass that may be mediated by (systemic or local) IGF-I (500). In fact, administration of GH increases the hepatic and renal IGF-I mRNA levels but does not affect the renal levels of IGF-I receptor mRNA (198). As a result, GH induces a local renal as well as systemic increase in serum IGF-I levels. Since in rats the renal IGF-I mRNA is confined to certain tubular segments but may not

TABLE 2. IGF-1 in different rat models of compensatory renal growth

Model	Compensatory growth	Extractable renal IGF-1	Renal IGF-1 mRNA	Serum IGF-1	Hepatic IGF-1	Reference
Potassium depletion	Hypertrophy and hyperplasia	↑ (d 1–7) ^a	↓	↔	↔	(554)
High protein diet → Uninephrectomy	Hypertrophy and hyperplasia	↑ (d 4)	Not done	↔	↔	(320)
High protein diet Uninephrectomy	Hyperplasia	Not done	↑ (d 1–2; MTAL)	Not done	↔	(229)
	↑ Renal weight	↑ (d 5)	Not done	↔ (d 5)	↔ (d 5)	(523)
		↔ (d 1)				
Uninephrectomy	↑ Renal weight	↑ (d 4)	↑ (d 1–7)	↔	↔	(522)
Uninephrectomy	↑ Renal weight	↑ (d 5–14) ^a	↔	↔	Not done	(532)
Uninephrectomy (immature/adult)	↑ Renal weight	Not done	↔ (d 1–2, adult)	Not done	Not done	(529)
			↑ (d 1–2, immature)			
Subtotal nephrectomy	Hypertrophy and hyperplasia	↑ (peak d 7, 90, 150)				(319)
GH-deficient + Uninephrectomy	Hypertrophy and hyperplasia	↑	Not done	↔	↔	(346)
Uninephrectomy	Hypertrophy and hyperplasia	↑ (d 1–4)	Not done	↓	Not done	(330)
Streptozotocin-induced diabetes	↑ Renal weight	↑ (d 2–4)	↔	↓ d 2	←	(537)
Streptozotocin diabetes (pre and post pubertal)	↑ Renal weight	Not done	↑ (d 1–2; post-pub)	↔ d 4	↔ (d 1, mRNA)	(538)
		(postpubertal)	↔ (prepubertal)	(postpub)	↓ (d 7, mRNA)	
		↑ (d 1–2; prepubertal)		↑ (prepubertal)		

MTAL, Medullary thick ascending limb; d, day of experimental intervention.

^a ↑ immunostaining of medullary collecting duct.

be expressed in glomeruli and glomeruli may not display GH receptors (Fig. 1), the GH-induced increase in glomerular size is most likely mediated by circulating IGF-I (198). Mice that over express GH endogenously due to expression of a (bovine) GH transgene also have increased kidney and glomerular size (207, 501–507). Similarly, transgenic mice overexpressing IGF-I have increased kidney and nephron size (508).

Studies in this laboratory have shown that similar degrees of renal growth (and an increase in GFR) can be induced in GH-deficient rats by administration of either GH or IGF-I (353), suggesting that IGF-I can mimic renal GH effects and may mediate much of the effects of GH on renal growth. Acromegalic patients have increased renal size (277, 291–293, 295, 509, 510), which is reduced by a reduction in GH secretion with either surgery or somatostatin.

The effects of exogenous GH or IGF-I on renal growth were also examined by Mehls *et al.* (511) who administered each of these peptides to female rats for up to 2 months. GH stimulated body growth as well as renal mass, whereas IGF-I raised renal mass more selectively. Several indices such as increased kidney DNA/protein ratio, mitosis, and increased proliferating cell nuclear antigen expression suggested the presence of hyperplasia in addition to hypertrophy in response to treatment with GH or IGF-I (511). Increased proliferation that contributes to renal growth was also demonstrated in mice transgenic for bovine GH (512) and is found in cultured glomerular cells when exposed to IGF-I (208, 210, 513, 514). However, other renal cells such as proximal tubule cells undergo hypertrophy, rather than hyperplasia, when exposed to IGF-I in culture (515). In adult rats, hypertrophy probably predominates, but hyperplasia in glomeruli and interstitium, especially in young rats, contributes significantly to the GH or IGF-I induced renal growth (516, 517). Maneuvers that prevent the increase in renal and systemic IGF-I that would normally result from administration of GH seem to prevent at least in part, the GH-induced renal growth. Flyvbjerg and associates (332) treated rats concomitantly with GH and octreotide, a somatostatin analog. Octreotide partially prevented the increase in extractable and immunoassayable renal IGF-I as well as renal growth.

GH deficiency causes reduced renal mass. In rats, hypophysectomy decreases renal tubular IGF-I mRNA levels and increases IGFBP-1 mRNA and administration of GH reverses these changes (198). However, hypophysectomy causes also other hormonal disturbances, and the reduced kidney (and body) size in hypophysectomized rats is more completely restored when GH and T_4 are both replaced.⁴ Administration of GH to hypophysectomized rats also increases the renal tubular expression of EGF, suggesting that some of the GH-induced nephron hypertrophy is mediated by EGF (235). Dwarf rats that are selectively deficient for GH have reduced renal mass and glomerular tuft volume compared to normal rats and both can be normalized by administration of GH or IGF-I (353).

⁴ In rats T_4 increases renal EGF-expression in distal tubules and collecting ducts as well as the extractable renal IGF-I (500, 518) and may contribute to renal growth through this mechanism. Since there is evidence for EGF to increase diuresis (452), reduced renal EGF levels in hypothyroidism may mediate the fluid retention in this condition.

In summary, increased GH levels due to increased endogenous secretion or pharmacological administration causes an increase in renal mass. Possibly, although unproven, GH induces these changes in part directly since its receptor is expressed in parts of the nephron (Fig. 1). Some of these effects of GH are mediated through increased renal IGF-I synthesis. However, the GH-induced growth of glomeruli cannot be explained by either direct effects of GH or by a local increase in IGF-I, since GH receptors and IGF-I mRNA have not been consistently demonstrated in (rat) glomeruli. Hence, the systemic induction of IGF-I by GH most likely mediates much of the GH effects on glomerular hypertrophy and may contribute to the GH-induced tubular growth.

B. Unilateral nephrectomy

Renal hypertrophy that is induced by unilateral nephrectomy is unique with regard to early transcriptional mechanisms that precede the onset of size increment (519). The cell protein accumulation in compensatory renal hypertrophy is regulated further by posttranscriptional mechanisms (515). There is circumstantial evidence that IGF-I and possibly GH (directly or via IGF-I) is involved in both the transcriptional as well as posttranscriptional regulation of compensatory renal growth. IGF-II may also be involved, primarily in weanling rats. Furthermore, other growth factors, such as EGF, also play some role in compensatory renal hypertrophy (454, 520, 521).

Fagin and Melmed (522) reported that IGF-I mRNA and peptide levels increase in remnant kidneys after experimental unilateral nephrectomy in rats, and this rise in renal IGF-I expression precedes the onset of hypertrophy. However, these investigators noted that the increased expression of IGF-I occurs only for the initial 7 days and then the levels decline to normal control ranges, despite the fact that the hypertrophic state of the remnant kidney is sustained for several months in this animal model (522).

Unilateral nephrectomy in rats was also shown to cause a rapid increase in extractable and immunoassayable IGF-I in the contralateral kidney that preceded the increase in renal mass. The renal tissue levels of IGF-I peaked at about 24 h and declined to baseline by day 4 (330). Flyvbjerg *et al.* (330) also observed a moderate decrease in serum IGF-I levels after unilateral nephrectomy, but this finding was not confirmed by other investigators and may reflect the postsurgical state rather than an effect of the reduction in renal mass *per se*. Evan *et al.* found that the renal levels of IGF-I and IGF-I mRNA were elevated on day 5 after nephrectomy, but in contrast to other investigators, these authors also indicated that the levels remained elevated by day 33. Prolonged (≤ 30 days) elevation of extractable renal IGF-I in unilaterally nephrectomized rats was also described by Stiles *et al.* (523). The reason for this discrepancy is unclear.

Hise *et al.* (524) demonstrated increased specific binding of IGF-I to glomerular membranes and basolateral tubular membranes *ex vivo* 1 month after unilateral nephrectomy in rats. Affinity labeling studies suggested an increase in IGF-I receptors as well as membrane-associated IGFBP-5. These changes in the IGF-I system occurred concomitantly with glomerular and proximal tubular hypertrophy. These find-

ings suggest that at this later stage after nephrectomy IGF-I may be trapped from the circulation and bound to IGF-I receptors and IGFBP-5 and may contribute to hypertrophy of the proximal part of the nephron (524).

At present, it is not known what signal mechanism induces a contralateral increase in the expression of IGF-I and renal compensatory growth after unilateral nephrectomy. GH has been thought to be involved as a systemic mediator of renal hypertrophy after reduction in renal mass. Haramati and associates (525) examined the pulsatile GH release at 24 and 48 h after unilateral nephrectomy in adult rats. At each time point multiple sequential serum GH measurements were performed. These investigators demonstrated an increase in pulsatile GH release early (24 h) after unilateral nephrectomy. At 48 h, the pulsatile GH release was somewhat decreased (525). The increased GH secretion at 24 h was associated with a rapid early rise in kidney mass. This initial rapid rate of compensatory renal growth of the contralateral kidney was somewhat reduced at 48 h, paralleling the reduced rate of pulsatile GH release at this later time. Administration of a GHRF antagonist reduced both the pulsatile GH release as well as the rate of compensatory renal growth (525). This experimental finding supports the hypothesis that GH plays a regulating or permissive role for the remnant kidney hypertrophic response after partial nephrectomy.

The mechanisms that cause compensatory contralateral renal growth after unilateral nephrectomy seem to be different in juvenile rats compared to adult animals. In juvenile rats, the initial rapid compensatory renal growth after unilateral nephrectomy appears to be GH independent. Mulroney and associates (526) did not find an early increase in pulsatile GH release in juvenile as compared to adult unilaterally nephrectomized rats. Furthermore, treatment with GHRF antagonist did not prevent compensatory renal growth in juvenile rats. Moreover, the expression of IGF-I and IGF-I mRNA, which were increased during remnant kidney hypertrophy, were not attenuated by the GH releasing hormone antagonist in juvenile rats (526). The latter studies suggest that renal growth after partial nephrectomy is GH dependent in adult, but not in juvenile, rats. Mulroney *et al.* (527) supported this differential finding in subsequent experimental studies in juvenile and adult rats that underwent unilateral nephrectomy. In the contralateral kidney, these investigators observed a 3- to 4-fold increase in mRNA encoding IGF-I, IGF-I receptors, and IGF-II receptors in young, but not in adult, rats. *In situ* hybridization localized the increase in IGF-I mRNA levels primarily to the thick ascending limb of the loop of Henle (527). Incubation of tubule membranes *ex vivo* with labeled IGF-I and IGF-II indicated increased ligand binding in young but not in adult rats (527). In weanling rats, renal IGF-II levels also increase transiently (528) and may contribute to compensatory nephron growth.

In contrast to the above findings by Mulroney *et al.* that in immature but not in adult rats there is a rise in renal IGF-I and IGF-I mRNA after unilateral nephrectomy, Flyvbjerg and associates as well as Fagin and Melmed found similar results in adult rats (498, 522, 527, 529, 530). Again, there is a disparity in the experimental results obtained from different laboratories, but it appears that, in adult as well as in immature rats, a transient increase in renal IGF-I and IGF-I

mRNA levels contributes to the initial period of rapid renal growth of the contralateral kidney after unilateral nephrectomy.

After unilateral nephrectomy other growth factors may also contribute to compensatory growth of the contralateral kidney. EGF expression and binding increases and appears to contribute to nephron hypertrophy, particularly in distal tubules (520, 531).

In summary, in adult rats, unilateral nephrectomy causes an early, transient rise in the renal expression of IGF-I mRNA and a transient rise in systemic GH activity, but the renal IGF-receptor levels may not change. Locally increased activity of IGF-I, which results from renal synthesis as well as binding of circulating IGF-I, may contribute to the early phase of rapid renal growth in adult unilaterally nephrectomized rats. In contrast, in immature rats, unilateral nephrectomy causes rapid early renal growth of the contralateral kidney by GH-independent mechanisms, which involve increased renal expression of IGF-I, IGF-II, and IGF-I receptors. These changes in the GH/IGF-system probably occur only during the initial period of rapid renal growth (<7 days) but some increase in renal IGF-I synthesis may persist for longer periods of time. Thus, GH and IGFs appear to contribute most to the early, rapid phase of compensatory renal growth after unilaterally nephrectomy and appear to be less important for subsequent slow growth and the long-term maintenance of increased renal mass after unilateral nephrectomy.

C. Subtotal nephrectomy

A more severe reduction in renal mass and nephron number than is achieved with unilateral nephrectomy causes even greater compensatory growth of remaining, remnant nephrons. The 5%-nephrectomy rat model has been used in many experimental studies to examine mechanisms and consequences of a large reduction in the nephron number. Experimental data derived from this rat model suggest a role of the IGF-system in the induction and possibly maintenance of nephron hypertrophy.

Muchaneta-Kubara *et al.* (319) studied the relationship between IGF-I and compensatory renal growth after subtotal nephrectomy in the rat. Renal compensatory growth occurred over the first 30 days after nephrectomy, and increased kidney mass is maintained thereafter. Both extractable renal IGF-I levels and immunostainable IGF-I in the cortical collecting ducts were elevated but returned to normal by day 30. A second peak was noted between 3 and 5 months after nephrectomy, but at this later time point the IGF-I was found in distal tubules and interstitium, which by now had accumulated fibrous scars (319). An increase in medullary collecting duct IGF-I expression was also noticed by other investigators after partial nephrectomy in the rat (202, 532).

Circumstantial evidence suggests that in adult rats the renal hypertrophy after subtotal nephrectomy is GH dependent. Yoshida and associates demonstrated a lesser degree of remnant kidney hypertrophy in dwarf rats with isolated GH deficiency as compared to normal rats undergoing five-sixths-nephrectomy (351).

D. Streptozotocin diabetes

In human diabetes as well as in streptozotocin-diabetes in rats, serum IGF-I levels are moderately reduced but IGF-II levels are elevated (56, 533). Serum IGFBP-1 and -2 levels are increased, but IGFBP-3 levels are reduced (170, 534). This suggests that the binding of IGFs is shifted from the 150-kDa to the 45 to 50-kDa carrier complex with a greater availability of the peptides to tissues. Serum IGFBP-4 may be normal in diabetic rats (535). These changes in the serum IGFBP-levels are remarkably similar to those found in the nephrotic syndrome (167).

In rats with streptozotocin-induced diabetes, renal hypertrophy is preceded by an increment in renal IGF-I levels which return to baseline 4 days after administration of streptozotocin (330, 536). In diabetic rats, extracted renal IGF-I mRNA levels were either unchanged (537) or increased (538). Flyvbjerg and associates (330) found an increase in renal extractable IGF-I levels in rats that received streptozotocin and were not treated with insulin. The rise occurred within 24 h and levels peaked at about 48 h. Renal growth was significant on day 4 and, hence, was preceded by the rise in renal IGF-I. In this study renal IGF-I concentrations decreased back to baseline after 4 days, despite continued but slower renal growth. Insulin treatment, however, prevented both the rise in renal IGF-I as well as kidney growth (330). The authors conclude that IGF-I may have a role in the initial, rapid growth phase but may not be necessary for the maintenance of renal hypertrophy. Phillip *et al.* (539) demonstrated such accumulation of extractable IGF-I only in diabetic kidneys from postpubertal rats that was associated with a seemingly paradoxical decline in renal IGF-I mRNA (539). As discussed further below, this finding suggests that the accumulation of IGF-I in the kidney in early diabetes results from increased binding rather than *in situ* synthesis. However, specific IGF-I receptor mRNA in postpubertal diabetic rat kidneys is not increased, suggesting increased renal uptake of IGF-I by non-IGF-I receptor binding. In prepubertal diabetic rats, IGF-I mRNA was unaltered, but the extractable IGF-I levels were much lower than in control rats. This occurred despite an increase in IGF-I receptor mRNA (539). To the authors it appears impossible to formulate a general hypothesis that would unify the experimental findings from these different groups of investigators.

Renal IGF-I mRNA levels were found to be decreased by *in situ* hybridization, and extractable renal IGF-I mRNA is also reduced in longstanding diabetes in rats (186, 540) (Table 3). Thus, the increase in renal extractable and immunoreactive IGF-I levels found by some investigators may result from binding of circulating IGF-I to renal IGF-I receptors or binding proteins.

Different stimuli for renal growth may be additive. For example, unilateral nephrectomy in rats with streptozotocin-induced diabetes further enhances renal growth, and the increase in renal mass with the combined lesion exceeds the renal hypertrophy that is achieved with either manipulation alone (530). In diabetic rats there seems to be a correlation between the severity of the diabetic metabolic disturbance (hyperglycemia, insulin deficiency), the rise in renal IGF-I levels, and the degree of renal hypertrophy (541).

TABLE 3. The IGF-I system in kidney in streptozotocin-diabetes in the rat

	Days of diabetes											
	2-7			7-30			90-180					
IGF-I mRNA	↓	↓	OS, MTAL	↓	↓	OS, MTAL	↓	↓	OS, MTAL			
BP-1 mRNA	↑	Cort	↓	↓	OM	↑	Cort	↓	↓	OM		
BP-2 mRNA			↓	OM			↓	OM				
BP-3 mRNA			↓	Cort								
BP-4 mRNA			↓	Cort			↓	↓	Cort			
BP-5 mRNA	↑	OM	↓	Cort			↓	Cort	↑	OM	↓	Cort

Compiled from published data (186). OS, Outer stripe of medulla; Cort, cortex; MTAL, medullary thick ascending limb of the loop of Henle; OM, outer medulla.

Menon and associates (542) demonstrated a tissue-specific increase in the GH receptor mRNA levels in streptozotocin diabetes in rats. These investigators found a decrease in mRNA levels encoding for GH receptors in liver and heart, but receptor expression increased in the kidney. This finding supports the notion that GH may contribute (directly or via IGF-I) to the renal hypertrophy in diabetes. In contrast, renal GH receptor mRNA levels were found to be similar in rats at 7 days of experimental diabetes compared to normal animals (543). Induction of diabetes with streptozotocin in GH-deficient rats causes only a lesser degree of renal hypertrophy. The increase in kidney extractable and immunostainable IGF-I is reduced compared to diabetic rats with normal GH hormone status (348, 349). Treatment of diabetic rats with a somatostatin analog that reduces endogenous GH activity, prevents both the hypertrophy as well as the rise in renal IGF-I (330). Thus, renal hypertrophy in diabetic rats may be GH dependent.

IGF-I receptor mRNA and receptor binding were both found to be increased 2.5-fold in diabetic rats (544), but other investigators did not confirm this finding (543). The reason for this discrepancy remains unclear.

In streptozotocin diabetes in rats Marshall *et al.* (329) found a transient decrease in binding of IGF-I to kidney membranes on the first day, but thereafter binding was not different from control (329). Other authors found increased binding of IGF-I to kidney membranes during the first 3 days of experimental diabetes either to IGF-I receptors or to binding proteins (538). "Specific binding" may refer to binding to IGF-I (or IGF-II) receptors, or to membrane-associated binding proteins. In this study the increase in renal IGF-I levels in rats with experimental diabetes is associated with a transient increase in extractable IGFBP-3 and an increase in a IGFBP of about 30 kDa by Western ligand blotting. This latter binding protein was thought to be IGFBP-2 (545) but possibly represents another binding protein, such as IGFBP-5. Whether the increase in the two binding proteins results from increased local synthesis has not been determined in these studies (545). Should the 30-kDa binding protein be indeed IGFBP-2, one may argue that this IGFBP may have been trapped by binding through its RGD-sequence to membrane integrins. This is further supported by the finding that IGFBP-2 mRNA levels in the kidney are unchanged after induction of diabetes in rats (186). In contrast, IGFBP-1 and -5 mRNAs in renal cortex increase significantly in diabetic rats compared to controls. Increased IGFBP-1 may contribute to kidney

growth through mitogenic actions via $\alpha_5\beta_2$ -integrins that are independent of IGFs, although there is no direct proof for this function of IGFBP-1 *in vivo*. In the medulla, there is a moderate decrease in IGFBP-1 mRNA. IGFBP-3 and -4 mRNAs change only modestly in diabetic rats (186). In concert, the 30-kDa IGFBP that was found by Flyvbjerg *et al.* to be increased is either trapped IGFBP-2 or locally synthesized IGFBP-1 or -5 (186, 545). Fervenza *et al.* (543) found a moderate increase in renal cortical IGFBP-1 mRNA and moderate decreases in IGFBP-2 and -4 mRNAs in diabetic rat kidneys, but IGFBP-1 mRNA in renal medulla was decreased. IGFBP-3 and -5 were not different from controls in these studies. Due to some discrepancies in the experimental data of renal IGFBPs in diabetic rats, an unifying picture as to the contribution of IGFBPs to diabetic nephron growth cannot be drawn.

In summary, there is evidence for a role of GH as well as IGF-I in the rapid renal growth that occurs early in the course of experimental diabetes. GH receptors in the kidney are increased, suggesting some dependence of the renal hypertrophy on GH. GH may induce increased synthesis of renal IGF-I, but trapping of circulating IGF-I is also suggested. Many of the experimental data described thus far are in conflict with each other, and it is virtually impossible to arrive at clear conclusions as to the involvement of the GH/IGF-I-system in diabetic renal growth. There are no data on the renal GH/IGF-I-system in kidney hypertrophy that occurs in the early stages of human diabetes.

E. Diet and renal growth

Short-term fasting as well as long-term starvation reduce serum IGF-I levels (23, 338, 340, 344, 546, 547). Long-term starvation or protein and/or calorie malnutrition also reduces kidney mass (341, 342, 548). Protein and calorie malnutrition or short-term starvation also reduce the IGF-I mRNA levels in several tissues, such as lung, liver, muscle, and kidney (175, 322, 549). Thus, there is circumstantial evidence that reduced serum and/or renal IGF-I levels contribute to the reduced kidney mass in states of protein or calorie malnutrition.

Kobayashi and associates (175) examined the expression of mRNAs encoding IGF-I and IGFBP-1 to -5 in GH deficient and in normal rats that were fasted for 48 h or received GH. In both groups of rats short-term fasting reduced the renal mRNA levels for IGF-I and increased the IGFBP-1 mRNA levels in the kidney. Administration of GH causes the exact opposite effects. Short-term fasting did not affect significantly the renal IGFBP-2, -3, -4, and -5 mRNA levels (175). Since the reduction in IGF-I and the rise in IGFBP-1 mRNA with fasting occurred in GH-deficient as well as in normal rats, this nutritional effect seems to be GH independent (175). However, fasting of short duration does not reduce kidney mass measurably.

The association between increased dietary protein and renal hypertrophy has received widespread attention. Normal rats pairfed a high-protein diet (36% protein) have greater body weight gain and increased renal mass compared to animals receiving a low-protein diet (9% protein) (233). The high-protein diet also causes a greater concentra-

tion of extractable IGF-I in liver and in isolated glomeruli (233). These findings suggest that the renal IGF-I may contribute to the nephron growth that is induced with high-protein diets. In rats receiving a high-protein diet, serum IGF-I levels are also elevated, giving rise to the possibility that circulating IGF-I may contribute to the renal hypertrophy. In fact, the increased extractable IGF-I levels in isolated glomeruli may reflect peptide that was bound from the circulation.

The additive effects of high-protein diets on nephron hypertrophy were also examined in rats with partial nephrectomy. Both the degree of nephron compensatory growth and renal tissue levels of IGF-I in partially nephrectomized rats increase further with administration of high-protein diets (320, 350, 497, 498). In some of these studies, serum and hepatic IGF-I levels were also measured, and both were found to be normal, suggesting a local intrarenal mechanism that increases renal IGF-I levels in response to partial nephrectomy with or without high-protein diets (320, 346). The latter investigators performed similar experiments in GH- (and IGF-I-) deficient Lewis dwarf rats. In these GH-deficient animals, as compared to appropriate controls, kidney hypertrophy and renal IGF-I levels were unaffected by dietary protein (346), suggesting that the dietary protein-induced renal growth is GH-dependent (350). However, Charlton *et al.* (550) demonstrated an increase in both renal IGF-I mRNA levels and renal growth in response to a high-protein diet in GH-deficient rats. This was also shown in partially nephrectomized normal rats receiving high-protein diets (551). Thus, there is a discrepancy in reported experimental data that is not readily explained. At present, it seems unclear, whether the protein-induced renal hypertrophy is GH dependent.

Increases in renal IGF-I secondary to high-protein diets or partial nephrectomy in the rat were not demonstrated universally. Caverzasio and co-workers (551) investigated the role of IGF-I in compensatory renal growth in rats after unilateral nephrectomy with or without concurrent parathyroidectomy followed by feeding high or moderate protein diets. These investigators found no change in renal IGF-I levels but demonstrated an increase in serum IGF-I levels in the rats fed the high-protein diet compared to those receiving the low-protein diet. This increase was present in both nephrectomized and nonnephrectomized rats (551). In unilaterally nephrectomized rats fed high-protein diets, the compensatory renal growth as well as the rise in serum IGF-I were both prevented by parathyroidectomy (551). Coxam and associates (552, 553) demonstrated that PTH can increase the hepatic IGF-I synthesis and IGF-I mRNA levels in liver. These experiments suggest an important role of liver-derived IGF-I in the renal hypertrophy in response to high-protein diets. Furthermore, the high-protein diet-induced renal hypertrophy may be PTH-dependent, although the mechanisms are unclear, and confirmation of this intriguing finding by other investigators has not yet been published.

In summary, high-protein diets increase liver, serum, and glomerular and renal extractable IGF-I levels and induce an increase in renal mass. IGF-I may contribute to the increase in renal mass that occurs in this setting. The dietary protein-induced renal hypertrophy may or may not be GH dependent, a question that is at present unsettled. Reduced IGF-I

activity in low-protein diets may contribute to a reduction in renal mass. Short-term fasting and probably long-term protein/calorie malnutrition reduce the synthesis of IGF-I in kidney and other tissues and increase the renal levels of IGFBP-1, which may further reduce local IGF-I activity.

F. Other experimental models

There are several other experimental animal models in which nephron hypertrophy may be mediated by IGF-I. Potassium depletion in rats causes rapid kidney growth. In this model the increase in kidney weight is preceded by an increase in immunoassayable IGF-I that was extracted from the kidney. The increase in renal IGF-I levels in this models is only transient, for about 4 days, and levels return to baseline by about 7 days after induction of the lesion, despite the kidney hypertrophy is maintained (554).

Acute, unilateral ureteral obstruction in rats and pigs results in a rapid decrease in renal IGF-I levels in the hydronephrotic kidney and in an increase in renal IGF-I in the contralateral kidney, where it precedes the onset of renal growth (555, 556).

Regional hypertrophy can develop in segments of the nephron during increased workload. Treatment of rats with furosemide blocks the sodium absorption in the thick ascending limb of the loop of Henle and increases distal tubular sodium delivery. Thus, in turn, furosemide increases the work load of distal tubules and cortical collecting ducts which undergo hypertrophy. This hypertrophy is associated with an early increase in the local IGFBP-1 mRNA expression but IGF-I mRNA is not increased. However, immunohistologically there is also increased IGF-I peptide present shortly after onset of treatment with furosemide. Unfortunately, the investigators did not examine IGF-I receptor expression in distal tubules in this study. Thus, the hypertrophy in this model may be caused by IGF-I, which is trapped by increased expression of IGFBP-1 in distal tubules and collecting ducts (557), but increased binding of circulating IGF-I to IGF-I receptors may also occur. This hypothesis assumes that IGFBP-1 does not neutralize IGF-I activity which may or may not be correct. Furthermore, intrinsic effects of IGFBP-1 to increase cell growth through an integrin-mediated mechanism may also contribute.

G. Summary of data on renal growth

There seems to be a sequential relationship between renal compensatory growth and the increase in renal IGF-I levels in different models of acute renal hypertrophy in the rat: partial nephrectomy, acute streptozotocin-induced diabetes, potassium depletion, and postrenal obstruction. In all these rat models, most but not all studies found that renal IGF-I levels increase within 1 to 2 days and precede the onset of renal growth. However, this rise in renal IGF-I seems to last between 4 and 7 days (although this is not without controversy), whereas hypertrophy is maintained for long periods of time (186, 329, 330, 349, 498, 530, 537, 541, 545, 554–556, 558, 559). Thus, renal as well as systemic IGF-I may be involved in the induction of hypertrophy and the early phase of rapid renal growth, but apparently does not contribute as

significantly to the maintenance of increased kidney mass. There is evidence for GH dependence of the compensatory renal growth after partial nephrectomy and in experimental diabetes, but this concept is not mutually accepted. Similarly, some but not all investigators have suggested that there are differences between juvenile and adult rats in the mechanism by which GH and IGF-I may contribute to renal growth. It is possible, although unproven, that IGFBP-1 contributes to kidney growth in some conditions, such as diabetes, through its interaction with integrins.

H. Compensatory renal growth and progressive glomerular sclerosis and interstitial fibrosis: is there a role for GH and IGF-I?

Renal growth (more specifically, glomerular hypertrophy and/or hyperplasia in experimental renal diseases in rats) has been implicated as a cause of progressive glomerular sclerosis and chronic renal failure (351, 493–496, 560). In aging rats there is a correlation between the severity of glomerular sclerosis and plasma GH levels (561). Moreover, administration of GH to rats with puromycin-aminonucleoside-induced nephrosis accelerates the development of glomerular sclerosis (562). Furthermore, administration of GH accelerates the development of glomerular sclerosis and the progression of renal failure in partially nephrectomized rats in some studies (560).

Several observations obtained from mice transgenic for bovine or human GH have suggested a strong link between GH induced hypertrophy/hyperplasia of the nephron and progressive glomerular sclerosis. Mice transgenic for GH develop mesangial proliferation, glomerular hypertrophy, and progressive glomerular sclerosis (207, 502, 503, 505, 508, 512, 563). Further experimental evidence suggests that different domains of the GH molecule might cause body growth as compared to the glomerular hypertrophy and sclerosis (564–566). In streptozotocin-induced diabetes in the rat, renal GH receptor mRNA levels were found to be increased in one study, giving rise to the possibility that increased action of GH may contribute to diabetic glomerular sclerosis (542). However, GH may not be expressed in rat glomeruli (Fig. 1), and a direct effect of GH on glomerular sclerosis is unlikely.

GH-deficient rats are somewhat protected from the development of glomerular sclerosis after partial nephrectomy (351). Similarly, mice transgenic for a GH antagonist are protected from streptocotozin-diabetes-induced progressive glomerular sclerosis (567). In these mice, glomeruli were normal in contrast to severe glomerular sclerosis seen in normal diabetic mice or in mice expressing the GH transgene.

To the authors' knowledge, there have been no reports indicating that acromegalic patients develop glomerular sclerosis and interstitial fibrosis prematurely or have an increased incidence of progressive renal failure. It will be difficult, if not impossible, to examine this question in a prospective trial, since acromegalics are treated either surgically or pharmacologically to improve their hypersomatotropic state.

There is also evidence that IGF-I may contribute to the development of glomerular sclerosis and interstitial fibrosis. In cultured rat glomerular mesangial cells, IGF-I stimulates

mRNA and secreted protein levels of type I and IV collagen (513). Moreover, rat kidneys that were perfused with IGF-I had increased expression of mRNA for fibronectin, laminin, heparan sulfate proteoglycan, collagen type II, and collagen type IV (568). In subtotally nephrectomized rats, administration of exogenous IGF-I augments glomerular procollagen $\alpha_1(\text{IV})$ mRNA levels (497). In transgenic mice overexpressing GH (which have also elevated IGF-I levels), glomeruli contain increased amounts of collagen type I and type IV, laminin, and heparan sulfate proteoglycan (563). The expression of these proteins and their mRNA somewhat resembles diabetic glomerular sclerosis in rats (563). Greatly increased single glomerular levels of procollagen $\alpha_1(\text{I})$ and $\alpha_1(\text{IV})$ mRNA above control were also demonstrated in isolated glomeruli from GH transgenic mice using a truly quantitative PCR technique (569). Thus, increased GH activity in rodents is associated with increased glomerular production of extracellular matrix proteins.

Mice transgenic for IGF-I also develop glomerular enlargement, similar to GH hormone transgenic mice, but do not develop accelerated glomerular sclerosis (207, 508), even though serum (and presumably renal) IGF-I levels in IGF-I-transgenic mice were greater than in GH transgenic animals (207, 508). Furthermore, administration of exogenous IGF-I to rats with reduced renal mass causes a similar degree of renal hypertrophy as can be achieved by giving high-protein diets. However, the degree of glomerular sclerosis and the increment in glomerular collagen type I and IV levels are less in rats receiving IGF-I compared to those in which similar nephron hypertrophy was induced with the high-protein diet (497). Mehls and associates (511) administered IGF-I to female rats resulting in increased renal growth and hyperplasia but not premature glomerular sclerosis (511). However, female rats are known to be less sensitive to various interventions that cause glomerular sclerosis compared to male animals (570). Thus, in rodents, GH and IGF-I may contribute to the premature development of glomerular sclerosis (and interstitial fibrosis) in chronic renal disease. Whether this is also true in human renal disease is presently unknown. In rodents and probably even more so in man, the contribution of IGF-I to the development of glomerular sclerosis and progression of renal failure, if any, may be rather modest.

VIII. GH and the IGF System in Selected Renal Diseases

Abnormal serum and/or tissue levels of IGF-I, IGFBPs, and GH have been described in several renal disease states. Furthermore, both GH and IGF-I may each have an impor-

tant clinical role as pharmaceutical agents in the treatment of sequelae of renal diseases. These issues will be discussed in this section as they apply to chronic renal failure, acute renal failure, and the nephrotic syndrome.

A. Chronic renal failure

In chronic renal failure the GFR as well as tubule function decline, and many metabolic and hormonal abnormalities become evident. Abnormalities in the systemic GH/IGF-system may contribute to growth failure in children and experimental animals (571) and net muscle protein catabolism (572–576). Poor nutritional status may contribute to many metabolic changes in pediatric and adult patients with chronic renal failure (577). The catabolic state of patients with chronic renal failure may also be caused, in part, by metabolic acidosis, which is present in most patients with advanced renal failure (576, 578). The intake of glucocorticosteroids, such as after renal transplantation, contributes to the catabolic state (578, 579).

1. *GH and IGF/IGFBP levels in chronic renal failure (Table 4).* The kidneys contribute significantly to the degradation of GH. Hence, serum GH levels should be increased in patients with chronic renal failure. Indeed, the serum half-life of GH is significantly increased in renal failure and the MCR of exogenous recombinant human GH is reduced (255). In concert, these abnormalities cause elevated serum levels of GH (255). Elevated levels of serum GH have also been found by several other investigators (580–583). There is also evidence for increased pulsatile release of GH in children with chronic renal failure as compared to normal children that contributes to the about 2.5-fold elevated serum GH levels in children with renal disease (584). Furthermore, the levels of serum GH binding protein which is the soluble form of the GH receptor, is reduced in chronic renal failure (248, 582). However, in uremic rats, serum binding of GH was found to be elevated (585). Serum GH levels are decreased after a hemodialysis session compared to the predialysis values in patients on chronic maintenance hemodialysis (580). This may result from removal of free serum GH.

Most investigators found normal serum IGF-I levels in patients with chronic renal failure (580, 586–591). In contrast to GH, IGF-I is not removed from serum during hemodialysis (580), possibly because of the molecular weight of the circulating IGF-I that is present in binding protein complexes with a molecular mass of ≥ 45 kDa. In contrast to most published reports, some investigators described elevated IGF-I serum levels in patients with chronic renal failure (266, 267, 592). This discrepancy may result from technical differences in the assays or sample preparation such as the extraction and separation method used to separate IGFs from the bind-

TABLE 4. The GH-IGF-I system in chronic renal failure in the rat

	GH-R	IGF-I	IGF-I R	BP-1	BP-2	BP-3	BP-4
Plasma		→		↑	↑	→	
Liver	↓	↓		↑	↑		
Lung		↓					
Muscle		↓		→	→		
Kidney		→	↓		↓	↑	
Heart		→					↑

Compiled from published data (625, 722, 723).

ing proteins (590, 591). IGF-I serum levels and bioactivity are both reduced in patients with malnutrition, which may be present in a subgroup of chronic renal failure patients (338, 546, 586, 593).

Fouque and associates (268) performed pharmacokinetic studies on rhIGF-I in adult patients undergoing maintenance hemodialysis or continuous ambulatory peritoneal dialysis as well as in normal subjects (268). No differences in the IGF-I pharmacokinetics were found between the dialysis patients and the normal subjects after a single subcutaneous dose of rhIGF-I, 50 $\mu\text{g}/\text{kg}$ (268). With a 100 $\mu\text{g}/\text{kg}$ injection, the hemodialysis and peritoneal dialysis patients displayed higher peak serum IGF-I concentrations and a decreased half-life and volume of distribution of IGF-I. The maximum increment in serum IGF-I in the dialysis patients was 72 to 88% greater compared to normal subjects (268). The T_{max} , area under the curve (AUC), and the serum clearance of IGF-I in the dialysis patients were all not different from normal. The greater peak serum IGF-I levels and the reduced volume of distribution of IGF-I may reflect increased plasma binding, presumably to IGFBPs or binding protein fragments. By 12–14 h after injection, serum IGF-I levels were not different in dialysis patients compared to normal subjects (268). Twice-daily injections of rhIGF-I in continuous peritoneal dialysis patients for 20 days does not lead to progressive accrual of IGF-I in serum (268, 594).

Although normal serum IGF-I levels in chronic renal failure were demonstrated in most studies, the liver synthesis of IGF-I may be decreased as suggested by reduced liver steady state IGF-I mRNA levels in rats with chronic renal failure compared to pair-fed normal controls (585, 595). The reduced IGF-I synthesis in liver may result from a reduction in the GH receptor expression in liver, which was demonstrated in uremic rats (585).

In chronic renal failure, serum IGFBP-levels have been reported to be elevated (586, 588, 596). This has been examined by Western ligand and immunoblot analysis as well as with immunoassays (RIA, IRMA). Western ligand blot analysis and RIA data correlate well if normal serum is examined, but falsely elevated levels are found if body fluids that contain IGFBP-protease activity are examined (313). This is particularly true for IGFBP-3. IGFBP-protease activity has been described in sera from pregnant women (182, 183, 310, 313–315, 597–606), patients with severe acute illness such as the sepsis syndrome (607), and in sera from patients with chronic renal failure (276, 608–610). Low levels of proteolytic activity toward IGFBP-3 are apparently also present in normal rat serum (611). In rat pregnancy the IGFBP-3 protease activity is expressed in several tissues, but not in the kidney (602). Proteases that degrade other IGFBPs are also known (5) but proteolysis of serum IGFBP-1, -2, or -4 apparently does not play a significant role in patients with renal failure.

In normal human serum, there is a positive correlation between the sum of the IGF-I and -II serum levels and the serum IGFBP-3 concentration, both of which are about 100 nM, suggesting that IGFBP-3 in the 150-kDa complex is nearly saturated (142, 152, 612). The IGFBP-3 protease activity degrades the binding protein into smaller proteolytic fragments, which have a 50-fold reduced binding activity but are retained in the 150-kDa complex (183, 184, 611, 613). Some

investigators have suggested that proteolytic IGFBP-3 fragments in serum from pregnant women still retain significant binding activity for IGF-I (182, 597). Much of the circulating IGF-I and -II is still present in the 150-kDa protein complex which would further support the fact that proteolytic fragments of IGFBP-3 still bind IGFs, albeit the affinity may be reduced (614). Other experimental evidence suggests that IGFBP-3-proteolysis liberates IGF-I from the binding protein complex and increases its bioactivity (611, 613). In serum from pregnant women, IGFBP-3 is almost undetectable by Western ligand blot, since the proteolytic fragments do not bind the tracer in this assay (312, 315). However, IGFBP-3 levels can be measured with RIAs, since the fragments still cross-react with the antibody (612). It is possible that the apparently increased serum IGFBP-3 levels that some investigators found in serum from patients with chronic renal failure results from the presence of multiple fragments that are recognized in the RIA (615). In chronic renal failure small molecular weight fragments of IGFBP-3 are also found in urine (615).

Valentini *et al.* (616) demonstrated that IGFBP-3 immunoreactivity is present in peritoneal dialysate outflow in children with end-stage renal disease undergoing continuous ambulatory peritoneal dialysis and in hemodialysate. In hemodialysis patients, the immunoreactive IGFBP-3 levels in dialysate were greater with high-flux dialyzers compared to regular dialysis membranes (616). Perhaps these investigators measured small molecular weight IGFBP-fragments that are dialyzable.

In chronic renal failure, serum IGFBP-1, -2, and -3 were found to be increased (142, 168, 586, 610, 617, 618). IGFBP-1 and -2 levels are truly increased, and there is no evidence that significant amounts of these binding proteins are degraded by specific proteases. In contrast, as demonstrated above, IGFBP-3 levels are elevated due to fragmentation, but the amounts of full-size IGFBP-3 are most likely decreased or are not different from normal (588). IGFBP-4 levels are also slightly increased in sera from patients with chronic renal failure (588). Circumstantial evidence suggests that in chronic renal failure patients, serum IGFBP-4 levels vary with nutritional status, particularly dietary protein intake, similar to IGF-I levels (588). During treatment with rhIGF-I, serum IGFBP-3 levels decrease greatly in patients with chronic renal failure (619–621).

There are less data available concerning the IGF-II serum levels in patients with chronic renal failure. Daughaday and co-workers (622) found elevated IGF-II levels in sera from hemodialysis patients. During treatment with recombinant human GH, serum IGF-II levels tend to increase (623).

2. *Resistance to GH and IGF-I in chronic renal failure.* Insensitivity to GH is apparent from several observations in patients (and laboratory animals) with chronic renal failure. Despite normal or elevated serum GH levels and increased pulsatile growth hormone release, hepatic IGF-I mRNA levels are reduced in rats with chronic renal failure (585, 595). Furthermore, children with chronic renal disease have normal or elevated serum GH levels but body growth failure. Moreover, some of the metabolic abnormalities of adult patients with chronic renal failure can be explained by resistance to

the action of GH. Notwithstanding the presence of this GH resistance, pharmacological administration of recombinant human GH will improve growth rates in children with chronic renal failure as well as nitrogen balance in adult patients.

The mechanisms causing the insensitivity to GH are less clear. Blum and associates (617) demonstrated reduced IGF-I production after GH administration. In rats with chronic renal failure, Tönshoff and associates (585) demonstrated reduced hepatic expression of GH receptors. It is not known whether a reduction in GH receptor number is also present in other GH target tissues. Possibly, patients with uremia are comparable to Laron dwarfs who have defective GH receptors or receptor-signaling mechanisms (624). Since many effects of GH are mediated by IGF-I, GH insensitivity may also be caused by IGF-I resistance, which is also present in chronic renal failure (*vide infra*).

There has long been evidence for resistance to IGF-I in chronic renal failure. Phillips and Kopple (593) found decreased sulfate incorporation activity in sera from hemodialysis patients as compared to normal subjects. In this study, the activity to incorporate sulfate into cartilage was partially restored after hemodialysis, suggesting the presence of inhibitors in uremic sera (593). The nature of such low mol wt inhibitors was not examined in this study. Moreover, it remains unclear from this report whether IGF-I, IGF-II, or other compounds in serum accounted for the sulfate incorporation activity (593). Thus, these earlier studies suggest, but do not prove, that IGF-I resistance is present in renal failure. Detailed metabolic studies by Fouque and associates (587, 594) clearly demonstrate that patients with chronic renal failure and maintenance dialysis patients have an IGF-I insensitivity syndrome. Similarly, IGF-I resistance has also been demonstrated in rats with experimental chronic renal failure (625). Blum *et al.* (617) suggested that increased levels of IGFBPs in uremic serum may account for IGF-I resistance. These investigators demonstrated that removal of IGFBPs by IGF-II-affinity chromatography restores IGF-I bioactivity. Small molecular weight binding protein fragments are removed by dialysis (616), which could retrospectively explain the improved serum sulfation activity after a dialysis session (593). However, the binding activity of IGFBP-3 fragments may be rather low (*vide supra*), and the efficient removal of full-sized IGFBP-3 by dialysis is unlikely. Valentini *et al.* (616) reported that IGFBP-3 levels (RIA) in hemodialysate are about 0.45 mg/liter. Since the flow rate of dialysate during a regular hemodialysis session is about 500 ml/min (120 liters in 4 h), the amounts of IGFBP-3 that would be removed during one 4-h dialysis session would exceed the total serum IGFBP-3 content, a value that is difficult to accept. Nevertheless, the elevated IGFBP-3/IGFBP-3-fragment levels in uremic serum may contribute to the IGF-I resistance in chronic renal failure (626).

Ding and associates (625) recently examined the effects of IGF-I on protein synthesis and degradation in rats made chronically uremic and in pair-fed control animals. Chronic renal failure rats received sodium bicarbonate to prevent metabolic acidosis, which is known to cause protein catabolism (578). Uremic rats treated with IGF-I had decreased protein catabolism and increased protein synthesis but, at

each dose of IGF-I, this response was less compared to normal controls (625). In uremic rats, as compared with control animals, skeletal muscle IGF-I levels and IGF-I mRNA levels were decreased, but IGF-I receptor mRNA was elevated. Furthermore, the receptor number was increased but the affinity of IGF-I to the receptor was normal (625). Semipurified IGF-I receptors from skeletal muscle from uremic rats had lesser degrees of autophosphorylation when incubated with IGF-I *in vitro*, and the receptor kinase activity was reduced, as was measured by the degree of *in vitro* IRS-I substrate phosphorylation (625). These elegant studies strongly suggest that the IGF-I resistance in chronic renal failure is caused, at least in part, by a (functional) IGF-I receptor defect. Furthermore, these findings provide evidence that (muscle) tissue IGF-I levels may be reduced in chronic renal failure without a concomitant reduction in serum IGF-I levels (625). Thus, reduced tissue action of IGF-I may contribute to the increased risk of poor nutritional status in patients with chronic renal failure.

3. Treatment of patients with chronic renal failure with recombinant human GH or IGF-I. There are several reasons to use GH or IGF-I as therapeutic agents in patients with chronic renal failure. Pharmacological dosages of each of the peptides have been shown to induce a variety of metabolic effects and override, to some extent, the state of insensitivity to GH and IGF-I. First, GH raises growth rates in children with chronic renal failure. Second, both GH and IGF-I may improve nitrogen balance and nutritional status in patients with renal failure. Third, IGF-I may raise the GFR and help to postpone the need for dialysis therapy. Each of these potential uses has undergone experimental study and clinical trials.

Growth failure is an important medical problem in children with chronic renal insufficiency, on hemo- or peritoneal dialysis, or after renal transplantation. Several metabolic abnormalities may contribute to the growth failure observed in uremic children, each of which is addressed by dietary, medical, or renal replacement therapy. These include chronic (renal tubular) acidosis, secondary hyperparathyroidism, and the uremic syndrome. Despite aggressive conventional treatments of these metabolic disorders, growth failure persists in most children with chronic renal disease. *In vivo* studies in experimental animals indicated that recombinant human GH when given pharmacologically has several metabolic effects and induces body growth (627, 628). These experimental observations led to controlled clinical trials that demonstrated that accelerated body growth is indeed achieved with exogenous administration of recombinant human GH (623, 629–637) (Table 5). In children with chronic renal failure, administration of recombinant human GH is associated with increased growth velocity, which is greatest during the first year of treatment. In pediatric patients with end-stage renal disease who are treated with continuous ambulatory peritoneal dialysis, GH can be added to the peritoneal dialysate to avoid the need for subcutaneous or intramuscular injections (638). Treatment with recombinant GH has also been used in pediatric patients who have undergone successful kidney transplantation, and this treatment is also recommended in the latter patient population (629, 639, 640). GH is now a recommended treatment mo-

TABLE 5. Prospective clinical studies examining the effects of rhGH in pediatric patients

Diagnosis	Number of subjects	Duration of treatment	Dose of recombinant human GH	Creatinine clearance (ml/min/1.73 m)	Complications	Reference
CRF	20	5 yr	Not given	32	1 pt-avascular necrosis of the femoral head	(636)
CRF	77	1 yr	0.1–0.2 IU/kg/day SQ	Not given	No complications	(637)
CRF	125	2 yr	0.05 mg/kg/day SQ	28	Postprandial I ↑ at 1 yr, not at 2 yr	(633)
CRF	13	1–3 yr	0.375 mg/kg/wk SQ ^a	66	Creat cl ↓ to 55 ml/min after 2 yr	(724)
CRF	20	6 months	4 IU/m ² /day SQ	15.2	7/16 on dialysis; no complications	(631)
CRF	11	4 yr	0.375 mg/kg/wk SQ ^a	14–64	Dialysis initiated in 1 pt	(629)
ESRD	9	1 yr	4 IU/m ² /day SQ	7–CAPD; 1–HD;	No complications	(630)
CAPD	8	2 yr	0.05 mg/kg/day IP	ESRD	No complications	(638)
Renal transplant	17	3 yr	4.3 IU/m ² /day SQ	60	1 pt ↑ creat after 6 weeks; ↑ fasting and stimulated insulin levels	(639)
Renal transplant	8	1 yr	0.05 mg/kg/day SQ	2 mg/dl ^b	4/8 pt had a significant increase in serum creatinine during rhGH treatment	(725)
Renal transplant	9	6–30 months	0.375 mg/kg/wk SQ ^a	Not listed	2 pts developed rejection episodes	(629)

All studies demonstrated a significant increase in growth velocity. CRF, chronic renal failure; ESRD, end-stage renal disease; CAPD, chronic ambulatory peritoneal dialysis; SQ, subcutaneous injection; IP, intraperitoneal injection; I, Insulin; pt, patient; creat cl, creatinine clearance; HD, hemodialysis.

^a Administered daily or three times/week.

^b Average serum creatinine.

TABLE 6. The IGF system in the nephrotic syndrome in the rat

	Serum	Urine	Glom UF	Liver	Kidney
IGF-I	↓	↑ ↑	↑ ↑	→	(↓ mRNA)
IGF-II	↓	↑ ↑			
BP-2	↑ ↑	↑		↑ ↑ mRNA + Protein	→
BP-3	↓	↑	None		(↓ mRNA)
BP-3-Fragment	↑	↑ ↑			
BP-3-Protease	↑ ?	↑ ↑ ↑			
BP-4	↓				

Glom UF, Glomerular ultrafiltrate.

dality to accelerate growth in children with chronic renal failure or end-stage renal disease (641). Despite this promising data, it is still not known whether increasing the rate of growth in uremic children will also lead to increased final adult height.

As outlined previously, in laboratory animal models with chronic renal failure, there has been evidence for a role of GH to accelerate the development of glomerular sclerosis and to increase the rate of progression of chronic renal failure to end-stage renal disease. This experimental evidence has been of great concern to many pediatricians, since it may infer that long-term treatments with exogenous GH to accelerate body growth in pediatric patients with small stature may cause accelerated progression of renal failure and an earlier need for dialysis or renal transplantation. Furthermore, anecdotal reports have suggested that this side effect of GH treatment may indeed occur (642). This important question has been addressed in more definitive studies by a number of investigators (634, 636, 643–646). Fine *et al.* (634) followed 125 patients with chronic renal failure undergoing long-term GH treatment for up to 5 yr and concluded that GH would not accelerate the progression of renal failure (634). Tönshoff and associates (643) compared the loss of renal function measured as creatinine clearance in pediatric patients before and after treatment with GH for 1 yr (643). These investigators concluded that exogenous treatment with GH will not ac-

celerate the loss of kidney function compared to the period before initiation of treatment (643, 645).

Glomerular hyperfiltration has been associated with increased progression of renal failure (337, 372, 374, 375), and GH has been demonstrated to induce increased glomerular ultrafiltration in normal subjects (289, 290, 293). However, the effect of GH to raise GFR is obliterated in patients with advanced renal failure (284, 285). This may be one of the reasons why GH may not increase the rate of progression of chronic renal failure in children. Riedl and associates (646) examined the renal functional reserve in a group of GH-deficient patients undergoing long-term treatment with GH and concluded that GH treatment does not adversely affect kidney function. However, it is of note that the patients in this latter study did not have renal disease at the onset. Maxwell *et al.* (647) examined this question in pediatric patients with chronic renal failure and concluded that GH may not accelerate the progression of chronic renal failure. Thus, in summary, at present there is no conclusive evidence that would link long-term treatment with exogenous GH in children with chronic renal failure to accelerated progression of renal disease.

Additional concern was raised whether long-term treatment of pediatric renal failure patients with GH might reduce carbohydrate tolerance or increase the frequency of malignancies. Occurrence of malignancy is of particular concern in

children who received renal allografts and require chronic immunosuppressive therapies (645). Tönshoff and associates (643) examined whether GH treatment would reduce glucose tolerance. These investigators found no significant change in the fasting or stimulated serum glucose levels but an increase in the fasting and stimulated serum insulin levels that was induced by long-term treatment with GH (643). Hence, as one might expect, some degree of glucose intolerance is induced by GH in patients with chronic renal failure, although this may not achieve clinical significance in the majority of patients.

Anecdotal case reports have mentioned the possibility that GH treatment bears a risk of increasing the incidence of malignancies, such as leukemia (648–650). It is also of note that GH is a progression factor for pre-T-acute lymphocytic leukemia cells *in vitro* (648). Furthermore, the chronic uremic state *per se* increases the risk for solid tumor malignancies, and hyperparathyroidism was implicated as a contributing cause (651). Rapaport suggested, after observation of six pediatric patients with GH deficiency, that there is an increased risk of leukemia in this group of patients, but this may possibly be associated with the GH deficiency rather than with exogenous GH treatment (652). The incidence of leukemia in pediatric patients who receive long-term GH was estimated as 5:100,000 as compared to 2:100,000 in a matched normal population (653). In 47 children who received GH treatment for radiation-induced GH deficiency, the tumor recurrence was comparable to that from a tumor registry control population (654). For each different tumor category there was an increased relative risk of tumor recurrence in patients who had received GH therapy (654). An increased risk of solid cancers, particularly colonic neoplasias, has been reported in acromegalic patients (655, 656). In a retrospective cohort study Buchanan *et al.* (657) examined 1246 male and 662 female patients who received pituitary GH for the treatment of short stature. This study suggested that GH may contribute to an increased incidence of tumor recurrence, but probably not to an increased frequency of new malignancies. The risk for Creutzfeld-Jacob disease that existed when extracted human GH was used has been eliminated by the sole use of recombinant GH preparations (658). Although there is no definitive prospective, controlled study that evaluates the risk of malignancy that may be associated with long-term GH treatment in patients with chronic renal failure, it is our impression that this risk is rather small, if it exists at all, but careful follow-up observation appears prudent. Similar conclusions from available data were recently published as guidelines for the use of GH in children with short stature by the Lawson Wilkins Pediatric Endocrine Society (641).

Due to GH resistance in pediatric and adult patients with chronic renal failure, IGF-I treatment may be used to accelerate growth rates in pediatric patients with chronic renal failure. To our knowledge, there are no studies to evaluate the use of rhIGF-I for this purpose. One reason may be the need for multiple daily injections of IGF-I. However, IGF-I is used in patients with short stature due to GH receptor defects, and these studies have shown that IGF-I accelerates growth rates (354, 624, 659).

Several surveys have indicated that some patients with

chronic renal failure and those undergoing maintenance dialysis therapy have a poor nutritional status and display clinical signs and symptoms of protein catabolism (660–662). Statistical evidence suggests that this contributes to the increased mortality of this patient population (663, 664). In addition to various modes of nutritional therapy, several investigators have examined whether supplemental administration of recombinant human GH and/or IGF-I may improve nitrogen balance and nutritional status. Ziegler *et al.* (665) demonstrated that exogenous GH can improve protein synthesis, decrease urea generation, and improve nitrogen balance in critically ill patients. Similar findings were obtained in short-term studies in patients on maintenance hemo- or ambulatory peritoneal dialysis (666–670). These studies indicate that in many patients GH hormone improves the nitrogen balance in dialysis patients, sometimes rather dramatically (670). However, some patients have only a poor response to this treatment, and this coincides with no or only a modest increase in serum IGF-I levels in response to the treatment with GH, possibly as a result of the GH insensitivity (670, 671).

IGF-I alone, or in combination with GH administration, also promotes anabolism and increases positive nitrogen balance in normal subjects (672–675). As was summarized above, adults with renal failure have a IGF-I insensitivity syndrome, and dialysis patients demonstrate a decreased metabolic response to exogenous IGF-I compared to normal subjects (587, 594), and the relative IGF-I (as well as GH) resistance in patients with chronic renal failure or on dialysis may contribute to the reduced nutritional status. Since not all chronic renal failure patients respond successfully to exogenous GH, there is a rationale to examine whether treatment with recombinant IGF-I may improve nitrogen balance and nutritional status in this group of patients (574, 670, 676). Shamir and associates (677) recently reported the effects of treatment of six malnourished maintenance peritoneal dialysis patients with IGF-I. Subjects underwent nitrogen balance measurements in a metabolic research unit before and during IGF-I administration, 50 or 100 $\mu\text{g}/\text{kg}$ every 12 h (677). Serum urea nitrogen levels fell significantly, and the nitrogen balance, which was not different from zero prior to the onset of IGF-I treatment, became positive. This resulted primarily from reduced urea formation and less urea-nitrogen losses with the peritoneal dialysate (677). In these patients, IGF-I induced a slight decline in serum phosphorus levels (possibly due to increased uptake into bone in these dialysis patients who are functionally nearly anephric) and a slight rise in serum calcium levels (677). These carefully performed studies demonstrate that various important metabolic parameters can be improved with short-term IGF-I therapy in malnourished dialysis patients. However, it will have to be determined whether such treatments given long-term will improve overall nutritional status and reduce mortality in the dialysis population. There has been evidence that IGF-I may also improve erythropoiesis in patients with chronic renal failure and renal anemia and may reduce the need for treatment with erythropoietin (678, 679).

In addition to its potential to increase body growth rate and to improve nutritional status in patients with chronic renal failure, IGF-I may also improve renal function and

delay the need for dialytic therapy. Miller and associates (680) examined rats with subtotal nephrectomy-induced chronic renal failure to determine whether treatment with GH or IGF-I would improve GFR. Although each hormonal treatment increased the GFR in normal control animals, both therapies failed to improve kidney function in rats with renal failure (680). Haffner and associates (284) confirmed this experimental finding by demonstrating that in patients with chronic renal failure, recombinant human GH would not increase GFR (284). Thus, in contrast to normal subjects and laboratory animals, GH does not augment renal function in rats or humans with chronic renal failure. This may, in part, result from the resistance to GH and the inability to mount an increase in systemic or renal IGF-I levels. O'Shea and associates (619) revisited the question of whether a short-term treatment course with recombinant IGF-I would increase GFR in a group of four patients with chronic renal disease and severely impaired renal function. The GFR was between 21 and 55 ml/min/1.73 m². Each subject received rhIGF-I, 100 µg/kg twice daily for 3 days. In each patient, GFR and renal plasma flow rose. This increase in renal function was associated with a small increase in kidney size (619). In a subsequent study, these investigators examined whether a more long-term treatment with IGF-I would achieve similar results and the rise in kidney function would be sustained (620). In this study nine patients with advanced renal failure (GFR ≤ 21 ml/min/1.73 m²) received a similar dose of IGF-I for a total of 20 days. However, the outcome was disappointing in several respects. First, significant side effects warranted discontinuation of the experimental treatment and withdrawal of some study participants. Second, although GFR rose initially, this rise was not sustained for the entire 20 days of study. The serum IGFBP-3 levels decreased substantially during the course of IGF-I treatment, which may have contributed to the failure of IGF-I to achieve a sustained rise in GFR (620). This group of investigators recently revisited the question as to whether IGF-I could be used in patients with chronic advanced renal failure (GFR ~8 ml/min/1.73 m²), to improve renal glomerular filtration (681). In this study, the protocol was changed in two important aspects: First, a lesser dose of IGF-I was used, namely 50 µg/kg; and second, the therapy was administered intermittently in cycles of 4 days of treatment interrupted by three treatment-free days (681). GFR (inulin clearance) increased by 42–81%. The filtration fraction remained unchanged, since the renal plasma flow rate also increased. IGFBP-2 serum levels increased with IGF-I treatment and tended to decline during off-drug days. Importantly, IGFBP-3 levels did not change significantly during the 4 week course of the study (681). Edema formation was observed in one of the nine subjects, and two patients had some irritation at the injection site. No other side effects were observed. In contrast, uremic symptoms (nausea, vomiting, pruritus) and exercise tolerance improved in most subjects. One of the subjects was maintained on IGF-I treatment for a total of 7 months with a sustained increase in GFR of 165% of baseline and remained free of uremic symptoms (681). Although this study is limited by the small number of patients, it suggests that IGF-I treatment may be useful in some patients with near-end-stage renal failure and frank clinical uremia when,

for one reason or another, the onset of definitive therapy, such as maintenance dialysis or kidney transplantation, has to be delayed.

B. Acute renal failure

Acute renal failure (ARF) may occur as a result of several acute renal disorders, but in a more narrow sense, the term is usually reserved for ischemic or toxic acute renal injury. In acute renal ischemia, after periods of reduced renal blood flow below the level necessary to maintain nephron integrity, proximal convoluted tubules (particularly the last third, referred to as the S₃-segment) and cells in the thick ascending limb of the loop of Henle endure damage, lose their brush borders, and may become necrotic (682–685). More recently, investigators found that some cells undergo apoptosis, rather than necrosis (686–689), and apoptosis in proximal tubules is associated with expression of the clusterin gene (690), which possibly encodes for an adhesion protein that facilitates subsequent repair (691).

The ARF is not caused by primary failure of the glomeruli to form an ultrafiltrate (682, 684, 685). Rather, renal failure is caused by tubular incompetence such as tubular obstruction with proteinaceous casts and cell debris, all causing backleak of glomerular ultrafiltrate through the injured tubule (692, 693).

Much of our understanding of the pathophysiology and the cellular and molecular events in ischemic ARF has been generated from a rat model in which ARF is induced by clamping of both renal arteries for periods up to 90 min to induce acute tubular injury, or from cell culture studies in which experimental damage is induced in primary proximal tubule cells with periods of anoxia (incubation under N₂/5%CO₂-conditions).

1. *Expression of GH receptors and the IGF-I system in the rat kidney in ischemic ARF.* Matejka and Jennische (217) examined the expression of IGF-I/IGF-I mRNA during the recovery in rats with ischemic ARF with *in situ* immunohistological and hybridization methods. On days 2 and 3 of reperfusion, when histological findings indicate active tissue repair, particularly in S₃-segments of proximal tubules, regenerative cells transiently express IGF-I peptide and IGF-I mRNA. Furthermore, invading macrophages also express IGF-I. Tissue-binding experiments further indicate that IGF-I receptors are up-regulated in proximal tubules for about 7 days after injury (217) and decline thereafter. These findings strongly suggest that IGF-I regulates tissue repair in injured tubules by autocrine/paracrine mechanisms in this rat model of ARF. In a separate study, Andersson and Jennische (203) demonstrated increased immunohistological staining for IGF-I in S₃-segments of proximal tubules in rats recovering from acute ischemic renal failure. This was found during the first 7 days of reperfusion and is apparently strictly localized to regenerating cells. Adjacent cells not undergoing regeneration did not express IGF-I. This distribution of IGF-I expression during tubule regeneration may indicate that IGF-I is expressed transiently during differentiation of newly formed cells.

Ding and associates (694) examined IGF-I and IGF-I re-

ceptor (mRNA) levels in rats before and after bilateral renal ischemia that was induced by clamping of the renal arteries for 60 min, and in sham-operated control rats on days 1, 3, 4, and 7 of reperfusion (694). Probably as a result of the anesthesia and the surgical stress, serum IGF-I levels fell in both groups during the first 3 days post injury, but more so in ARF rats compared to controls. In kidney of ARF rats, extracted IGF-I peptide and mRNA were decreased in both renal cortex and medulla during the entire 7 days. Renal cortical IGF-I receptor mRNA decreased transiently during the first 5 days, but was increased on day 7 (694). In the renal medulla, IGF-I receptor mRNA was decreased during the initial 3 days after injury, but was increased on the seventh day. In ARF rats compared to sham-controls, hepatic extractable IGF-I peptide and mRNA levels were also decreased for the first 3 days after injury and returned subsequently to baseline. Small decreases in IGF-I, IGF-I mRNA, and IGF-I receptor mRNA were also found to occur transiently in myocardium and in skeletal muscle in rats after acute ischemic renal injury. Thus, IGF-I and IGF-I receptor expression are transiently decreased during ARF in the kidney and in extrarenal organs and this may contribute to the catabolic state that is associated with ARF (694). The studies by Andersson, Matejka, and Ding (203, 217, 694), in concert, indicate that IGF-I and IGF-I receptors are down-regulated in the kidney during the initial phase of recovery from acute ischemic renal failure, except in recovering cells in segments of major injury, namely the S₃-segment of the proximal convoluted tubule, where IGF-I/IGF-I receptors are up-regulated.⁵

Tsao and co-workers (698) examined the renal expression of GH receptors and the IGF-I system during the natural repair process in rat kidneys after 60 min of acute renal ischemia. During the initial 2 days after injury, extracted renal GH receptor mRNA and IGF-I mRNA as well as IGF-I peptide were decreased below control values. These investigators found no change in renal IGF-I receptor mRNA levels but found increased receptor binding, suggesting up-regulation of receptor affinity (698). The renal tissue mRNA levels for IGFBP-2 through -5 were all decreased. IGFBP-3 was most markedly decreased and remained reduced for prolonged periods of time during regeneration. IGFBP-1 mRNA was unchanged compared to controls throughout the course of the disease. GH receptor mRNA levels were also decreased to about 20% of control levels during the initial several days after renal injury (698).

2. *Treatment with rhIGF-I to accelerate the recovery from ARF.* The transient expression of IGF-I in proximal tubules during regeneration after acute renal injury in the rat provides a rationale for the treatment of rats with ARF with IGF-I to accelerate the recovery of tubule integrity and renal function.

⁵ Epidermal growth factor (EGF), which is normally expressed in abundance in distal tubules/collecting segments, virtually disappears in ischemic ARF in the rat for >7 days. Thus, EGF is probably not acting as a mitogen during the natural course of regeneration after ischemic acute renal injury (453). However, EGF receptors in proximal tubules seem to be up-regulated (695). Hepatocyte growth factor (HGF) is normally also expressed in great abundance in the kidney and may also contribute to the recovery after ischemic ARF (696, 697).

Several investigators have studied this question using similar models of acute renal ischemia in the rat (621, 695, 699–703).

Ding *et al.* (700) administered either rhIGF-I or vehicle subcutaneously three times daily to rats with ischemic ARF or to sham control animals (700). Most notably, treatment with rhIGF-I was begun in this study at 5 h after induction of renal injury (clamping of both renal arteries for 60 min) to examine whether a true therapeutic effect of the peptide would occur. Administration of rhIGF-I resulted in doubling of the serum IGF-I levels. During the initial 3 days, serum creatinine (Fig. 14) and urea nitrogen rose less steeply and to lower peak values in ARF rats receiving IGF-I as compared to those receiving vehicle. At 72 h after induction of renal injury, GFR, RPF, the filtration fraction, and the renal extraction of *p*-aminohippuric acid (PAH) were measured and were all much greater in IGF-I treated ARF rats. The extraction of PAH may serve as a marker for the functional integrity of the proximal tubules, since PAH is extracted from serum mainly by proximal tubular secretion through the organic acid transport system. On the third day after injury, IGF-I-treated rats showed a greater rate of [³H]thymidine incorporation into DNA which was extracted from dissected renal tissues or as demonstrated by *in situ* autoradiography (700). The [³H]thymidine uptake was increased 6-fold in proximal tubules (mainly S₃-segments) in IGF-I compared to vehicle-treated animals. The expression of the proliferating cell nuclear antigen (PCNA), a 36-kDa protein in the DNA-polymerase- δ complex that becomes activated during the G₁-, S-, and G₂-phases of the cell cycle, was measured immunohistologically. PCNA was greatly increased in rats receiving rhIGF-I compared to both ARF rats receiving vehicle and sham-operated control animals that were also treated with either IGF-I or vehicle, respectively (700). Histological examination of the kidney indicated a trend toward an increased mitosis index in IGF-I-treated ARF rats.

Since ARF is a catabolic disease, Ding and collaborators (700) also examined whether treatment of ARF rats with

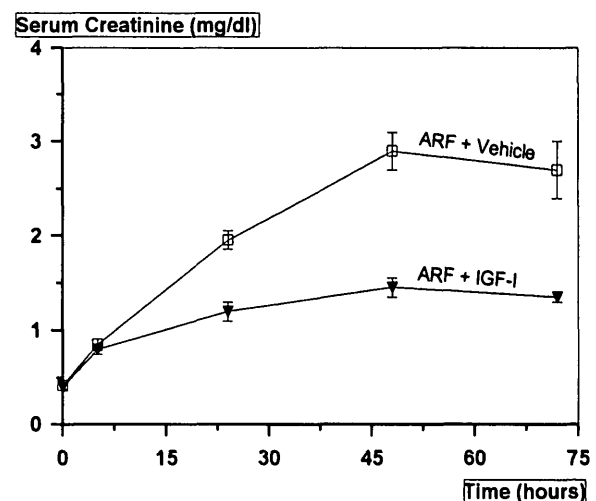


FIG. 14. Serum creatinine in rats with ischemic acute renal failure (ARF) receiving vehicle or IGF-I. The serum creatinine at 0 h depicts the baseline creatinine before inducing ARF. Treatment with vehicle or IGF-I, respectively, was begun after obtaining the serum creatinine at 5 h. [Data derived from (700).]

IGF-I would reduce protein catabolism. Indeed, pair-fed IGF-I-treated ARF rats have reduced nitrogen excretion compared to ARF rats not receiving IGF-I. Moreover, protein synthesis was increased and protein degradation was decreased in excised epitrochlearis muscle from IGF-I-treated as compared to vehicle-treated rats (700).

The laudable effects of rhIGF-I in ARF of the rat were also shown by other investigators. Miller and associates (701) demonstrated a similar acceleration of recovery from ischemic ARF in rats receiving rhIGF-I, and this was associated with increased rates of bromodeoxyuridine incorporation into proximal tubules. Similar results were obtained by Noguchi *et al.* (704). Clark *et al.* (699) demonstrated beneficial effects of des(1-3)IGF-I in using a particularly severe model of ischemic ARF in rats. In this study, administration of the IGF-I variant also significantly reduced mortality. Martin and co-workers (705) found similar effects of IGF-I-treatment in rats with ARF. These investigators also studied two variants of IGF-I, namely des(1-3)IGF-I and LR₃IGF-I. These two peptides might have further advantage, since they do not bind to IGF-BPs and have been shown to have greater activity with regard to body weight gain and nitrogen balance in rats with renal failure (45, 46, 575). Although both IGF-I variants had a greater effect on weight gain in ARF rats compared to IGF-I, there was no advantage in the rate of recovery of renal function with des(1-3)IGF-I, and the outcome was worse compared to vehicle-treated control ARF rats when LR₃IGF-I was administered (705).

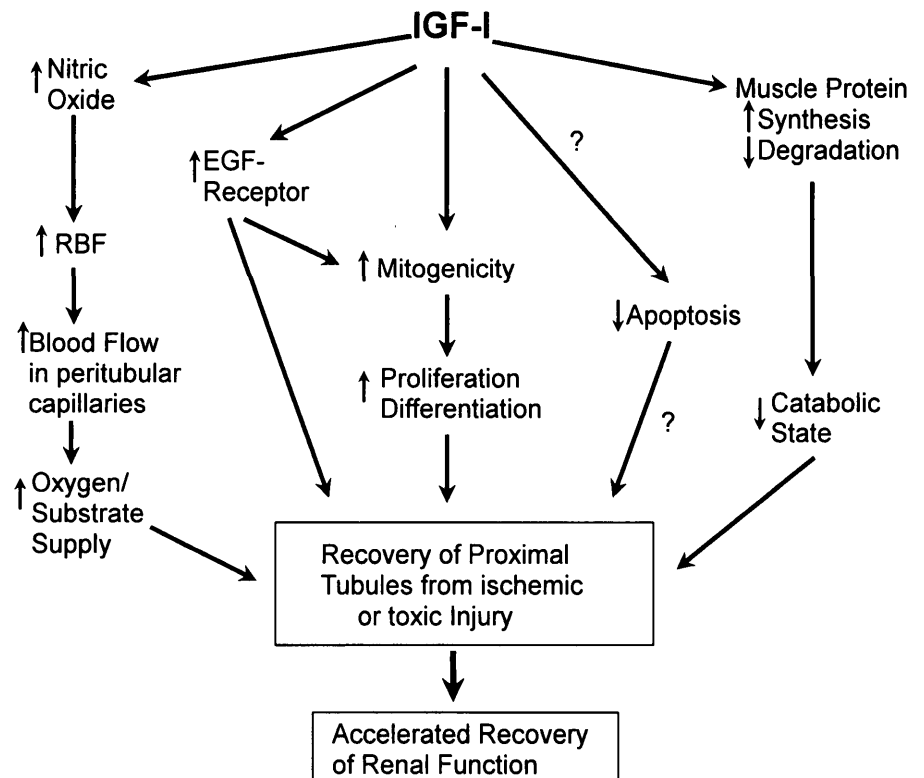
The beneficial effects of rhIGF-I to accelerate the recovery from ARF has also been demonstrated in rat models of (mercury chloride-induced) toxic ARF (695, 706). Administration of other recombinant human growth factor peptides, such as EGF and HGF, have also been demonstrated to be beneficial

in rats with ischemic or toxic acute renal injury and accelerate renal recovery (453, 621, 695, 707-710).

There is direct or circumstantial evidence for several different mechanisms of action by which endogenous or exogenous IGF-I may accelerate the recovery from acute ischemic or toxic renal failure in rats (Fig. 15). First, IGF-I may act as a vasoactive agent that improves renal blood flow, thus increasing the oxygen and substrate supply to proximal tubules, the segment of the nephron sustaining most of the injury. Second, IGF-I may act on tubule cells as a mitogen to accelerate the cell cycle turn-around and improve the rate of mitosis and differentiation of newly ingrowing cells. Third, IGF-I may promote the "healing" of injured but not yet necrotic cells. Fourth, IGF-I may reduce the rate of apoptosis. These different effects may result directly from IGF-I or indirectly by interfering with other local growth factor systems, such as the EGF-system. In fact, there is supportive evidence for each of these different mechanisms either from *in vivo* or from cell culture experiments.

Treatment of ARF rats with IGF-I results in improved renal plasma and blood flow rates (700, 701). As pointed out earlier in this review, the dilation of the renal resistance microvasculature by IGF-I is apparently mediated by NO (*vide supra*). Consequently, Noguchi *et al.* (704) examined whether the effects of treatment with IGF-I to accelerate recovery in rats with ischemic ARF would be ameliorated by an inhibitor to NO synthase (N^G-nitro-L-arginine) that would be administered concurrently. Indeed, in these studies the IGF-I-induced improvement in GFR was inhibited by the NO blocker, suggesting that vasodilation is an important mediating event. However, NO may have only a permissive function, and reduced blood flow may not allow other beneficial events on tubule cells to take place.

FIG. 15. Algorithm summarizing the potential mechanisms of action of IGF-I that contribute to the accelerated recovery of renal function in ARF. The contribution of tubule cell apoptosis to proximal tubule damage and the action of IGF-I on apoptosis are not yet established.



In ARF rats, IGF-I increases the mitosis index and the rate of [³H]-thymidine or bromodeoxyuridine incorporation into tubule cell DNA (700, 701). These effects could result merely from improved blood flow, but cell culture studies suggest a direct effect of IGF-I on proximal tubule cells. In primary cultures of rat proximal tubule cells, anoxic injury and subsequent reoxygenation can be used as an *in vitro* model of acute ischemic tubular injury to examine mechanisms that are independent of blood flow. In this cell culture model, the expression of IGF-I receptor mRNA decreases, not immediately after anoxia, but at 8 and 24 h of reoxygenation, and incubation with IGF-I does not change the expression of the IGF-I receptor (R. Hirschberg, own preliminary observations). However, brief periods of incubation of the cells after anoxia-reoxygenation injury with IGF-I results in activation of the (remaining) receptors as demonstrated by increased receptor autophosphorylation (R. Hirschberg, own unpublished observation). These findings suggest that some effects of IGF-I on proximal tubules may be induced by direct interaction between tubule cell IGF-I receptors and the peptide. IGF-I increases the rate of [³H]thymidine and bromodeoxyuridine incorporation into cell DNA in anoxia-damaged cells, if added to the medium during reoxygenation (R. Hirschberg, own unpublished observation). In this cell culture model, IGF-I also tends to reduce the rate of apoptosis during reoxygenation as demonstrated by end-labeling of DNA-strands using the TUNEL method (R. Hirschberg, own unpublished observation). IGF-I also reduces the incidence of apoptosis in other cell culture models (711). Apoptosis also occurs *in vivo* in rats with ischemic ARF (686, 690), and IGF-I may facilitate the recovery from ARF by reducing the incidence of apoptosis. However, it is unclear whether apoptosis in proximal tubules is an important biological event in human ARF (712).

After acute ischemic renal injury in rats there is an early increase in the renal osteopontin mRNA and protein expression that is localized to the thick ascending limb of the loop of Henle and to distal tubules (713). Administration of IGF-I further increases the osteopontin expression, which is thought to contribute to the healing process, although the mechanisms are unknown. However, much of the damage and action of IGF-I appear to occur in the proximal tubules where osteopontin is not expressed. Thus, osteopontin-mediated effects seem to be less important in the IGF-I-induced acceleration of recovery after experimental ARF in rats.

There is further evidence that some of the beneficial effects of IGF-I in ARF are mediated through the EGF-system.⁶ In rats with ARF, treatment with rhIGF-I increases the EGF

⁶ EGF is normally expressed in the distal nephron (234, 455), and EGF receptors are present in proximal tubule cell membranes (518, 714). In acute ischemic or mercury chloride-induced tubular injury in rats, EGF mRNA and peptide levels decrease rapidly and remain decreased for more than 7 days (453, 715-717), although some investigators found elevated renal immunoreactive EGF (707, 718). Postmortem immunohistochemical staining of human kidneys from patients with ARF and those without ARF demonstrated increased EGF levels in distal tubules and some expression of EGF in proximal tubules in ARF. EGF was found only in distal tubules but not in proximal tubules in patients without ARF (719). EGF receptor immunoreactivity was similar in human kidneys with or without ARF (719). Induction of EGF in proximal tubules was also demonstrated in rats with ARF but not in control animals (716).

receptor phosphorylation as well as specific EGF binding to renal cell membranes suggesting EGF receptor up-regulation (695). Thus, IGF-I may not only act through its own receptor but also by increasing (proximal tubule cell) EGF receptor expression allowing for increased mitogenicity through EGF. Whether there is further action of IGF-I through HGF is possible but at present unknown. rhHGF also accelerates tubule regeneration after ARF (708, 709).

Whether rhIGF-I will also accelerate the recovery from ARF or will reduce the incidence of ARF in high-risk patient populations is currently (May 1996) the subject of two ongoing clinical trials. One such study, a multicenter, randomized, double-blind, placebo-controlled trial has recently been terminated and results have not yet been published. In another study, a single center blinded trial, patients undergoing surgical repair of aortic aneurysms received treatment with rhIGF-I or placebo. In this study, treatment with rhIGF-I was initiated immediately after surgery, before ARF could be diagnosed. Thus, in this study, rhIGF-I was evaluated in its ability to prevent the onset of (clinically apparent) ARF. A preliminary announcement indicates a reduction in the rate of occurrence of ARF in this high-risk patient population by about one-third, although only a very small number of patients acquired ARF.⁷ Thus, rhIGF-I may be a useful therapeutic agent in patients with ARF. In addition to its effects on renal recovery, the anabolic effects of rhIGF-I may also be beneficial, since most patients with ARF are quite catabolic due to the renal disease itself as well as owing to the often severe underlying diseases.

C. The IGF system in the nephrotic syndrome

The nephrotic syndrome results from many different primary or secondary glomerular diseases such as minimal change disease, focal segmental glomerular sclerosis, various glomerulonephritides, diabetic glomerular sclerosis, and others. Owing to the glomerular insult, (selective or nonselective) glomerular ultrafiltration of plasma proteins causes gross proteinuria and urinary losses of many proteins and compounds that are bound to serum proteins. As a result, hypoproteinemia and hypoalbuminemia are invariably present. Hypercholesterolemia and/or hypertriglyceridemia are also found and may be caused by increased hepatic synthesis of lipoproteins as well as increased proximal tubular generation of triglycerides from fatty acids that are associated with ultrafiltered and absorbed albumin. The edema of the nephrotic syndrome results from increased renal sodium retention that is thought to be caused in response to signals arising from an underfilled circulation due to hypoalbuminemia-induced extravasation of plasma water, or by increased intrinsic renal sodium absorption, possibly caused by a defect in distal tubule atrial natriuretic peptide receptor signaling due to increased cGMP-phosphodiesterase activity (720).

Studies in patients with glomerular proteinuria have

Administration of EGF to rats with toxic ARF increases the rate of recovery of renal function (710).

⁷ As communicated during a platform presentation by Steven Miller, M.D. (St. Louis, MO) at the Annual Meeting of the American Society of Nephrology, San Diego, CA, November 1995.

shown that the urinary excretion of IGF-I, IGF-II, and IGFBPs is increased (261, 275). In patients with the nephrotic syndrome, serum levels of both IGF-I and -II are reduced by about 50% and 60%, respectively (275). IGFs are excreted in urine primarily in the 45 kDa-molecular form, but IGFs present in the 150-kDa complex also appear in urine in some nephrotic subjects (275).

This laboratory has recently performed a series of experimental studies in rats with the adriamycin-induced nephrotic syndrome (167, 257–259, 721). In these rats, serum IGF-I levels are reduced by about one-third compared to controls. This is most likely caused by urinary losses of IGF-I (0.61 ± 0.15 pg/mg urinary protein). The liver does not compensate for these losses and does not seem to increase the IGF-I synthesis as indicated by normal liver IGF-I mRNA levels in nephrotic rats (167). Nephrotic rat serum IGFBP-3 levels are reduced. This is probably caused by proteolytic degradation of IGFBP-3 *in vivo*, since a ~27 kDa immunoreactive but not ligand-binding fragment is found in serum from nephrotic but not from control animals (167). Moreover, nephrotic but not normal rat urine contains proteolytic activity at high levels that appears to be Ca-dependent. Nephrotic rat serum may contain some IGFBP-3 proteolytic activity, but certainly far less compared to urine (167). Possibly, the protease or proteases is (are) ultrafiltered by glomeruli with other plasma proteins and then concentrated in nephrotic urine due to tubular water absorption. Alternatively, the protease may be primarily expressed in the kidney and serum IGFBP-3 proteolysis, which occurs during the passage of blood through the kidneys in the nephrotic syndrome. Such selective tissue-specific expression of IGFBP-3 protease activity has been shown in rat pregnancy, although not in the kidney (602). In nephrotic rats IGFBP-3 and IGFBP-3 proteolytic fragments are excreted with the urine, but IGFBP-3 cannot be detected by Western ligand blot analysis in proximal tubule fluid (167, 257, 258). This finding may suggest that urinary IGFBP-3 is primarily of tubular origin, as has also been suggested by Gargosky and associates (261) based on findings in nonnephrotic humans.

IGFBP-4 serum levels are also slightly reduced in nephrotic rats. In contrast, serum IGFBP-2 levels are greatly increased, despite the fact that this binding protein is ultrafiltered in glomeruli and excreted in urine (167, 257). This is probably due to increased synthesis of IGFBP-2 in liver as suggested by 5-fold greater hepatic IGFBP-2 mRNA levels as compared to normal control animals. Furthermore, IGFBP-2 can be immunoprecipitated from nephrotic but not normal rat liver lysates (167). The reduction of IGFBP-3 and the increase of serum IGFBP-2 levels suggests that more IGFs are distributed in the 45-kDa protein complex as compared to the 150-kDa binding protein complex. This may be of importance with regard to the tissue availability of bound IGFs.

Nephron micropuncture studies demonstrated that IGF-I is present in glomerular ultrafiltrate in nephrotic rats at 1.35 nM in association (but probably not bound to) IGFBP-2. IGF-I in tubule fluid may act through apical tubule IGF-I receptors and may contribute to increased phosphate absorption and extracellular matrix protein synthesis. Indeed, nephrotic rat urine extracts increase the collagen type I and IV secretion in cultured proximal tubular cells which is partly ameliorated

by coincubation with neutralizing IGF-I receptor antibody (258). Thus, the ultrafiltration of IGF-I in the nephrotic syndrome may contribute to interstitial fibrosis, which is commonly found in patients with chronic glomerular diseases causing heavy proteinuria and contributes substantially to the progression of chronic renal failure.

IX. Summary

GH receptors, IGFs, and IGF-receptors are expressed in the kidney. Their location in the different parts of the nephron suggests autocrine or paracrine as well as endocrine modes of action. A lack of GH receptors and probably of IGF-I synthesis in glomeruli *in vivo* suggest that all glomerular GH and IGF-I effects are mediated by circulating IGF-I through endocrine modes. GH and IGF-I increase GFR in normal rats and humans, and increase phosphate and possibly sodium reabsorption in normal and diabetic subjects. During normal renal development GH, IGF-I, and IGF-II appear to play a role. GH and IGF-I cause kidney growth, and circulating and/or renal IGF-I appear to contribute to renal hypertrophy and compensatory renal growth in experimental animal models. GH may contribute also to glomerular sclerosis and progression of renal failure in experimental models. In patients with chronic renal failure such a role of endogenous or exogenous GH has not yet been convincingly proven.

In chronic or acute renal failure and in the nephrotic syndrome there are complex abnormalities in the systemic and renal IGF/IGFBP-system. In chronic renal failure there is resistance to GH and IGF-I that can be overridden by pharmacological administration of each of the peptides. GH is used therapeutically in children with chronic renal failure to accelerate growth. GH and IGF-I may be useful agents to improve nitrogen balance and nutritional status in patients with chronic renal failure. In rats with ARF, administration of IGF-I accelerates the recovery of renal function. Whether this treatment is also successful in patients with ARF remains to be demonstrated by ongoing clinical trials.

References

1. Hirschberg R, Kopple J 1995 Insulin-like growth factor I and renal function. *Diabetes Rev* 3:177–195
2. O'Shea M, Miller SB, Hammerman MR 1993 Insulin-like growth factor I and the kidney. *Semin Nephrol* 13:96–108
3. Hammerman M 1989 The growth hormone-insulin-like growth factor axis in kidney. *Am J Physiol* 257:F503–F514
4. Hammerman M, Miller S 1993 The growth hormone insulin-like growth factor axis in kidney revisited. *Am J Physiol* 265:F1–F14
5. Jones JI, Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev* 16:3–34
6. Chen E, Liao Y, Smith D, Barrera-Saldana H, Gelinas R, Seeburg P 1989 The human growth hormone locus: nucleotide sequence, biology, and evolution. *Genomics* 4:479–497
7. Roskam WG, Rougeon F 1979 Molecular cloning and nucleotide sequence of the human growth hormone structural gene. *Nucleic Acids Res* 7:305–320
8. Goodman HM, Tai LR, Ray J, Cooke NE, Liebhaber SA 1991 Human growth hormone variant produces insulin-like and lipolytic responses in rat adipose tissue. *Endocrinology* 129:1779–1783
9. Cooke N, Ray J, Emery J, Liebhaber S 1988 Two distinct species of human growth hormone-variant mRNA in the human placenta

- predict the expression of novel growth hormone proteins. *J Biol Chem* 263:9001-9006
10. Harel Z, Tannenbaum GS 1993 Dietary protein restriction impairs both spontaneous and growth hormone-releasing factor-stimulated growth hormone release in the rat. *Endocrinology* 133:1035-1043
 11. Van Cauter E, Kerkhofs M, Caufriez A, Van Onderbergen A, Thorner MO, Copinschi G 1992 A quantitative estimation of growth hormone secretion in normal man: reproducibility and relation to sleep and time of day. *J Clin Endocrinol Metab* 74:1441-1450
 12. Winer LM, Shaw MA, Baumann G 1990 Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. *J Clin Endocrinol Metab* 70:1678-1686
 13. Rogol A 1991 Growth hormone physiology and pathophysiology. *Growth* 7:1-4
 14. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI 1987 Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature* 330:537-543
 15. Smith WC, Kuniyoshi J, Talamantes F 1989 Mouse serum growth hormone (GH) binding protein has GH receptor extracellular and substituted transmembrane domains. *Mol Endocrinol* 3:984-990
 16. Edens A, Southard JN, Talamantes F 1994 Mouse growth hormone-binding protein and growth hormone receptor transcripts are produced from a single gene by alternative splicing. *Endocrinology* 135:2802-2805
 17. Barnard R, Thordarson G, Lopez MF, Yamaguchi M, Edens A, Cramer SD, Ogren L, Talamantes F 1994 Expression of growth hormone-binding protein with a hydrophilic carboxyl terminus by the mouse placenta: studies *in vivo* and *in vitro*. *J Endocrinol* 140:125-135
 18. Barnard R, Southard JN, Edens A, Talamantes F 1993 Growth hormone receptor and growth hormone-binding protein messages in mouse placenta contain the exon analogous to human exon 3. *Endocrinology* 133:1474-1477
 19. Mathews LS, Enberg B, Norstedt G 1989 Regulation of rat growth hormone receptor gene expression. *J Biol Chem* 264:9905-9910
 20. Postel-Vinay MC, Fontoura M 1991 Regulation of growth hormone binding proteins in human plasma. *Acta Paediatr Scand Suppl* 379:79-83
 21. Baumbach WR, Horner DL, Logan JS 1989 The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. *Genes Dev* 3:1199-205
 22. Smith WC, Linzer DJ, Talamantes F 1988 Detection of two growth hormone receptor mRNAs and primary translation products in the mouse. *Proc Natl Acad Sci USA* 85:9576-9579. [published erratum appears in *Proc Natl Acad Sci USA* 1989:3664, 1986].
 23. Smith IF, Taiwo O, Payne-Robinson HM 1989 Plasma somatomedin-C in Nigerian malnourished children fed a vegetable protein rehabilitation diet. *Eur J Clin Nutr* 43:705-713
 24. Ymer SI, Herington AC 1992 Developmental expression of the growth hormone receptor gene in rabbit tissues. *Mol Cell Endocrinol* 83:39-49
 25. Baumann G, Shaw MA 1990 Plasma transport of the 20,000-dalton variant of human growth hormone (20K): evidence for a 20K-specific binding site. *J Clin Endocrinol Metab* 71:1339-1343
 26. Schwander JC, Hauri C, Zapf J, Froesch ER 1983 Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. *Endocrinology* 113:297-305
 27. 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. *Proc Natl Acad Sci USA* 81:935-939
 28. D'Ercole A, Underwood LE 1987 Estimation of tissue concentrations of somatomedin C/insulin-like growth factor I. *Methods Enzymol* 146:227-233
 29. D'Ercole AJ, Hill DJ, Strain AJ, Underwood LE 1986 Tissue and plasma somatomedin-C/insulin-like growth factor I concentrations in the human fetus during the first half of gestation. *Pediatr Res* 20:253-255
 30. Rinderknecht E, Humbel RE 1978 The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J Biol Chem* 253:2769-2776
 31. Blundell TL, Bedarkar S, Rinderknecht E, Humbel RE 1978 Insulin-like growth factor: a model for tertiary structure accounting for immunoreactivity and receptor binding. *Proc Natl Acad Sci USA* 75:180-184
 32. Rinderknecht E, Humbel RE 1976 Amino-terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like and cell-growth-promoting activities: evidence for structural homology with insulin B chain. *Proc Natl Acad Sci USA* 73:4379-4381
 33. Rinderknecht E, Humbel RE 1978 Primary structure of human insulin-like growth factor II. *FEBS Lett* 89:283-286
 34. Shimatsu A, Rotwein P 1987 Sequence of two rat insulin-like growth factor I mRNAs differing within the 5' untranslated region. *Nucleic Acids Res* 15:7196
 35. Shimatsu A, Rotwein P 1987 Mosaic evolution of the insulin-like growth factors. Organization, sequence, and expression of the rat insulin-like growth factor I gene. *J Biol Chem* 262:7894-7900
 36. Bell GI, Merryweather JP, Sanchez-Pescador R, Stempien MM, Priestley L, Scott J, Rall LB 1984 Sequence of a cDNA clone encoding human preproinsulin-like growth factor II. *Nature* 310:775-777
 37. Rotwein P, Pollock KM, Didier DK, Krivi GG 1986 Organization and sequence of the human insulin-like growth factor I gene. Alternative RNA processing produces two insulin-like growth factor I precursor peptides. *J Biol Chem* 261:4828-4832
 38. Rotwein P 1986 Two insulin-like growth factor I messenger RNAs are expressed in human liver. *Proc Natl Acad Sci USA* 83:77-81
 39. Adamo ML, Lanau F, Neuenschwander S, Werner H, LeRoith D, Roberts Jr CT 1993 Distinct promoters in the rat insulin-like growth factor-I (IGF-I) gene are active in CHO cells [published erratum appears in *Endocrinology* 133:792, 1993]. *Endocrinology* 132:935-937
 40. Adamo ML, Neuenschwander S, LeRoith D, Roberts Jr CT, 1993 Structure, expression, and regulation of the IGF-I gene. *Adv Exp Med Biol* 343:1-11
 41. Oh Y, Muller HL, Zhang H, Ling N, Rosenfeld RG 1993 Synthesis and characterization of IGF-II analogs: applications in the evaluation of IGF receptor function and IGF-independent actions of IGF-BPs. *Adv Exp Med Biol* 343:41-54
 42. Hizuka N, Takano K, Asakawa K, Sukegawa I, Fukuda I, Demura H, Iwashita M, Adachi T, Shizume K 1991 Measurement of free form of insulin-like growth factor I in human plasma. *Growth Regul* 1:51-55
 43. Takano K, Hizuka N, Shizume K, Asakawa K, Fukuda I, Demura H 1991 Repeated sc administration of recombinant human insulin-like growth factor I (IGF-I) to human subjects for 7 days. *Growth Regul* 1:23-28
 44. Frystyk J, Skjaerbaek C, Dinesen B, Orskov H 1994 Free insulin like growth factor (IGF-I and IGF-II) in human serum. *FEBS Lett* 348:185-191
 45. Ballard F, Francis G, Bagley C, Szabo L, Wallace J 1989 Effects of insulin-like growth factors on protein metabolism: Why are some molecular variants more potent? *Biochem Soc Symp* 55:91-104
 46. Ross M, Francis G, Szabo L, Wallace J, Ballard F 1989 Insulin-like growth factor (IGF)-binding proteins inhibit the biological activities of IGF-I and IGF-2 but not des-(1-3)-IGF-I. *Biochem J* 258:267-272
 47. Cascieri MA, Chicchi GG, Bayne ML 1991 Characterization of the biological activity of IGF I analogs with reduced affinity for IGF receptors and binding proteins. *Adv Exp Med Biol* 293:23-30
 48. Cascieri MA, Bayne ML 1993 Analysis of the interaction of IGF-I analogs with the IGF-I receptor and IGF binding proteins. *Adv Exp Med Biol* 343:33-40
 49. Saunders JC, Dickson MC, Pell JM, Gilmour RS 1991 Expression of a growth hormone-responsive exon of the ovine insulin-like growth factor-I gene. *J Mol Endocrinol* 7:233-240
 50. Pell JM, Gilmour RS 1993 Differential regulation of IGF-I leader exon transcription. *Adv Exp Med Biol* 343:13-23
 51. Mathews LS, Norstedt G, Palmiter RD 1986 Regulation of insulin-like growth factor I gene expression by growth hormone. *Proc Natl Acad Sci USA* 83:9343-9347
 52. Bortz JD, Rotwein P, DeVol D, Bechtel PJ, Hansen VA,

- Hammerman MR 1988 Focal expression of insulin-like growth factor I in rat kidney collecting duct. *J Cell Biol* 107:811-819
53. Miller SB, Rotwein P, Bortz JD, Bechtel PJ, Hansen VA, Rogers SA, Hammerman MR 1990 Renal expression of IGF I in hypersomatotropic states. *Am J Physiol* 259:F251-F257
 54. Schimpff RM, Donnadieu M, Duval M 1980 Serum somatomedin activity measured as sulphation factor in peripheral, hepatic and renal veins in normal mongrel dogs: early effects of intravenous injection of growth hormone. *Acta Endocrinol (Copenh)* 93:155-161
 55. Schimpff RM, Donnadieu M, Duval M 1980 Serum somatomedin activity measured as sulphation factor in peripheral, hepatic and renal veins of mongrel dogs: basal levels. *Acta Endocrinol (Copenh)* 93:67-72
 56. Bach MA, Chin E, Bondy CA 1994 The effects of subcutaneous insulin-like growth factor-I infusion in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 79:1040-1045
 57. Tada H, Watanabe Y, Futakuchi Y, Amino N 1994 Change in serum concentration of insulin-like growth factor II (IGF-II) in patients with thyroid disease. *Endocr J* 41:541-545
 58. Tally M, Eriksson U, Thoren M, Brismar K, Hall K 1994 Immunoreactive proinsulin-like growth factor-II levels in healthy subjects, patients with growth hormone deficiency, and patients with type 1 diabetes: effects of insulin-like growth factor-I and insulin. *J Clin Endocrinol Metab* 79:1576-1581
 59. de Pagter-Holthuisen P, Jansen M, van Schaik FM, van der Kammen R, Oosterwijk C, Van den Brande JL, Süßenbach JS 1987 The human insulin-like growth factor II gene contains two development-specific promoters. *FEBS Lett* 214:259-264
 60. De Pagter-Holthuisen P, Jansen M, Bovenberg W, Van den Brande JL, Süßenbach JS 1989 The human somatomedin/insulin-like growth factor genes: organization and development-specific expression. *J Intern Med* 225:37-42
 61. de Pagter-Holthuisen P, Jansen M, van der Kammen RA, van Schaik FM, Süßenbach JS 1988 Differential expression of the human insulin-like growth factor II gene. Characterization of the IGF-II mRNAs and an mRNA encoding a putative IGF-II-associated protein. *Biochim Biophys Acta* 950:282-295
 62. Süßenbach JS, Rodenburg RJ, Scheper W, Holthuisen P 1993 Transcriptional and post-transcriptional regulation of the human IGF-II gene expression. *Adv Exp Med Biol* 343:63-71
 63. Steele-Perkins G, Turner J, Edman JC, Hari J, Pierce SB, Stover C, Rutter WJ, Roth RA 1988 Expression and characterization of a functional human insulin-like growth factor I receptor. *J Biol Chem* 263:11486-11492
 64. Germain-Lee EL, Janicot M, Lammers R, Ullrich A, Casella SJ 1992 Expression of a type I insulin-like growth factor receptor with low affinity for insulin-like growth factor II. *Biochem J* 281:413-417
 65. Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J, Fujita-Yamaguchi Y 1986 Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J* 5:2503-2512
 66. Wada J, Liu ZZ, Alvares K, Kumar A, Wallner E, Makino H, Kanwar YS 1993 Cloning of cDNA for the alpha subunit of mouse insulin-like growth factor I receptor and the role of the receptor in metanephric development. *Proc Natl Acad Sci USA* 90:10360-10364
 67. Werner H, Woloschak M, Adama M, Shen-Orr Z, Roberts C, LeRoith D 1989 Developmental regulation of the rat insulin-like growth factor I receptor gene. *Proc Natl Acad Sci USA* 86:7451-7455
 68. Frattali AL, Treadway JL, Pessin JE 1992 Transmembrane signaling by the human insulin receptor kinase. Relationship between intramolecular beta subunit trans- and cis-autophosphorylation and substrate kinase activation. *J Biol Chem* 267:19521-19528
 69. Frattali AL, Pessin JE 1993 Relationship between alpha subunit ligand occupancy and beta subunit autophosphorylation in insulin/insulin-like growth factor-1 hybrid receptors. *J Biol Chem* 268:7393-7400
 70. Soos MA, Field CE, Lammers R, Ullrich A, Zhang B, Roth RA, Andersen AS, Kjeldsen T, Siddle K 1992 A panel of monoclonal antibodies for the type I insulin-like growth factor receptor. Epitope mapping, effects on ligand binding, and biological activity. *J Biol Chem* 267:12955-12963
 71. Xiong L, Kasuya J, Li SL, Kato J, Fujita-Yamaguchi Y 1992 Growth-stimulatory monoclonal antibodies against human insulin-like growth factor I receptor. *Proc Natl Acad Sci USA* 89:5356-5360
 72. Gronborg M, Wulff BS, Rasmussen JS, Kjeldsen T, Gammeltoft S 1993 Structure-function relationship of the insulin-like growth factor-I receptor tyrosine kinase. *J Biol Chem* 268:23435-23440
 73. Kato H, Faria TN, Stannard B, Roberts Jr CT, LeRoith D 1993 Role of tyrosine kinase activity in signal transduction by the insulin-like growth factor-I (IGF-I) receptor. Characterization of kinase-deficient IGF-I receptors and the action of an IGF-I-mimetic antibody (alpha IR-3). *J Biol Chem* 268:2655-2661
 74. Kato H, Faria TN, Stannard B, Roberts Jr CT, LeRoith D 1994 Essential role of tyrosine residues 1131, 1135, and 1136 of the insulin-like growth factor-I (IGF-I) receptor in IGF-I action. *Mol Endocrinol* 8:40-50
 75. Murakami MS, Rosen OM 1991 The role of insulin receptor autophosphorylation in signal transduction. *J Biol Chem* 266:22653-22660
 76. Stannard B, Blakesley V, Kato H, Roberts C, LeRoith D 1995 Single tyrosine substitution in the insulin-like growth factor I receptor inhibits ligand-induced receptor autophosphorylation and internalization, but not mitogenesis. *Endocrinology* 136:4918-4924
 77. MacDonald RG, Pfeffer SR, Coussens L, Tepper MA, Brocklebank CM, Mole JE, Anderson JK, Chen E, Czech MP, Ullrich A 1988 A single receptor binds both insulin-like growth factor II and mannose-6-phosphate. *Science* 239:1134-1137
 78. Oshima A, Nolan CM, Kyle JW, Grubb JH, Sly WS 1988 The human cation-independent mannose 6-phosphate receptor. Cloning and sequence of the full-length cDNA and expression of functional receptor in COS cells. *J Biol Chem* 263:2553-2562
 79. Pohlmann R, Nagel G, Schmidt B, Stein M, Lorkowski G, Krentler C, Cully J, Meyer HE, Grzeschik KH, Mersmann G, Haslik A, von Figura K 1987 Cloning of a cDNA encoding the human cation-dependent mannose 6-phosphate-specific receptor. *Proc Natl Acad Sci USA* 84:5575-5579
 80. Nielsen FC 1992 The molecular and cellular biology of insulin-like growth factor II. *Prog Growth Factor Res* 4:257-290
 81. Czech MP 1989 Signal transmission by the insulin-like growth factors. *Cell* 59:235-238
 82. Rechler MM, Nissley SP 1985 The nature and regulation of the receptors for insulin-like growth factors. *Annu Rev Physiol* 47:425-442
 83. Corvera S, Whitehead RE, Mottola C, Czech MP 1986 The insulin-like growth factor II receptor is phosphorylated by a tyrosine kinase in adipocyte plasma membranes. *J Biol Chem* 261:7675-7679
 84. Corvera S, Roach PJ, DePaoli-Roach AA, Czech MP 1988 Insulin action inhibits insulin-like growth factor-II (IGF-II) receptor phosphorylation in H-35 hepatoma cells. IGF-II receptors isolated from insulin-treated cells exhibit enhanced *in vitro* phosphorylation by casein kinase II. *J Biol Chem* 263:3116-3122
 85. Rogers SA, Purchio AF, Hammerman MR 1990 Mannose 6-phosphate-containing peptides activate phospholipase C in proximal tubular basolateral membranes from canine kidney. *J Biol Chem* 265:9722-9727
 86. Kyle JW, Nolan CM, Oshima A, Sly WS 1988 Expression of human cation-independent mannose 6-phosphate receptor cDNA in receptor-negative mouse P388D1 cells following gene transfer. *J Biol Chem* 263:16230-16235
 87. Willingham MC, Pastan IH, Sahagian GG, Jourdain GW, Neufeld EF 1981 Morphologic study of the internalization of a lysosomal enzyme by the mannose 6-phosphate receptor in cultured Chinese hamster ovary cells. *Proc Natl Acad Sci USA* 78:6967-6971
 88. Geuze HJ, Slot JW, Strous GJ, Peppard J, von Figura K, Hasilik A, Schwartz AL 1984 Intracellular receptor sorting during endocytosis: comparative immunoelectron microscopy of multiple receptors in rat liver. *Cell* 37:195-204
 89. Geuze HJ, Slot JW, Strous GJ, Lodish HF, Schwartz AL 1983 Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis. *Cell* 32:277-287

90. Matsunaga H, Nishimoto I, Kojima I, Yamashita N, Kurokawa K, Ogata E 1988 Activation of a calcium-permeable cation channel by insulin-like growth factor II in BALB/c 3T3 cells. *Am J Physiol* 255:C442-C446
91. Kojima I, Nishimoto I, Iiri T, Ogata E, Rosenfeld R 1988 Evidence that type II insulin-like growth factor receptor is coupled to calcium gating system. *Biochem Biophys Res Commun* 154:9-19
92. Deleted in proof
93. Nishimoto I, Murayama Y, Katada T, Ui M, Ogata E 1989 Possible direct linkage of insulin-like growth factor-II receptor with guanine nucleotide-binding proteins. *J Biol Chem* 264:14029-14038
94. Okamoto T, Katada T, Murayama Y, Ui M, Ogata E, Nishimoto I 1990 A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. *Cell* 62:709-717
95. Takahashi K, Murayama Y, Okamoto T, Yokota T, Ikezu T, Takahashi S, Giambarella U, Ogata E, Nishimoto I 1993 Conversion of G-protein specificity of insulin-like growth factor II/mannose 6-phosphate receptor by exchanging of a short region with beta-adrenergic receptor. *Proc Natl Acad Sci USA* 90:11772-11776
96. Nishimoto I 1993 The IGF-II receptor system: a G protein-linked mechanism. *Mol Reprod Dev* 35:398-407
97. Ikezu T, Okamoto T, Giambarella U, Yokota T, Nishimoto I 1995 *In vivo* coupling of insulin-like growth factor II/mannose 6-phosphate receptor to heteromeric G proteins. Distinct roles of cytoplasmic domains and signal sequestration by the receptor. *J Biol Chem* 270:29224-29228
98. Hari J, Pierce SB, Morgan DO, Sara V, Smith MC, Roth RA 1987 The receptor for insulin-like growth factor II mediates an insulin-like response. *EMBO J* 6:3367-3371
99. Nishimoto I, Hata Y, Ogata E, Kojima I 1987 Insulin-like growth factor II stimulates calcium influx in competent BALB/c 3T3 cells primed with epidermal growth factor. Characteristics of calcium influx and involvement of GTP-binding protein. *J Biol Chem* 262:12120-12126
100. Nishimoto I, Ohkuni Y, Ogata E, Kojima I 1987 Insulin-like growth factor II increases cytoplasmic free calcium in competent BALB/c 3T3 cells treated with epidermal growth factor. *Biochem Biophys Res Commun* 142:275-286
101. Hirschberg R, Ding H, Wanner C 1995 Effects of insulin-like growth factor I on phosphate transport in cultured proximal tubule cells. *J Lab Clin Med* 126:428-434
102. Hirai K, Miyamasu M, Yamaguchi M, Nakajima K, Ohtoshi T, Koshino T, Takaishi T, Morita Y, Ito K 1993 Modulation of human basophil histamine release by insulin-like growth factors. *J Immunol* 150:1503-1508
103. Adashi EY, Resnick CE, Rosenfeld RG 1990 Insulin-like growth factor-I (IGF-I) and IGF-II hormonal action in cultured rat granulosa cells: mediation via type I but not type II IGF receptors. *Endocrinology* 126:216-222
104. DeChiara TM, Efstratiadis A, Robertson EJ 1990 A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* 345:78-80
105. Nissley P, Kiess W, Sklar M 1993 Developmental expression of the IGF-II/mannose 6-phosphate receptor. *Mol Reprod Dev* 35:408-413
106. Bobek G, Scott CD, Baxter RC 1991 Secretion of soluble insulin-like growth factor-II/mannose 6-phosphate receptor by rat tissues in culture. *Endocrinology* 128:2204-2206
107. Bobek G, Scott CD, Baxter RC 1992 Radioimmunoassay of soluble insulin-like growth factor-II/mannose 6-phosphate receptor: developmental regulation of receptor release by rat tissues in culture. *Endocrinology* 130:3387-3394
108. Clairmont KB, Czech MP 1991 Extracellular release as the major degradative pathway of the insulin-like growth factor II/mannose 6-phosphate receptor. *J Biol Chem* 266:12131-12134
109. Binkert C, Landwehr J, Mary JL, Schwander J, Heinrich G 1989 Cloning, sequence analysis and expression of a cDNA encoding a novel insulin-like growth factor binding protein (IGFBP-2). *EMBO J* 8:2497-2502
110. Brinkman A, Groffen C, Kortleve DJ, Geurts van Kessel A, Drop SL 1988 Isolation and characterization of a cDNA encoding the low molecular weight insulin-like growth factor binding protein (IBP-1). *EMBO J* 7:2417-2423
111. Brinkman A, Groffen CA, Kortleve DJ, Drop SL 1988 Organization of the gene encoding the insulin-like growth factor binding protein IBP-1. *Biochem Biophys Res Commun* 157:898-907
112. Brown AL, Chiariotti L, Orłowski CC, Mehman T, Burgess WH, Ackerman EJ, Bruni CB, Rechler MM 1989 Nucleotide sequence and expression of a cDNA clone encoding a fetal rat binding protein for insulin-like growth factors. *J Biol Chem* 264:5148-5154
113. Brewer MT, Stetler GL, Squires CH, Thompson RC, Busby WH, Clemmons DR 1988 Cloning, characterization, and expression of a human insulin-like growth factor binding protein [published erratum appears in *Biochem Biophys Res Commun* 1988 Sep 30; 155(3):1485]. *Biochem Biophys Res Commun* 152:1289-1297
114. Albiston AL, Herington AC 1990 Cloning and characterization of the growth hormone-dependent insulin-like growth factor binding protein (IGFBP-3) in the rat. *Biochem Biophys Res Commun* 166:892-897
115. Agarwal N, Hsieh CL, Sills D, Swaroop M, Desai B, Francke U, Swaroop A 1991 Sequence analysis, expression and chromosomal localization of a gene, isolated from a subtracted human retina cDNA library, that encodes an insulin-like growth factor binding protein (IGFBP2). *Exp Eye Res* 52:549-561
116. Albiston AL, Herington AC 1992 Tissue distribution and regulation of insulin-like growth factor (IGF)-binding protein-3 messenger ribonucleic acid (mRNA) in the rat: comparison with IGF-I mRNA expression. *Endocrinology* 130:497-502
117. Albiston AL, Saffery R, Herington AC 1995 Cloning and characterization of the promoter for the rat insulin-like growth factor-binding protein-3 gene. *Endocrinology* 136:696-704
118. Dai J, Baxter RC 1992 Molecular cloning of the acid-labile subunit of the rat insulin-like growth factor binding protein complex. *Biochem Biophys Res Commun* 188:304-309
119. Drop SL, Schuller AG, Lindenbergh-Kortleve DJ, Groffen C, Brinkman A, Zwarthoff EC 1992 Structural aspects of the IGFBP family. *Growth Regul* 2:69-79
120. Kiefer MC, Masiarz FR, Bauer DM, Zapf J 1991 Identification and molecular cloning of two new 30-kDa insulin-like growth factor binding proteins isolated from adult human serum. *J Biol Chem* 266:9043-9049
121. Kiefer MC, Ioh RS, Bauer DM, Zapf J 1991 Molecular cloning of a new human insulin-like growth factor binding protein. *Biochem Biophys Res Commun* 176:219-225
122. LaTour D, Mohan S, Linkhart TA, Baylink DJ, Strong DD 1990 Inhibitory insulin-like growth factor-binding protein: cloning, complete sequence, and physiological regulation. *Mol Endocrinol* 4:1806-1814
123. Margot JB, Binkert C, Mary JL, Landwehr J, Heinrich G, Schwander J 1989 A low molecular weight insulin-like growth factor binding protein from rat: cDNA cloning and tissue distribution of its messenger RNA. *Mol Endocrinol* 3:1053-1060
124. Mohn KL, Melby AE, Tewari DS, Laz TM, Taub R 1991 The gene encoding rat insulin-like growth factor-binding protein 1 is rapidly and highly induced in regenerating liver. *Mol Cell Biol* 11:1393-1401
125. Murphy LJ, Seneviratne C, Ballejo G, Croze F, Kennedy TG 1990 Identification and characterization of a rat decidua insulin-like growth factor-binding protein complementary DNA. *Mol Endocrinol* 4:329-336
126. Julkunen M, Koistinen R, Aalto-Setälä K, Seppälä M, Janne OA, Kontula K 1988 Primary structure of human insulin-like growth factor-binding protein/placental protein 12 and tissue-specific expression of its mRNA. *FEBS Lett* 236:295-302
127. Luthman H, Soderling-Barros J, Persson B, Engberg C, Stern I, Lake M, Franzen SA, Israelsson M, Raden B, Lindgren B, Hjelmqvist L, Enobäck S, Carlsson P, Bjuosell G, Pouva G, Holl K, Jörnvoll H 1989 Human insulin-like growth-factor-binding protein. Low-molecular-mass form: protein sequence and cDNA cloning. *Eur J Biochem* 180:259-265
128. Shimasaki S, Emoto N, Koba A, Mercado M, Shibata F, Cooksey K, Baird A, Ling N 1988 Complementary DNA cloning and sequencing of rat ovarian basic fibroblast growth factor and tissue distribution study of its mRNA. *Biochem Biophys Res Commun* 157:256-263
129. Shimasaki S, Koba A, Mercado M, Shimonaka M, Ling N 1989

- Complementary DNA structure of the high molecular weight rat insulin-like growth factor binding protein (IGFBP3) and tissue distribution of its mRNA. *Biochem Biophys Res Commun* 165:907-912
130. Shimasaki S, Shimonaka M, Ui M, Inouye S, Shibata F, Ling N 1990 Structural characterization of a follicle-stimulating hormone action inhibitor in porcine ovarian follicular fluid. Its identification as the insulin-like growth factor-binding protein. *J Biol Chem* 265: 2198-2202
 131. Shimasaki S, Uchiyama F, Shimonaka M, Ling N 1990 Molecular cloning of the cDNAs encoding a novel insulin-like growth factor-binding protein from rat and human. *Mol Endocrinol* 4:1451-1458
 132. Shimasaki S, Ling N 1991 Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5, and -6). *Prog Growth Factor Res* 3:243-266
 133. Shimasaki S, Gao L, Shimonaka M, Ling N 1991 Isolation and molecular cloning of insulin-like growth factor-binding protein-6. *Mol Endocrinol* 5:938-948
 134. Shimasaki S, Shimonaka M, Zhang HP, Ling N 1991 Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a novel IGFBP-5 in rat and human. *J Biol Chem* 266:10646-10653
 135. Shimonaka M, Schroeder R, Shimasaki S, Ling N 1989 Identification of a novel binding protein for insulin-like growth factors in adult rat serum. *Biochem Biophys Res Commun* 165:189-195
 136. Schuller AG, Groffen C, van Neck JW, Zwarthoff EC, Drop SL 1994 cDNA cloning and mRNA expression of the six mouse insulin-like growth factor binding proteins. *Mol Cell Endocrinol* 104:57-66
 137. Spratt SK, Tatsuno GP, Sommer A 1991 Cloning and characterization of bovine insulin-like growth factor binding protein-3 (bIGFBP-3). *Biochem Biophys Res Commun* 177:1025-32
 138. Wood WI, Cachianes G, Henzel WJ, Winslow GA, Spencer SA, Hellmiss R, Martin JL, Baxter RC 1988 Cloning and expression of the growth hormone-dependent insulin-like growth factor-binding protein. *Mol Endocrinol* 2:1176-1185
 139. Zapf J, Kiefer M, Merryweather J, Musiarz F, Bauer D, Born W, Fischer JA, Froesch ER 1990 Isolation from adult human serum of four insulin-like growth factor (IGF) binding proteins and molecular cloning of one of them that is increased by IGF I administration and in extrapancreatic tumor hypoglycemia. *J Biol Chem* 265:14892-14898
 140. Thweatt R, Fleischmann R, Goldstein S 1993 Analysis of the primary structure of insulin-like growth factor binding protein-3 cDNA from Werner Syndrome fibroblasts. *DNA Seq* 4:43-46
 141. Bautista CM, Baylink DJ, Mohan S 1991 Isolation of a novel insulin-like growth factor (IGF) binding protein from human bone: a potential candidate for fixing IGF-II in human bone. *Biochem Biophys Res Commun* 176:756-763
 142. Holly J 1993 Insulin-like growth factor binding proteins in diabetic and non-diabetic states. In: Flyvbjerg A, Orskov H, Alberti K (eds) *Growth Hormone and Insulin-Like Growth Factor I in Human and Experimental Diabetes*. John Wiley & Sons, Chichester, UK, pp 47-76
 143. Mohan S, Bautista CM, Wergedal J, Baylink DJ 1989 Isolation of an inhibitory insulin-like growth factor (IGF) binding protein from bone cell-conditioned medium: a potential local regulator of IGF action. *Proc Natl Acad Sci USA* 86:8338-8342
 144. Rechler MM, Brown AL 1992 Insulin-like growth factor binding proteins: gene structure and expression. *Growth Regul* 2:55-68
 145. Roghani M, Hossenlopp P, Lepage P, Ballard A, Binoux M 1989 Isolation from human cerebrospinal fluid of a new insulin-like growth factor-binding protein with a selective affinity for IGF-II. *FEBS Lett* 255:253-258
 146. Hynes RO 1992 Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69:11-25
 147. Ruoslahti E, Pierschbacher MD 1987 New perspectives in cell adhesion: RGD and integrins. *Science* 238:491-497
 148. Jones JI, Gockerman A, Busby Jr WH, Wright G, Clemmons DR 1993 Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. *Proc Natl Acad Sci USA* 90:10553-10557
 149. Jones JI, D'Ercole AJ, Camacho-Hubner C, Clemmons DR 1991 Phosphorylation of insulin-like growth factor (IGF)-binding protein 1 in cell culture and *in vivo*: effects on affinity for IGF-I. *Proc Natl Acad Sci USA* 88:7481-7485
 150. Jones JI, Busby Jr WH, Wright G, Smith CE, Kimack NM, Clemmons DR 1993 Identification of the sites of phosphorylation in insulin-like growth factor binding protein-1. Regulation of its affinity by phosphorylation of serine 101. *J Biol Chem* 268:1125-1131
 151. Jones JI, Busby Jr WH, Wright G, Clemmons DR 1993 Human IGFBP-1 is phosphorylated on 3 serine residues: effects of site-directed mutagenesis of the major phosphoserine. *Growth Regul* 3:37-40
 152. Baxter R, Martin J 1989 Structure of the M_r 140,000 growth hormone-dependent insulin-like growth factor binding protein complex: determination by reconstitution and affinity-labeling. *Proc Natl Acad Sci USA* 86:6898-6902
 153. Lewitt MS, Saunders H, Phuyal JL, Baxter RC 1994 Complex formation by human insulin-like growth factor-binding protein-3 and human acid-labile subunit in growth hormone-deficient rats. *Endocrinology* 134:2404-2409
 154. Baxter RC 1993 IGF binding protein-3 and the acid-labile subunit: formation of the ternary complex *in vitro* and *in vivo*. *Adv Exp Med Biol* 343:237-244
 155. Liu F, Baxter RC, Hintz RL 1992 Characterization of the high molecular weight insulin-like growth factor complex in term pregnancy serum. *J Clin Endocrinol Metab* 75:1261-1267
 156. Baxter RC, Bayne ML, Cascieri MA 1992 Structural determinants for binary and ternary complex formation between insulin-like growth factor-I (IGF-I) and IGF binding protein-3. *J Biol Chem* 267:60-65
 157. Dai J, Baxter RC 1994 Regulation *in vivo* of the acid-labile subunit of the rat serum insulin-like growth factor-binding protein complex. *Endocrinology* 135:2335-2341
 158. Dai J, Scott CD, Baxter RC 1994 Regulation of the acid-labile subunit of the insulin-like growth factor complex in cultured rat hepatocytes. *Endocrinology* 135:1066-1072
 159. Baxter RC 1990 Circulating levels and molecular distribution of the acid-labile (alpha) subunit of the high molecular weight insulin-like growth factor-binding protein complex. *J Clin Endocrinol Metab* 70:1347-1353
 160. Hodgkinson S, Spencer G, Bass J, Davis S, Gluckman P 1991 Distribution of circulating insulin-like growth factor-I (IGF-I) into tissues. *Endocrinology* 129:2085-2093
 161. Bassett NS, Breier BH, Hodgkinson SC, Davis SR, Henderson HV, Gluckman PD 1990 Plasma clearance of radiolabelled IGF-I in the late gestation ovine fetus. *J Dev Physiol* 14:73-79
 162. Hodgkinson SC, Napier JR, Spencer GS, Bass JJ 1994 Glycosaminoglycan binding characteristics of the insulin-like growth factor-binding proteins. *J Mol Endocrinol* 13:105-112
 163. Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S, Binoux M 1986 Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the method for titration of the binding proteins and competitive binding studies. *Anal Biochem* 154:138-143
 164. Hossenlopp P, Seurin D, Segovia B, Portolan G, Binoux M 1987 Heterogeneity of insulin-like growth factor binding proteins between structure and affinity. 2. Forms released by human and rat liver in culture. *Eur J Biochem* 170:133-142
 165. Hardouin S, Hossenlopp P, Segovia B, Seurin D, Portolan G, Lassarre C, Binoux M 1987 Heterogeneity of insulin-like growth factor binding proteins and relationships between structure and affinity. 1. Circulating forms in man. *Eur J Biochem* 170:121-132
 166. Hardouin S, Gourmelen M, Noguez P, Seurin D, Roghani M, Le Bouc Y, Povoas G, Merimee TJ, Hossenlopp P, Binoux M 1989 Molecular forms of serum insulin-like growth factor (IGF)-binding proteins in man: relationships with growth hormone and IGFs and physiological significance. *J Clin Endocrinol Metab* 69:1291-1301
 167. Hirschberg R, Kaysen GA 1995 Insulin-like growth factor I and its binding proteins in the experimental nephrotic syndrome. *Endocrinology* 136:1565-1571
 168. Blum W, Breier B 1994 Radioimmunoassays for IGFs and IGFBPs. *Growth Regul* 4 [Suppl 1]:11-19
 169. Bicsak TA, Nakatani A, Shimonaka M, Malkowski M, Ling N 1990 Insulin-like growth factor binding protein measurement: so-

- dium dodecyl sulfate-stable complexes with insulin-like growth factor in serum prevent accurate assessment of total binding protein content by ligand blotting. *Anal Biochem* 191:75-79
170. Ooi GT, Tseng LY, Rechler MM 1993 Transcriptional regulation of the rat IGFBP-1 and IGFBP-2 genes. *Growth Regul* 3:14-17
 171. Ooi GT, Tseng L, Brown A, Orlowski C, Rechler M 1992 Rat insulin-like growth factor-binding protein-1 (IGFBP-1) mRNA is increased by dexamethasone and decreased by insulin in H4-11-E cells. Unpublished: EMBL/GenBank/NCBI Accession Number M89791.
 172. Ooi GT, Orlowski CC, Brown AL, Becker RE, Unterman TG, Rechler MM 1990 Different tissue distribution and hormonal regulation of messenger RNAs encoding rat insulin-like growth factor-binding proteins-1 and -2. *Mol Endocrinol* 4:321-328
 173. Murphy LJ, Seneviratne C, Moreira P, Reid RE 1991 Enhanced expression of insulin-like growth factor-binding protein-I in the fasted rat: the effects of insulin and growth hormone administration. *Endocrinology* 128:689-696
 174. Seneviratne C, Luo JM, Murphy LJ 1990 Transcriptional regulation of rat insulin-like growth factor-binding protein-1 expression by growth hormone. *Mol Endocrinol* 4:1199-1204
 175. Kobayashi S, Nogami H, Ikeda T 1995 Growth hormone and nutrition interact to regulate expressions of kidney IGF-I and IGFBP mRNAs. *Kidney Int* 48:65-71
 176. Nogami H, Watanabe T, Kobayashi S 1994 IGF-I and IGF-binding protein gene expressions in spontaneous dwarf rat. *Am J Physiol* 267:E396-401
 177. Oh Y, Beukers MW, Pham HM, Smanik PA, Smith MC, Rosenfeld RG 1991 Altered affinity of insulin-like growth factor II (IGF-II) for receptors and IGF-binding proteins, resulting from limited modifications of the IGF-II molecule. *Biochem J* 278:249-254
 178. Camacho-Hubner C, Clemmons DR, D'Ercole AJ 1991 Regulation of insulin-like growth factor (IGF) binding proteins in transgenic mice with altered expression of growth hormone and IGF-I. *Endocrinology* 129:1201-1206
 179. Wolf E, Kramer R, Blum WF, Foll J, Brem G 1994 Consequences of postnatally elevated insulin-like growth factor-II in transgenic mice: endocrine changes and effects on body and organ growth. *Endocrinology* 135:1877-1886
 180. Evan AP, Henry DP, Connors BA, Summerlin P, Lee WH 1995 Analysis of insulin-like growth factors (IGF)-I, and -II, type II IGF receptor and IGF-binding protein-2 mRNA and peptide levels in normal and nephrectomized rat kidney. *Kidney Int* 48:1517-1529
 181. Feyen JH, Evans DB, Binkert C, Heinrich GF, Geisse S, Kocher HP 1991 Recombinant human [Cys281]insulin-like growth factor-binding protein 2 inhibits both basal and insulin-like growth factor I-stimulated proliferation and collagen synthesis in fetal rat calvariae. *J Biol Chem* 266:19469-19474
 182. Suikkari AM, Baxter RC 1991 Insulin-like growth factor (IGF) binding protein-3 in pregnancy serum binds native IGF-I but not iodo-IGF-I. *J Clin Endocrinol Metab* 73:1377-1379
 183. Hossenlopp P, Segovia B, Lassarre C, Roghani M, Bredon M, Binoux M 1990 Evidence of enzymatic degradation of insulin-like growth factor-binding proteins in the 150K complex during pregnancy. *J Clin Endocrinol Metab* 71:797-805
 184. Baxter RC, Skriver L 1993 Altered ligand specificity of proteolysed insulin-like growth factor binding protein-3. *Biochem Biophys Res Commun* 196:1267-1273
 185. Matsell DG, Delhanty PJ, Stepaniuk O, Goodyear C, Han VK 1994 Expression of insulin-like growth factor and binding protein genes during nephrogenesis. *Kidney Int* 46:1031-1042
 186. Landau D, Chin E, Bondy C, Domene H, Roberts Jr CT, Gronbaek 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
 187. Wang E, Wang J, Chin E, Zhou J, Bondy CA 1995 Cellular patterns of insulin-like growth factor system gene expression in murine chondrogenesis and osteogenesis. *Endocrinology* 136:2741-2751
 188. Jones JL, Gockerman A, Busby Jr WH, Camacho-Hubner C, Clemmons DR 1993 Extracellular matrix contains insulin-like growth factor binding protein-5: potentiation of the effects of IGF-I. *J Cell Biol* 121:679-687
 189. 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 Int* 48:402-411
 190. Hise MK, Mantzouris NM, Lahn JS, Sheikh MS, Shao ZM, Fontana JA 1994 Low-protein diet regulates a proximal nephron insulin-like growth factor binding protein. *Am J Kidney Dis* 23:849-855
 191. Bach LA, Hsieh S, Brown AL, Rechler MM 1994 Recombinant human insulin-like growth factor (IGF)-binding protein-6 inhibits IGF-II-induced differentiation of L6A1 myoblasts. *Endocrinology* 135:2168-2176
 192. Bach LA, Salemi R, Leeding KS 1995 Roles of insulin-like growth factor (IGF) receptors and IGF-binding proteins in IGF-II-induced proliferation and differentiation of L6A1 rat myoblasts. *Endocrinology* 136:5061-5069
 193. Chin E, Bondy C 1992 Insulin-like growth factor system gene expression in the human kidney. *J Clin Endocrinol Metab* 75:962-968
 194. Bondy CA, Chin E, Zhou J 1993 Significant species differences in local IGF-I and -II gene expression. *Adv Exp Med Biol* 343:73-77
 195. Chin E, Michels K, Bondy CA 1994 Partition of insulin-like growth factor (IGF)-binding sites between the IGF-I and IGF-II receptors and IGF-binding proteins in the human kidney. *J Clin Endocrinol Metab* 78:156-164
 196. D'Ercole A, Hill DJ, Strain AJ, Underwood LE 1986 Tissue and plasma somatomedin-C/insulin-like growth factor I concentrations in the human fetus during the first half of gestation. *Pediatr Res* 20:253-255
 197. Chin E, Zhou J, Bondy CA 1992 Renal growth hormone receptor gene expression: relationship to renal insulin-like growth factor system. *Endocrinology* 131:3061-3066
 198. 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
 199. Gröne HJ, Neumann P, Fuchs E 1992 Localization and characterization of IGF-I receptors in fetal and adult human kidneys. *Miner Electrolyte Metab* 18:256-263
 200. Rabkin R, Brody M, Lu L, Chan C, Shaheen A, Gillett N 1995 Expression of genes encoding the rat renal insulin-like growth factor-I system. *J Am Soc Nephrol* 6:1511-1518
 201. Kobayashi S, Clemmons DR, Venkatachalam MA 1991 Colocalization of insulin-like growth factor-binding protein with insulin-like growth factor I. *Am J Physiol* 261:F22-F28
 202. Andersson GL, Skottner A, Jennische E 1988 Immunocytochemical and biochemical localization of insulin-like growth factor I in the kidney of rats before and after uninephrectomy. *Acta Endocrinol (Copenh)* 119:555-560
 203. Andersson G, Jennische E 1988 IGF-I immunoreactivity is expressed by regenerating renal tubular cells after ischaemic injury in the rat. *Acta Physiol Scand* 132:453-457
 204. Andersson GL, Ericson LE, Jennische E 1990 Ultrastructural localization of IGF-I in the rat kidney; an immunocytochemical study. *Histochemistry* 94:263-267
 205. 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
 206. Conti FG, Striker LJ, Elliot SJ, Andreani D, Striker GE 1988 Synthesis and release of insulin-like growth factor I by mesangial cells in culture. *Am J Physiol* 255:F1214-F1219
 207. Doi T, Striker LJ, Quaipe C, Conti FG, Palmiter R, Behringer R, Brinster R, Striker GE 1988 Progressive glomerulosclerosis develops in transgenic mice chronically expressing growth hormone and growth hormone releasing factor but not in those expressing insulin-like growth factor-1. *Am J Pathol* 131:398-403
 208. Conti FG, Striker LJ, Lesniak MA, MacKay K, Roth J, Striker GE 1988 Studies on binding and mitogenic effect of insulin and insulin-like growth factor I in glomerular mesangial cells. *Endocrinology* 122:2788-2795
 209. Conti FG, Elliot SJ, Striker LJ, Striker GE 1989 Binding of insulin-like growth factor-I by glomerular endothelial and epithelial cells: further evidence for IGF-I action in the renal glomerulus. *Biochem Biophys Res Commun* 163:952-958

210. Aron DC, Rosenzweig JL, Abboud HE 1989 Synthesis and binding of insulin-like growth factor I by human glomerular mesangial cells. *J Clin Endocrinol Metab* 68:585-591
211. Abboud HE 1992 Growth factors and the mesangium. *J Am Soc Nephrol* 2:S185-S189
212. Kwok CF, Ho LT, Jap TS 1993 Insulin-like growth factor-I receptor increases in aortic endothelial cells from diabetic rats. *Metabolism* 42:1381-1385
213. Taylor WR, Nerem RM, Alexander RW 1993 Polarized secretion of IGF-I and IGF-I binding protein activity by cultured aortic endothelial cells. *J Cell Physiol* 154:139-142
214. Delafontaine P, Bernstein KE, Alexander RW 1991 Insulin-like growth factor I gene expression in vascular cells. *Hypertension* 17:693-699
215. Valentino KL, Pham H, Ocrant I, Rosenfeld RG 1988 Distribution of insulin-like growth factor II receptor immunoreactivity in rat tissues. *Endocrinology* 122:2753-2763
216. Chin E, Zhou J, Dai J, Baxter RC, Bondy CA 1994 Cellular localization and regulation of gene expression for components of the insulin-like growth factor ternary binding protein complex. *Endocrinology* 134:2498-2504
217. Matejka GL, Jennische E 1992 IGF-I binding and IGF-I mRNA expression in the post-ischemic regenerating rat kidney. *Kidney Int* 42:1113-1123
218. Hammerman MR, Rogers S 1987 Distribution of IGF receptors in the plasma membrane of proximal tubular cells. *Am J Physiol* 253:F841-F847
219. Cui S, Flyvbjerg A, Nielsen S, Kiess W, Christensen EI 1993 IGF-II/Man-6-P receptors in rat kidney: apical localization in proximal tubule cells. *Kidney Int* 43:796-807
220. Jacobsen C, Jessen H, Flyvbjerg A 1995 IGF-II receptors in luminal and basolateral membranes isolated from pars convoluta and pars recta of rabbit proximal tubule. *Biochim Biophys Acta* 1235:85-92
221. Flyvbjerg A, Nielsen S, Sheikh MI, Jacobsen C, Orskov H, Christensen EI 1993 Luminal and basolateral uptake and receptor binding of IGF-I in rabbit renal proximal tubules. *Am J Physiol* 265:F624-F633
222. Quigley R, Baum M 1991 Effects of growth hormone and insulin-like growth factor I on rabbit proximal convoluted tubule transport. *J Clin Invest* 88:368-374
223. Rogers SA, Karl IE, Hammerman MR 1989 Growth hormone directly stimulates gluconeogenesis in canine renal proximal tubule. *Am J Physiol* 257:E751-E756
224. Rogers SA, Miller SB, Hammerman MR 1990 Growth hormone stimulates IGF I gene expression in isolated rat renal collecting duct. *Am J Physiol* 259:F474-F479
225. Rogers SA, Miller SB, Hammerman MR 1991 Insulin-like growth factor I gene expression in isolated rat renal collecting duct is stimulated by epidermal growth factor. *J Clin Invest* 87:347-351
226. Hise M, Liu L, Rohan R 1995 Gene expression of the insulin-like growth factor (IGF) system in the hypersomatotrophic rat. *J Am Soc Nephrol* 6:768 (Abstract)
227. Hise MK, Mantzouris NM, Lahn JS, Sheikh MS, Shao ZM, Fontana JA 1994 IGF binding protein 5 and IGF-I receptor regulation in hypophysectomized rat kidneys. *Am J Physiol* 266:F147-F154
228. Hammerman MR, Miller SB 1991 Renal cellular biology of growth hormone and insulin-like growth factor I. *Pediatr Nephrol* 5:505-508
229. Chin E, Bondy CA 1994 Dietary protein-induced renal growth: correlation between renal IGF-I synthesis and hyperplasia. *Am J Physiol* 266:C1037-C1045
230. Lemozy S, Pucilowska JB, Underwood LE 1994 Reduction of insulin-like growth factor-I (IGF-I) in protein-restricted rats is associated with differential regulation of IGF-binding protein messenger ribonucleic acids in liver and kidney, and peptides in liver and serum. *Endocrinology* 135:617-623
231. Trinh-Trang-Tan MM, Antras J, Levillain O, Bankir L 1993 Adaptation of the medullary thick ascending limb to dietary protein intake. *Exp Nephrol* 1:158-167
232. Bankir L, Kriz W 1995 Adaptation of the kidney to protein intake and to urine concentrating activity: similar consequences in health and CRF (editorial). *Kidney Int* 47:7-24
233. Hirschberg R, Kopple JD 1991 Response of insulin-like growth factor I and renal hemodynamics to a high- and low-protein diet in the rat. *J Am Soc Nephrol* 1:1034-1040
234. Salido EC, Lakshmanan J, Fisher DA, Shapiro LJ, Barajas L 1991 Expression of epidermal growth factor in the rat kidney. An immunocytochemical and *in situ* hybridization study. *Histochemistry* 96:65-72
235. Rogers SA, Rasmussen J, Miller SB, Hammerman MR 1994 Effects of growth hormone on rat renal epidermal growth factor expression. *Am J Physiol* 267:F208-F214
236. Hammerman MR, Ryan G, Miller SB 1992 Expression of insulin-like growth factor in adult and embryonic kidney. *Miner Electrolyte Metab* 18:253-255
237. Hirschberg R, Brunori G, Kopple JD, Guler HP 1993 Effects of insulin-like growth factor I on renal function in normal men. *Kidney Int* 43:387-397
238. Maack T 1975 Renal handling of low molecular weight proteins. *Am J Med* 58:57-64
239. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D 1979 Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. *Kidney Int* 16:251-270
240. Camargo MJ, Sumpio BE, Maack T 1984 Renal hydrolysis of absorbed protein: influence of load and lysosomal pH. *Am J Physiol* 247:F656-664
241. Wall DA, Maack T 1985 Endocytic uptake, transport, and catabolism of proteins by epithelial cells. *Am J Physiol* 248:C12-20
242. Maack T, Park CH 1990 Endocytosis and lysosomal hydrolysis of proteins in proximal tubules. *Methods Enzymol* 191:340-354
243. Wallace A, Stacy B 1975 Disappearance of 125I-labeled rat growth hormone in nephrectomized and sham operated rats. *Horm Metab Res* 7:135-138
244. Wallace A, Stacy B, Thorburn G 1972 The fate of radioiodinated sheep growth hormone in intact and nephrectomized sheep. *Pfluegers Arch* 331:25-37
245. Samaan N, Freeman R 1970 Growth hormone levels in severe renal failure. *Metabolism* 19:102-113
246. Wright A, Lowy C, Fraser T, Spitz I, Rubinstein A, Bersohn I 1968 Serum growth hormone and glucose tolerance in renal failure. *Lancet* 2:798-801
247. Schaefer F, Hamill G, Stanhope R, Preece MA, Scharer K 1991 Pulsatile growth hormone secretion in peripubertal patients with chronic renal failure. Cooperative Study Group on Pubertal Development in Chronic Renal Failure. *J Pediatr* 119:568-577
248. Postel-Vinay MC, Tar A, Crosnier H, Broyer M, Rappaport R, Tönshoff B, Mehls O 1991 Plasma growth hormone-binding activity is low in uraemic children. *Pediatr Nephrol* 5:545-547
249. Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. *J Clin Endocrinol Metab* 71:688-695
250. Quattrin T, Albin CH, Vandlen RL, MacGillivray MH 1987 Quantitation of urinary somatomedin-C in children with normal and abnormal growth. *J Clin Endocrinol Metab* 65:1168-1171
251. Quattrin T, Albin CH, Cara JF, Vandlen RL, Mills BJ, MacGillivray MH 1988 Quantitation of urinary somatomedin-C and growth hormone in preterm and full-term infants and normal children. *J Clin Endocrinol Metab* 66:792-797
252. Albin CH, Quattrin T, Vandlen RL, MacGillivray MH 1988 Quantitation of urinary growth hormone in children with normal and abnormal growth. *Pediatr Res* 23:89-92
253. Johnson V, Maack T 1977 Renal extraction, filtration, absorption, and catabolism of growth hormone. *Am J Physiol* 233:F185-F196
254. Kau ST, Maack T 1986 Mechanism of tubular uptake on human growth hormone in perfused rat kidneys. *J Pharmacol Exp Ther* 236:596-601
255. Haffner D, Schaefer F, Girard J, Ritz E, Mehls O 1994 Metabolic clearance of recombinant human growth hormone in health and chronic renal failure. *J Clin Invest* 93:1163-1171
256. Krogsgaard Thomsen M, Friis C, Sehested Hansen B, Johansen P, Eschen C, Nowak J, Poulsen K 1994 Studies on the renal kinetics

- of growth hormone (GH) and on the GH receptor and related effects in animals. *J Pediatr Endocrinol* 7:93-105
257. **Hirschberg R** 1993 IGF-I is ultrafiltered into the urinary space and may exert biological effects in proximal tubules in the nephrotic syndrome. *J Am Soc Nephrol* 4:771 (Abstract)
 258. **Hirschberg R** 1996 Bioactivity of glomerular ultrafiltrate during heavy proteinuria may contribute to renal tubulo-interstitial lesions: evidence for a role for insulin-like growth factor I. *J Clin Invest* 98:116-124
 259. **Feld S, Hirschberg R** 1996 IGF-I and IGF-binding proteins in the nephrotic syndrome. *Pediatr Nephrol* 10:355-358
 260. **Hizuka N, Takano K, Tanaka I, Asakawa K, Miyakawa M, Horikawa R, Shizume K** 1987 Demonstration of insulin-like growth factor I in human urine. *J Clin Endocrinol Metab* 64:1309-1312
 261. **Gargosky SE, Hasegawa T, Tapanainen P, MacGillivray M, Hasegawa Y, Rosenfeld RG** 1993 Urinary insulin-like growth factors (IGF) and IGF-binding proteins in normal subjects, growth hormone deficiency, and renal disease. *J Clin Endocrinol Metab* 76:1631-1637
 262. **Yokoya S, Suwa S, Maesaka H, Tanaka T** 1988 Immunoreactive somatomedin C/insulin-like growth factor I in urine from normal subjects, pituitary dwarfs, and acromegalics. *Pediatr Res* 23:151-154
 263. **Yokoya S, Suwa S, Maesaka H, Tanaka T** 1988 Age, sex and growth hormone dependent changes in urinary IGF-I. *Acta Paediatr Scand Suppl* 347:134-135
 264. **Yokoya S, Suwa S, Maesaka H, Tanaka T** 1988 Age-related changes in IGF I/SmC levels in plasma and urine—glomerular filtration of the unbound form. *Acta Paediatr Jpn* 30 Suppl:50-54
 265. **Rabkin R, Fervenza F, Maidment H, Ike J, Jamison R, Hintz R, Bukar J, Arcila P, Gesundheit N** 1994 IGF-I pharmacokinetics in chronic renal failure. *J Am Soc Nephrol* 5:340 (Abstract)
 266. **Fouque D, Joly MO, Laville M, Beaufrere B, Goudable J, Pozet N, Chatelain P, Zech P** 1992 Increase of circulating insulin-like growth factor-I in chronic renal failure is reduced by low-protein diet. *Miner Electrolyte Metab* 18:276-279
 267. **Fouque D, Joly M, Laville M, Beaufrere B, Goudable J, Pozet N, Labeuw M, Chatelain P, Zech P** 1992 Increase of serum insulin-like growth factor I (IGF 1) during chronic renal failure is reduced by low protein diet. *Miner Electrolyte Metab* 18:276-279
 268. **Fouque D, Peng SC, Kopple JD** 1995 Pharmacokinetics of recombinant human insulin-like growth factor-1 in dialysis patients. *Kidney Int* 47:869-875
 269. **D'Ercole A, Decedue CJ, Furlanetto RW, Underwood LE, Van Wyk JJ** 1977 Evidence that somatomedin-C is degraded by the kidney and inhibits insulin degradation. *Endocrinology* 101:577-586
 270. **Fawcett J, Fielder P, Keller G, Rabkin R** 1994 Uptake and processing of IGF-I by cultured kidney cells. *J Am Soc Nephrol* 5:691 (Abstract)
 271. **Fawcett J, Rabkin R** 1994 Insulin-like growth factor binding protein-3 alters IGF-I processing by cultured kidney cells. Program of the 76th annual meeting The Endocrine Society, Anaheim, CA, 1994, p 299 (Abstract 396)
 272. **Zumkeller W, Hall K** 1990 Immunoreactive insulin-like growth factor II in urine. *Acta Endocrinol (Copenh)* 123:499-503
 273. **Hasegawa Y, Cohen P, Yorgin P, Rosenfeld RG** 1992 Characterization of urinary insulin-like growth factor binding proteins. *J Clin Endocrinol Metab* 74:830-835
 274. **Arany E, Zabel P, Freeman D, Hill DJ** 1993 Elimination of radiolabelled recombinant human insulin-like growth factor binding protein-3 from the circulation, and its distribution amongst organs and tissues in adult male rats. *Regul Pept* 48:133-143
 275. **Garin E, Grant M, Silverstein J** 1989 Insulin-like growth factors in patients with active nephrotic syndrome. *Am J Dis Child* 143:865-867
 276. **Lee DY, Cohen P, Krensky AM, Rosenfeld RG, Yorgin PD** 1993 Insulin-like growth factor binding protein-3 protease activity in the urine of children with chronic renal failure. *Pediatr Nephrol* 7:416-423
 277. **Ikkos D, Ljunggren H, Luft R** 1956 Glomerular filtration rate and renal plasma flow in acromegaly. *Acta Endocrinol (Copenhagen)* 21:226-236
 278. **Corvilain J, Abramow M, Bergans A** 1962 Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man. *J Clin Invest* 41:1230-1235
 279. **Falkheden T** 1963 Renal function following hypophysectomy in man. *Acta Endocrinol (Copenhagen)* 42:571-590
 280. **Falkheden T, Wickbom I** 1965 Renal function and kidney size following hypophysectomy in man. *Acta Endocrinol (Copenhagen)* 48:348-354
 281. **Hirschberg R, Kopple JD** 1987 Role of growth hormone in the amino acid-induced acute rise in renal function in man. *Kidney Int* 32:382-387
 282. **Ruilope L, Rodicio J, Miranda B, Robles R, Sancho-Rof J, Romero C** 1988 Renal effects of amino acid infusion in patients with pan-hypopituitarism. *Hypertension* 11:557-559
 283. **Kleinman K, Glassock R** 1986 Glomerular filtration rate fails to increase following protein ingestion in hypothalamo-hypophyseal-deficient adults. *Am J Nephrol* 6:169-174
 284. **Haffner D, Zacharewicz S, Mehls O, Heinrich U, Ritz E** 1989 The acute effect of growth hormone on GFR is obliterated in chronic renal failure. *Clin Nephrol* 32:266-269
 285. **Haffner D, Ritz E, Mehls O, Rosman J, Blum W, Heinrich U, Hubinger A** 1990 Growth hormone induced rise in glomerular filtration rate is not obliterated by angiotensin-converting enzyme inhibitors. *Nephron* 55:63-68
 286. **Parving H, Noer I, Mogensen C, Svendsen P** 1978 Kidney function in normal man during short-term growth hormone infusion. *Acta Endocrinol (Copenhagen)* 89:796-800
 287. **Westby R, Goldfarb M, Goldberg M, Agus Z** 1977 Acute effects of bovine growth hormone on renal calcium and phosphate excretion. *Metabolism* 26:525-530
 288. **Christiansen JS, Gammelgaard J, Orskov H, Andersen AR, Telmer S, Parving HH** 1981 Kidney function and size in normal subjects before and during growth hormone administration for one week. *Eur J Clin Invest* 11:487-490
 289. **Hirschberg RR, Kopple JD** 1988 Increase in renal plasma flow and glomerular filtration rate during growth hormone treatment may be mediated by insulin-like growth factor I. *Am J Nephrol* 8:249-254
 290. **Hirschberg R, Rabb H, Bergamo R, Kopple JD** 1989 The delayed effect of growth hormone on renal function in humans. *Kidney Int* 35:865-870
 291. **Dullaart RP, Meijer S, Marbach P, Sluiter WJ** 1992 Effect of a somatostatin analogue, octreotide, on renal haemodynamics and albuminuria in acromegalic patients. *Eur J Clin Invest* 22:494-502
 292. **Gershberg H, Heinemann H, Stumpf H** 1957 Renal function studies and autopsy report in a patient with gigantism and acromegaly. *J Clin Endocrinol Metab* 17:377-385
 293. **Hirschberg R, Kopple JD** 1989 Effects of growth hormone and IGF-I on renal function. *Kidney Int Suppl* 27:S20-S26
 294. **Hoogenberg K, ter Wee PM, Lievever AG, Sluiter WJ, Dullaart RP** 1994 Insulin-like growth factor I and altered renal hemodynamics in growth hormone deficiency, acromegaly, and type I diabetes mellitus. *Transplant Proc* 26:505-507
 295. **Ogle GD, Rosenberg AR, Kainer G** 1992 Renal effects of growth hormone. I. Renal function and kidney growth. *Pediatr Nephrol* 6:394-398
 296. **Hirschberg R, Kopple JD** 1987 Effects of growth hormone on GFR and renal plasma flow in man. *Kidney Int Suppl* 22:S21-S24
 297. **Kopple JD, Hirschberg R** 1990 Physiological effects of growth hormone and insulin-like growth factor I on the kidney. *Miner Electrolyte Metab* 16:82-88
 298. **Hoogenberg K, Sluiter WJ, Dullaart RP** 1993 Effect of growth hormone and insulin-like growth factor I on urinary albumin excretion: studies in acromegaly and growth hormone deficiency. *Acta Endocrinol (Copenh)* 129:151-157
 299. **Mulrone SE, Lumpkin MD, Haramati A** 1989 Antagonist to GH-releasing factor inhibits growth and renal Pi reabsorption in immature rats. *Am J Physiol* 257:F29-F34
 300. **Lumpkin MD, Mulrone SE, Haramati A** 1989 Inhibition of pulsatile growth hormone (GH) secretion and somatic growth in im-

- mature rats with a synthetic GH-releasing factor antagonist. *Endocrinology* 124:1154-1159
301. **Lumpkin MD, McDonald JK** 1989 Blockade of growth hormone-releasing factor (GRF) activity in the pituitary and hypothalamus of the conscious rat with a peptidic GRF antagonist. *Endocrinology* 124:1522-1531
 302. **Hirschberg R, Kopple JD** 1989 Evidence that insulin-like growth factor I increases renal plasma flow and glomerular filtration rate in fasted rats. *J Clin Invest* 83:326-330
 303. **Baumann U, Eisenhauer T, Hartmann H** 1992 Increase of glomerular filtration rate and renal plasma flow by insulin-like growth factor-I during euglycaemic clamping in anaesthetized rats. *Eur J Clin Invest* 22:204-209
 304. **Hirschberg R, Zipser R, Slomowitz L, Kopple J** 1988 Glucagon and prostaglandins are mediators of amino acid-induced rise in renal hemodynamics. *Kidney Int* 33:1147-1155
 305. **Wada L, Don BR, Schambelan M** 1991 Hormonal mediators of amino acid-induced glomerular hyperfiltration in humans. *Am J Physiol* 260:F787-F792
 306. **O'Shea M, Layish D** 1992 Growth hormone and the kidney: a case presentation and reviews of the literature. *J Am Soc Nephrol* 3:157-161
 307. **Hall K, Enberg G, Hellem E, Lundin G, Ottosson-Seeberger A, Sara V, Trygstad O, Ofverholm U** 1984 Somatomedin levels in pregnancy: longitudinal study in healthy subjects and patients with growth hormone deficiency. *J Clin Endocrinol Metab* 59:587-594
 308. **D'Ercole A, Bose CL, Underwood LE, Lawson EE** 1984 Serum somatomedin-C concentrations in a rabbit model of diabetic pregnancy. *Diabetes* 33:590-595
 309. **Davenport ML, D'Ercole AJ, Azizkhan JC, Lund PK** 1988 Somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) and insulin-like growth factor II (IGF-II) mRNAs during lung development in the rat. *Exp Lung Res* 14:607-618
 310. **Davenport ML, Clemmons DR, Miles MV, Camacho-Hubner C, D'Ercole AJ, Underwood LE** 1990 Regulation of serum insulin-like growth factor-I (IGF-I) and IGF binding proteins during rat pregnancy. *Endocrinology* 127:1278-1286
 311. **Donovan SM, Giudice LC, Murphy LJ, Hintz RL, Rosenfeld RG** 1991 Maternal insulin-like growth factor-binding protein messenger ribonucleic acid during rat pregnancy. *Endocrinology* 129:3359-3366
 312. **Gargosky SE, Moyses KJ, Walton PE, Owens JA, Wallace JC, Robinson JS, Owens PC** 1990 Circulating levels of insulin-like growth factors increase and molecular forms of their serum binding proteins change with human pregnancy. *Biochem Biophys Res Commun* 170:1157-1163
 313. **Gargosky SE, Pham HM, Wilson KF, Liu F, Giudice LC, Rosenfeld RG** 1992 Measurement and characterization of insulin-like growth factor binding protein-3 in human biological fluids: discrepancies between radioimmunoassay and ligand blotting. *Endocrinology* 131:3051-3060
 314. **Gargosky SE, Nanto-Salonen K, Tapanainen P, Rosenfeld RG** 1993 Pregnancy in growth hormone-deficient rats: assessment of insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and IGFBP protease activity. *J Endocrinol* 136:479-489
 315. **Giudice LC, Farrell EM, Pham H, Lamson G, Rosenfeld RG** 1990 Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the puerperium: effects of a pregnancy-associated serum protease activity. *J Clin Endocrinol Metab* 71:806-816
 316. **Lund PK, Moats-Staats BM, Hynes MA, Simmons JG, Jansen M, D'Ercole AJ, Van Wyk JJ** 1986 Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues. *J Biol Chem* 261: 14539-14544
 317. **Mills NC, D'Ercole AJ, Underwood LE, Ilan J** 1986 Synthesis of somatomedin C/insulin-like growth factor I by human placenta. *Mol Biol Rep* 11:231-236
 318. **Vileisis RA, D'Ercole AJ** 1986 Tissue and serum concentrations of somatomedin-C/insulin-like growth factor I in fetal rats made growth retarded by uterine artery ligation. *Pediatr Res* 20:126-130
 319. **Muchaneta-Kubara EC, Cope GH, el Nahas AM** 1995 Biphasic expression of IGF-I following subtotal renal ablation. *Exp Nephrol* 3:165-172
 320. **El Nahas AM, Le Carpentier JE, Bassett AH, Hill DJ** 1989 Dietary protein and insulin-like growth factor-I content following unilateral nephrectomy. *Kidney Int Suppl* 27:S15-S19
 321. **Fliesen T, Maiter D, Gerard G, Underwood LE, Maes M, Ketelslegers JM** 1989 Reduction of serum insulin-like growth factor-I by dietary protein restriction is age dependent. *Pediatr Res* 26:415-419
 322. **Kobayashi S, Venkatachalam MA** 1992 Differential effects of calorie restriction on glomeruli and tubules of the remnant kidney. *Kidney Int* 42:710-717
 323. **Maes M, Amand Y, Underwood LE, Maiter D, Ketelslegers JM** 1988 Decreased serum insulin-like growth factor I response to growth hormone in hypophysectomized rats fed a low protein diet: evidence for a postreceptor defect. *Acta Endocrinol (Copenh)* 117: 320-326
 324. **Maiter D, Maes M, Underwood LE, Fliesen T, Gerard G, Ketelslegers JM** 1988 Early changes in serum concentrations of somatomedin-C induced by dietary protein deprivation in rats: contributions of growth hormone receptor and post-receptor defects. *J Endocrinol* 118:113-120
 325. **Maiter D, Fliesen T, Underwood LE, Maes M, Gerard G, Davenport ML, Ketelslegers JM** 1989 Dietary protein restriction decreases insulin-like growth factor I independent of insulin and liver growth hormone binding. *Endocrinology* 124:2604-2611
 326. **Prewitt TE, AJ DE, Switzer BR, Van Wyk JJ** 1982 Relationship of serum immunoreactive somatomedin-C to dietary protein and energy in growing rats. *J Nutr* 112:144-150
 327. **Bosch J, Saccaggi A, Lauer A, Ronco C, Belledonne M, Glabman S** 1983 Renal functional research in humans. Effect of protein intake on glomerular filtration rate. *Am J Med* 75:943-950
 328. **Pullman T, Alving A, Dern R, Landowne M** 1954 The influence of dietary protein intake of specific renal function in normal man. *J Lab Clin Med* 44:320-332
 329. **Marshall SM, Flyvbjerg A, Frystyk J, Korsgaard L, Orskov H** 1991 Renal insulin-like growth factor I and growth hormone receptor binding in experimental diabetes and after unilateral nephrectomy in the rat. *Diabetologia* 34:632-639
 330. **Flyvbjerg A, Thorlacius-Ussing O, Naeraa R, Ingerslev J, Orskov H** 1988 Kidney tissue somatomedin C and initial renal growth in diabetic and uninephrectomized rats. *Diabetologia* 31:310-314
 331. **Dullaart RP, Meijer S, Marbach P, Sluiter WJ** 1992 Renal reserve filtration capacity in growth hormone deficient subjects. *Eur J Clin Invest* 22:562-568
 332. **Flyvbjerg A, Jorgensen KD, Marshall SM, Orskov H** 1991 Inhibitory effect of octreotide on growth hormone-induced IGF-I generation and organ growth in hypophysectomized rats. *Am J Physiol* 260:E568-E574
 333. **Flyvbjerg A, Marshall SM, Frystyk J, Hansen KW, Harris AG, Orskov H** 1992 Octreotide administration in diabetic rats: effects on renal hypertrophy and urinary albumin excretion. *Kidney Int* 41: 805-812
 334. **Gronbaek H, Nielsen B, Osterby R, Harris A, Orskov H, Flyvbjerg A** 1995 Effect of octreotide and insulin on manifest renal and glomerular hypertrophy and urinary albumin excretion in long-term experimental diabetes in rats. *Diabetologia* 38:135-144
 335. **Pedersen MM, Christensen SE, Christiansen JS, Pedersen EB, Mogensen CE, Orskov H** 1990 Acute effects of a somatostatin analogue on kidney function in type 1 diabetic patients. *Diabetic Med* 7:304-309
 336. **Bolze MS, Reeves RD, Lindbeck FE, Elders MJ** 1985 Influence of selected amino acid deficiencies on somatomedin, growth and glycosaminoglycan metabolism in weanling rats. *J Nutr* 115:782-787
 337. **Brenner B, Meyer T, Hostetter T** 1982 Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 307:652-659
 338. **Isley WL, Underwood LE, Clemmons DR** 1983 Dietary components that regulate serum somatomedin-C concentrations in humans. *J Clin Invest* 71:175-182
 339. **Alleyne G** 1967 The effect of severe protein calorie malnutrition on the renal function of Jamaican children. *Pediatrics* 39:400-411
 340. **Clemmons DR, Underwood LE, Dickerson RN, Brown RO, Hak**

- LJ, MacPhee RD, Heizer WD 1985 Use of plasma somatomedin-C/insulin-like growth factor I measurements to monitor the response to nutritional repletion in malnourished patients. *Am J Clin Nutr* 41:191-198
341. Ichikawa I, Purkerson ML, Klahr S, Troy JL, Martinez-Maldonado M, Brenner BM 1980 Mechanism of reduced glomerular filtration rate in chronic malnutrition. *J Clin Invest* 65:982-988
342. Klahr S, Alleyne G 1973 Effects of chronic protein-calorie malnutrition on the kidney. *Kidney Int* 3:129-141
343. Maase C, Zondek H 1917 Ueber eigenartige Oedeme. *Dtsch Med Wochenschr* 43:484-485
344. Payne-Robinson H, Smith I, Golden M 1986 Plasma somatomedin-C in Jamaican children recovering from severe malnutrition. *Clin Res* 34:866A (Abstract)
345. Klahr S, Tripathy K 1966 Evaluation of renal function in malnutrition. *Arch Intern Med* 118:323-325
346. El Nahas AM, Le Carpentier JE, Bassett AH 1990 Compensatory renal growth: role of growth hormone and insulin-like growth factor-I [see comments]. *Nephrol Dial Transplant* 5:123-129
347. Hirschberg R, Kopple JD 1992 The growth hormone-insulin-like growth factor I axis and renal glomerular function. *J Am Soc Nephrol* 2:1417-1422
348. Muchaneta-Kubara EC, Sayed-Ahmed N, Besbas N, Zhang G, Cope GH, el Nahas AM 1994 Experimental diabetic renal growth: role of growth hormone and insulin-like growth factor-I. *Nephrol Dial Transplant* 9:1395-1401
349. Flyvbjerg A, Frystyk J, Osterby R, Orskov H 1992 Kidney IGF-I and renal hypertrophy in GH-deficient diabetic dwarf rats. *Am J Physiol* 262:E956-E962
350. El Nahas AM 1991 The role of growth hormone and insulin-like growth factor-I in experimental renal growth and scarring. *Am J Kidney Dis* 17:677-679
351. Yoshida H, Mitarai T, Kitamura M, Suzuki T, Ishikawa H, Fogo A, Sakai O 1994 The effect of selective growth hormone defect in the progression of glomerulosclerosis. *Am J Kidney Dis* 23:302-312
352. El Nahas AM, Bassett AH, Cope GH, Le Carpentier JE 1991 Role of growth hormone in the development of experimental renal scarring. *Kidney Int* 40:29-34
353. Hirschberg R 1993 Effects of growth hormone and IGF-I on glomerular ultrafiltration in growth hormone-deficient rats. *Regul Pept* 48:241-250
354. Laron Z, Klinger B 1994 IGF-I treatment of adult patients with Laron syndrome: preliminary results. *Clin Endocrinol (Oxf)* 41:631-638
355. Guler HP, Eckardt KU, Zapf J, Bauer C, Froesch ER 1989 Insulin-like growth factor I increase glomerular filtration rate and renal plasma flow in man. *Acta Endocrinol (Copenh)* 121:101-106
356. Guler HP, Schmid C, Zapf J, Froesch ER 1989 Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects. *Proc Natl Acad Sci USA* 86:2868-2872
357. Giordano M, DeFronzo R 1995 Acute effects of human recombinant insulin-like growth factor I on renal function in humans. *Nephron* 71:10-15
358. Hirschberg R, Kopple JD, Blantz RC, Tucker BJ 1991 Effects of recombinant human insulin-like growth factor I on glomerular dynamics in the rat. *J Clin Invest* 87:1200-1206
359. Haylor J, Singh I, el Nahas AM 1991 Nitric oxide synthesis inhibitor prevents vasodilation by insulin-like growth factor I. *Kidney Int* 39:333-335
360. Myers B, Deen W, Brenner B 1975 Effects of norepinephrine and angiotensin II on the determinants of glomerular ultrafiltration and proximal tubule fluid reabsorption in the rat. *Circ Res* 37:101-110
361. Tolins J, Palmer R, Moncada S, Raji L 1990 Role of endothelium-derived relaxation factor in regulation of renal hemodynamic responses. *Am J Physiol* 258:H655-H662
362. Palmer R, Ferrige A, Moncada S 1989 Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327:524-525
363. Furchgott R, Cherry P, Zawadzki J, Jothianandan D 1984 Endothelial cells as mediators of vasodilation of arteries. *J Cardiovasc Pharmacol* 6:S336-S343
364. Furchgott R, Zawadzki J 1980 The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-376
365. Kon V, Harris R, Ichikawa I 1990 A regulatory role for large vessels in organ circulation. *J Clin Invest* 85:1728-1733
366. Shultz P, Shorer A, Raji L 1990 Effects of endothelium-derived relaxation factor and nitric oxide on rat mesangial cells. *Am J Physiol* 258:F161-F168
367. Burton GA, MacNeil S, de Jonge A, Haylor J 1990 Cyclic GMP release and vasodilatation induced by EDRF and atrial natriuretic factor in the isolated perfused kidney of the rat. *Br J Pharmacol* 99:364-368
368. Hirschberg R 1989 Die aminosäure- und hormoninduzierte Modulation der Nierenfunktion und ihre mögliche Bedeutung für die Progression der chronischen Niereninsuffizienz. Free University of Berlin, Berlin
369. Tsukahara H, Gordienko DV, Tönshoff B, Gelato MC, Goligorsky MS 1994 Direct demonstration of insulin-like growth factor-I-induced nitric oxide production by endothelial cells. *Kidney Int* 45:598-604
370. Inishi Y, Okuda T, Arakawa T, Kurokawa K 1994 Insulin attenuates intracellular calcium responses and cell contraction caused by vasoactive agents. *Kidney Int* 45:1318-1325
371. Tucker BJ, Anderson CM, Thies RS, Collins RC, Blantz RC 1992 Glomerular hemodynamic alterations during acute hyperinsulinemia in normal and diabetic rats. *Kidney Int* 42:1160-1168
372. Zatz R, Dunn B, Meyer T, Anderson S, Rennke H, Brenner B 1986 Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925-1930
373. Brenner BM, Meyer TW, Hostetter TH 1982 Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 307:652-659
374. Anderson S, Meyer T, Rennke H, Brenner B 1985 Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612-619
375. Anderson S, Rennke H, Garcia D, Brenner B 1989 Short- and long-term effects of antihypertensive therapy in the diabetic rat. *Kidney Int* 36:526-536
376. Haylor J, McKillop I, El Nahas M 1995 IGF-I antibody inhibits hyperfiltration following unilateral nephrectomy. *J Am Soc Nephrol* 6:679 (Abstract)
377. Elahi D, McAllon-Dyke M, Fukagawa N, Wong G, Minaker K, Seaman J, Good W, Vandepol C, Shannon R, Miles J, Wolfe R 1991 Hemodynamic and metabolic responses to human insulin-like growth factor I (IGF-I) in men. In: Spencer E (ed) *Modern Concepts of Insulin-Like Growth Factors*. Elsevier, New York, pp 219-224
378. Fryburg D, Jahn L, Hill S, Oliveras D, Barrett E 1995 Insulin and insulin-like growth factor-I enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. *J Clin Invest* 96:722-729
379. Corvilain J, Abramow M 1964 Effect of growth hormone on tubular transport of phosphate in normal and parathyroidectomized dogs. *J Clin Invest* 43:1608-1612
380. Corvilain J, Abramow M 1972 Growth and renal control of plasma phosphate. *J Clin Endocrinol Metab* 34:452-459
381. Bengtsson BA, Eden S, Lonn L, Kvist H, Stokland A, Lindstedt G, Bosaeus I, Tolli J, Sjostrom L, Isaksson OG 1993 Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *J Clin Endocrinol Metab* 76:309-317
382. Clemmons DR 1993 Use of growth hormone and insulin-like growth factor I in catabolism that is induced by negative energy balance. *Horm Res* 40:62-67
383. Marcus R, Butterfield G, Holloway L, Gilliland L, Baylink DJ, Hintz RL, Sherman BM 1990 Effects of short term administration of recombinant human growth hormone to elderly people. *J Clin Endocrinol Metab* 70:519-527
384. van Renen MJ, Hogg RJ, Sweeney AL, Henning PH, Penfold JL, Jureidini KF 1992 Accelerated growth in short children with chronic renal failure treated with both strict dietary therapy and recombinant growth hormone. *Pediatr Nephrol* 6:451-458
385. Lee D, Kurokawa K 1987 Divalent ion metabolism. In: Maxwell M,

- Kleeman C, Narins R (eds) *Clinical Disorders of Fluid and Electrolyte Metabolism*. McGraw-Hill Book Co, New York, pp 245–295
386. **Wesson L** 1964 Electrolyte excretion in relation to diurnal cycles of renal function. Plasma electrolyte concentrations and aldosterone secretion before and during salt and water balance changes in normotensive subjects. *Medicine* 43:547–592
387. **Knox G, Haramati A** 1985 Renal regulation of phosphate excretion. In: Seldin D, Giebisch G (eds) *The Kidney: Physiology and Pathophysiology*. Raven Press, New York, pp 1381–1396
388. **Chase L, Melson G, Aurbach G** 1969 Pseudohypoparathyroidism: defective excretion of 3',5'-AMP in response to parathyroid hormone. *J Clin Invest* 48:1832–1844
389. **Halden A, Eisenberg E, Gordan G** 1964 Parathyroid hormone and phosphate homeostasis in man. *Acta Endocrinol (Copenh)* 46:285–291
390. **Murer H, Werner A, Reshkin S, Wuarin F, Biber J** 1991 Cellular mechanisms in proximal tubular reabsorption of inorganic phosphate. *Am J Physiol* 260:C885–C899
391. **Murer H** 1992 Cellular mechanisms in proximal tubular Pi reabsorption: some answers and more questions. *J Am Soc Nephrol* 2:1649–1665
392. **Biber J, Murer H** 1993 Towards a molecular view of renal proximal tubular reabsorption of phosphate. *Renal Physiol Biochem* 16:37–47
393. **Barac-Nieto M, Spitzer A** 1994 NMR-visible intracellular P(i) and phosphoesters during regulation of Na(+)-P(i) cotransport in opossum kidney cells. *Am J Physiol* 267:C915–C919
394. **Diez JJ, Gomez-Pan A** 1993 New physiological and pharmacological aspects of the growth hormone (I). The effects in adults. *Rev Clin Esp* 192:280–287
395. **Caverzasio J, Montessuit C, Bonjour JP** 1990 Stimulatory effect of insulin-like growth factor-1 on renal Pi transport and plasma 1,25-dihydroxyvitamin D₃. *Endocrinology* 127:453–459
396. **Caverzasio J, Bonjour JP** 1992 Tyrosine phosphorylation selectively regulates renal cellular phosphate transport. Evidence that it mediates the stimulatory effect of insulin-like growth factor-1. *Endocrinology* 130:373–380
397. **Caverzasio J, Bonjour JP** 1992 IGF-1 and phosphate homeostasis during growth (editorial). *Nephrologie* 13:109–113
398. **Harbison MD, Gertner JM** 1990 Permissive action of growth hormone on the renal response to dietary phosphorus deprivation. *J Clin Endocrinol Metab* 70:1035–1040
399. **Nishiyama S, Ikuta M, Nakamura T, Tomoeda S, Matsuda I** 1992 Renal handling of phosphate can predict height velocity during growth hormone therapy for short children. *J Clin Endocrinol Metab* 74:906–909
400. **Ogle GD, Rosenberg AR, Kainer G** 1992 Renal effects of growth hormone. II. Electrolyte homeostasis and body composition. *Pediatr Nephrol* 6:483–489
401. **Caverzasio J, Faundez R, Fleisch H, Bonjour JP** 1981 Tubular adaptation to Pi restriction in hypophysectomized rats. *Pflugers Arch* 392:17–21
402. **Haramati A, Mulrone SE, Lumpkin MD** 1990 Regulation of renal phosphate reabsorption during development: implications from a new model of growth hormone deficiency. *Pediatr Nephrol* 4:387–391
403. **Bonjour J, Caverzasio J** 1991 IGF-I a key controlling element in phosphate homeostasis during growth. In: Spencer E (ed) *Modern Concepts of Insulin-Like Growth Factors*. Elsevier, New York, pp 193–198
404. **Caverzasio J, Bonjour JP** 1993 Growth factors and renal regulation of phosphate transport. *Pediatr Nephrol* 7:802–806
405. **Ernest S, Coureau C, Escoubet B** 1995 Deprivation of phosphate increases IGF-II mRNA in MDCK cells but IGFs are not involved in phosphate transport adaptation to phosphate deprivation. *J Endocrinol* 145:325–331
406. **Gray RW** 1987 Evidence that somatomedins mediate the effect of hypophosphatemia to increase serum 1,25-dihydroxyvitamin D₃ levels in rats. *Endocrinology* 121:504–512
407. **Halloran BP, Spencer EM** 1988 Dietary phosphorus and 1,25-dihydroxyvitamin D metabolism: influence of insulin-like growth factor I. *Endocrinology* 123:1225–1229
408. **Bonjour J, Caverzasio J, Muhlbauer R, Trechsel U, Troehler U** 1982 Are 1,25(OH)₂D₃ production and tubular phosphate transport regulated by one common mechanism which would be defective in x-linked hypophosphatemic rickets? In: Norman A, Schaefer K, Grigoleit H, von Herrath D (eds) *Vitamin D: Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism*. Walter de Gruyter & Co., Berlin, pp 427–433
409. **Muhlbauer RC, Bonjour JP, Fleisch H** 1982 Abnormal tubular adaptation to dietary Pi restriction in X-linked hypophosphatemic mice. *Am J Physiol* 242:F353–F359
410. **Bonjour J, Caverzasio J** 1984 Phosphate transport in the kidney. *Rev Physiol Biochem Pharmacol* 100:162–214
411. **Condamine L, Vztovsnik F, Friedlander G, Mena C, Garabedian M** 1994 Local action of phosphate depletion and insulin-like growth factor 1 on *in vitro* production of 1,25-dihydroxyvitamin D by cultured mammalian kidney cells. *J Clin Invest* 94:1673–1679
412. **Nesbitt T, Drezner MK** 1993 Insulin-like growth factor-I regulation of renal 25-hydroxyvitamin D-1-hydroxylase activity. *Endocrinology* 132:133–138
413. **Lund B, Eskildsen PC, Lund B, Norman AW, Sorensen OH** 1981 Calcium and vitamin D metabolism in acromegaly. *Acta Endocrinol (Copenh)* 96:444–450
414. **Chipman JJ, Zerwekh J, Nicar M, Marks J, Pak CY** 1980 Effect of growth hormone administration: reciprocal changes in serum 1,25-dihydroxyvitamin D and intestinal calcium absorption. *J Clin Endocrinol Metab* 51:321–324
415. **Gertner JM, Horst RL, Broadus AE, Rasmussen H, Genel M** 1979 Parathyroid function and vitamin D metabolism during human growth hormone replacement. *J Clin Endocrinol Metab* 49:185–188
416. **Bouillon R** 1992 Diabetic bone disease. Low turnover osteoporosis related to decreased IGF-I production. *Verh K Acad Geneesk Belg* 54:365–392
417. **Brixen K, Kassem M, Eriksen EF, Nielsen HK, Flyvbjerg A, Mosekilde L** 1993 Growth hormone (GH) and adult bone remodeling: the potential use of GH in treatment of osteoporosis. *J Pediatr Endocrinol* 6:65–71
418. **Burstein S, Chen IW, Tsang RC** 1983 Effects of growth hormone replacement therapy on 1,25-dihydroxyvitamin D and calcium metabolism. *J Clin Endocrinol Metab* 56:1246–1251
419. **Binz K, Schmid C, Bouillon R, Froesch ER, Jurgensen K, Hunziker EB** 1994 Interactions of insulin-like growth factor I with dexamethasone on trabecular bone density and mineral metabolism in rats. *Eur J Endocrinol* 130:387–393
420. **Ebeling PR, Jones JD, WM OF, Janes CH, Riggs BL** 1993 Short-term effects of recombinant human insulin-like growth factor I on bone turnover in normal women. *J Clin Endocrinol Metab* 77:1384–1387
421. **Eriksen EF, Kassem M, Brixen K** 1993 Growth hormone and insulin-like growth factors as anabolic therapies for osteoporosis. *Horm Res* 40:95–98
422. **Inzucchi SE, Robbins RJ** 1994 Clinical review 61: effects of growth hormone on human bone biology. *J Clin Endocrinol Metab* 79:691–694
423. **Johansson AG, Burman P, Westermark K, Ljunghall S** 1992 The bone mineral density in acquired growth hormone deficiency correlates with circulating levels of insulin-like growth factor I. *J Intern Med* 232:447–452
424. **Kassem M, Brixen K, Blum WF, Mosekilde L, Eriksen EF** 1994 Normal osteoclastic and osteoblastic responses to exogenous growth hormone in patients with postmenopausal spinal osteoporosis. *J Bone Miner Res* 9:1365–1370
425. **Kumar R, Merimee T, Silva P, Epstein F** 1979 The effect of growth hormone excess or deficiency of plasma 1,25-dihydroxyvitamin D levels in man. In: Norman A, Schaefer K, Grigoleit H, von Herrath D (eds) *Vitamin D. Basic Research and Its Clinical Application*. de Gruyter, Berlin, pp 1005–1009
426. **Ljunghall S, Johansson AG, Burman P, Kampe O, Lindh E, Karlsson FA** 1992 Low plasma levels of insulin-like growth factor 1 (IGF-1) in male patients with idiopathic osteoporosis. *J Intern Med* 232:59–64
427. **Moriwake T, Tanaka H, Kanzaki S, Higuchi J, Seino Y** 1992 1,25-Dihydroxyvitamin-D stimulates the secretion of insulin-like growth factor binding protein 3 (IGFBP-3) by cultured human osteosarcoma cells. *Endocrinology* 130:1071–1073

428. Nicolas V, Prewett A, Bettica P, Mohan S, Finkelman RD, Baylink DJ, Farley JR 1994 Age-related decreases in insulin-like growth factor-I and transforming growth factor-beta in femoral cortical bone from both men and women: implications for bone loss with aging (see comments). *J Clin Endocrinol Metab* 78:1011-1016
429. Rosen CJ, Donahue LR, Hunter SJ 1994 Insulin-like growth factors and bone: the osteoporosis connection. *Proc Soc Exp Biol Med* 206:83-102
430. Rubin CD, Reed B, Sakhaee K, Pak CY 1994 Treating a patient with the Werner syndrome and osteoporosis using recombinant human insulin-like growth factor. *Ann Intern Med* 121:665-668
431. Saggese G, Federico G, Cinquanta L 1993 *In vitro* effects of growth hormone and other hormones on chondrocytes and osteoblast-like cells. *Acta Paediatr Scand Suppl* [Suppl 82] 391:54-60
432. Adler RA, Farrell ME, Deiss WP, Krieg RJ, MacLeod RM 1991 Hypercalciuria in a new rat model of hyperprolactinemia. *Metabolism* 40:292-296
433. Nutting DF, Schriock EA, Palmieri GM, Bittle JB, Elmendorf BJ, Horner LH, Edwards MC, Griffin JW, Sacks HS, Bertorini TE 1995 Octreotide enhances positive calcium balance in Duchenne muscular dystrophy. *Am J Med Sci* 310:91-98
434. Koppie J, Ding H, Hirschberg R 1995 Effects of recombinant human insulin-like growth factor I on renal handling of phosphorus, calcium and sodium in normal humans. *Am J Kidney Dis* 26:818-824
435. Jabri N, Schalch DS, Schwartz SL, Fischer JS, Kipnes MS, Radnik BJ, Turman NJ, Marcisin VS, Guler HP 1994 Adverse effects of recombinant human insulin-like growth factor I in obese insulin-resistant type II diabetic patients. *Diabetes* 43:369-374
436. Clemmons D, Young S, Morrow L, O'Brien M, Moses A, Insulin-like growth factor I increases insulin sensitivity and improves glycemic control in type II diabetes mellitus. Program of the 77th Annual Meeting of The Endocrine Society, Washington, DC, 1995 (Abstract S42-3)
437. Beck J, McGarry E, Dyrenfurth I, Venning E 1958 The metabolic effects of human and monkey growth hormone in man. *Ann Intern Med* 49:1090-1094
438. Bergenstal D, Lipsett M 1960 Metabolic effects of human growth hormone and growth hormone of other species in man. *J Clin Endocrinol Metab* 20:1424-1436
439. Ho KY, Kelly JJ 1991 Role of growth hormone in fluid homeostasis. *Horm Res* 36 [Suppl 1]:44-48
440. Thompson JL, Butterfield GE, Marcus R, Hintz RL, Van Loan M, Ghiron L, Hoffman AR 1995 The effects of recombinant human insulin-like growth factor-I and growth hormone on body composition in elderly women. *J Clin Endocrinol Metab* 80:1845-1852
441. Valk NK, v.d. Lely AJ, de Herder WW, Lindemans J, Lamberts SW 1994 The effects of human growth hormone (GH) administration in GH-deficient adults: a 20-day metabolic ward study. *J Clin Endocrinol Metab* 79:1070-1076
442. Kaiser FE, Silver AJ, Morley JE 1991 The effect of recombinant human growth hormone on malnourished older individuals. *J Am Geriatr Soc* 39:235-240
443. Clemmons DR, Jones JI, Busby WH, Wright G 1993 Role of insulin-like growth factor binding proteins in modifying IGF actions. *Ann NY Acad Sci* 692:10-21
444. Gesek FA, Schoolwerth AC 1991 Insulin increases Na(+)-H+ exchange activity in proximal tubules from normotensive and hypertensive rats. *Am J Physiol* 260:F695-F703
445. Yanagawa N, Sheikh-Hamad D, Jo O 1991 Insulin-like growth factor II directly increases renal brush border membrane sodium transport. *J Am Soc Nephrol* 2:447 (Abstract)
446. Matsumoto PS, Ohara A, Duchatelle P, Eaton DC 1993 Tyrosine kinase regulates epithelial sodium transport in A6 cells. *Am J Physiol* 264:C246-250
447. Blazer-Yost BL, Cox M, Furlanetto R 1989 Insulin and IGF I receptor-mediated Na+ transport in toad urinary bladders. *Am J Physiol* 257:C612-20
448. Blazer-Yost BL, Cox M 1988 Insulin-like growth factor I stimulates renal epithelial Na+ transport. *Am J Physiol* 255:C413-C417
449. Gallego M, Chai Q, Marrero M, Ling B 1995 Stimulation of Na channels by IGF-I in A6 cells. Possible role of tyrosine phosphorylation. *J Am Soc Nephrol* 6:337 (Abstract)
450. Vehaskari VM, Hering-Smith KS, Moskowitz DW, Weiner ID, Hamm LL 1989 Effect of epidermal growth factor on sodium transport in the cortical collecting tubule. *Am J Physiol* 256:F803-F809
451. Gow CB, Phillips PA, Chandler KD, Moore GP 1993 Hormonal, fluid, and electrolyte responses of sheep during chronic intravenous infusion of epidermal growth factor. *Am J Physiol* 265:R203-R210
452. Gow CB, Phillips PA 1994 Epidermal growth factor as a diuretic in sheep. *J Physiol (Lond)* 477:27-33
453. Nouwen EJ, Verstrepen WA, De Broe ME 1994 Epidermal growth factor in acute renal failure. *Ren Fail* 16:49-60
454. Harris RC 1991 Potential physiologic roles for epidermal growth factor in the kidney. *Am J Kidney Dis* 17:627-630
455. Lakshmanan J, Salido EC, Lam R, Barajas L, Fisher DA 1990 Identification of pro-epidermal growth factor and high molecular weight epidermal growth factors in adult mouse urine. *Biochem Biophys Res Commun* 173:902-911
456. Moller S, Juul A, Becker U, Flyvbjerg A, Skakkebaek NE, Henriksen JH 1995 Concentrations, release, and disposal of insulin-like growth factor (IGF)-binding proteins (IGFBP), IGF-I, and growth hormone in different vascular beds in patients with cirrhosis. *J Clin Endocrinol Metab* 80:1148-1157
457. Chobanian MC, Julin CM, Molteni KH, Brazzy PC 1992 Growth hormone regulates ammoniogenesis in canine renal proximal tubule segments. *Am J Physiol* 262:F878-F884
458. Welbourne TC, Fuseler J 1993 Growth hormone-enhanced acid production and glutamate and glutamine utilization in LLC-PK-F+ cells. *Am J Physiol* 265:E874-E879
459. Welbourne TC, Mu X 1995 Extracellular glutamate flux regulates intracellular glutaminase activity in LLC-PK1-F+ cells. *Am J Physiol* 268:C1418-C1424
460. Sutton JR, Jones NL, Toews CJ 1976 Growth hormone secretion in acid-base alterations at rest and during exercise. *Clin Sci Mol Med* 50:241-247
461. Cappon J, Brasel JA, Mohan S, Cooper DM 1994 Effect of brief exercise on circulating insulin-like growth factor I. *J Appl Physiol* 76:2490-2496
462. Zanonato S, Moromisato DY, Moromisato MY, Woods J, Brasel JA, Leroith D, Roberts Jr CT, Cooper DM 1994 Effect of training and growth hormone suppression on insulin-like growth factor I mRNA in young rats. *J Appl Physiol* 76:2204-2209
463. Cooper DM, Moromisato D, Zanonato S, Moromisato M, Jensen S, Brasel JA 1994 Effect of growth hormone suppression on exercise training and growth responses in young rats. *Pediatr Res* 35:223-227
464. Zhou X, Maniar S, Martini J, Van Nack J, Postel-Vinay M, Kleinknecht C 1995 Plasma and liver mRNA levels of IGF binding proteins in experimental uremia and acidosis. *J Am Soc Nephrol* 6:751 (Abstract)
465. Tisher C, Madsen K 1985 Anatomy of the kidney. In: Brenner B, Rector F (eds) *The Kidney*. W.B. Saunders Co, Philadelphia, pp 3-60
466. Osathanondh V, Potter E 1963 Development of the human kidney as shown by microdissection. III. Formation and interrelationship of collecting tubules and nephrons. *Arch Pathol* 76:290
467. Osathanondh V, Potter E 1963 Development of human kidney shown by microdissection. II. Renal pelvis, calyces, and papillae. *Arch Pathol* 76:227
468. Horster M, Valtin H 1971 Postnatal development of renal function: micropuncture and clearance studies in the dog. *J Clin Invest* 50:779
469. Rogers SA, Ryan G, Hammerman MR 1991 Insulin-like growth factors I and II are produced in the metanephros and are required for growth and development *in vitro*. *J Cell Biol* 113:1447-1453
470. Hammerman MR, Rogers SA, Ryan G 1992 Growth factors and metanephrogenesis. *Am J Physiol* 262:F523-F532
471. Rogers SA, Ryan G, Hammerman MR 1992 Metanephric transforming growth factor- α is required for renal organogenesis *in vitro*. *Am J Physiol* 262:F533-F539
472. Hammerman MR, Rogers SA, Ryan G 1993 Growth factors and kidney development. *Pediatr Nephrol* 7:616-620
473. Rogers SA, Ryan G, Purchio AF, Hammerman MR 1993 Metanephric transforming growth factor-beta 1 regulates nephrogenesis *in vitro*. *Am J Physiol* 264:F996-F1002

474. **Hammerman MR** 1995 Growth factors in renal development. *Semin Nephrol* 15:291-299
475. **Liu ZZ, Kumar A, Wallner EL, Wada J, Carone FA, Kanwar YS** 1994 Trophic effect of insulin-like growth factor-I on metanephric development: relationship to proteoglycans. *Eur J Cell Biol* 65:378-391
476. **Avner ED, Sweeney Jr WE**, 1990 Polypeptide growth factors in metanephric growth and segmental nephron differentiation. *Pediatr Nephrol* 4:372-377
477. **Weller A, Sorokin L, Illgen EM, Ekblom P** 1991 Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factor. *Dev Biol* 144:248-261
478. **Kanwar YS, Liu ZZ, Wada J** 1994 Insulin-like growth factor-I receptor in metanephric development. *Contrib Nephrol* 107:168-173
479. **Liu ZZ, Wada J, Alvares K, Kumar A, Wallner EI, Kanwar YS** 1993 Distribution and relevance of insulin-like growth factor-I receptor in metanephric development. *Kidney Int* 44:1242-1250
480. **Shemer J, Adamo ML, Roberts Jr CT, LeRoith D** 1992 Tissue-specific transcription start site usage in the leader exons of the rat insulin-like growth factor-I gene: evidence for differential regulation in the developing kidney. *Endocrinology* 131:2793-2799
481. **Chin E, Zhou J, Bondy C** 1991 Gene expression of insulin-like growth factors and their receptors in nephrogenesis. Program of the 73rd Ann Meeting of The Endocrine Society Washington DC, 1991 (Abstract 449)
482. **O'Mahoney J, Brandon MR, Adams TE** 1991 Developmental and tissue-specific regulation of ovine insulin-like growth factor II (IGF-II) mRNA expression. *Mol Cell Endocrinol* 78:87-96
483. **Lee WH, Bowsher RR, Apathy JM, Smith MC, Henry DP** 1991 Measurement of insulin-like growth factor-II in physiological fluids and tissues. II. Extraction quantification in rat tissues. *Endocrinology* 128:815-822
484. **Latimer AM, Hausman GJ, McCusker RH, Buonomo FC** 1993 The effects of thyroxine on serum and tissue concentrations of insulin-like growth factors (IGF-I and -II) and IGF-binding proteins in the fetal pig. *Endocrinology* 133:1312-1319
485. **Delhanty PJ, Hill DJ, Shimasaki S, Han VK** 1993 Insulin-like growth factor binding protein-4, -5 and -6 mRNAs in the human fetus: localization to sites of growth and differentiation? *Growth Regul* 3:8-11
486. **Delhanty PJ, Han VK** 1993 The expression of insulin-like growth factor (IGF)-binding protein-2 and IGF-II genes in the tissues of the developing ovine fetus. *Endocrinology* 132:41-52
487. **Hammerman MR, M OS, Miller SB** 1993 Role of growth factors in regulation of renal growth. *Annu Rev Physiol* 55:305-321
488. **Ballesteros M, Scott CD, Baxter RC** 1990 Developmental regulation of insulin-like growth factor-II/ mannose 6-phosphate receptor mRNA in the rat. *Biochem Biophys Res Commun* 172:775-779
489. **Glasscock GF, Gelber SE, Lamson G, McGee-Tekula R, Rosenfeld RG** 1990 Pituitary control of growth in the neonatal rat: effects of neonatal hypophysectomy on somatic and organ growth, serum insulin-like growth factors (IGF)-I and -II levels, and expression of IGF binding proteins. *Endocrinology* 127:1792-1803
490. **Funk B, Kessler U, Eisenmenger W, Hansmann A, Kolb HJ, Kiess W** 1992 The expression of insulin-like growth factor binding proteins is tissue specific during human fetal life and early infancy. *Acta Endocrinol (Copenh)* 127:107-114
491. **Lee CY, Chung CS, Simmen FA** 1993 Ontogeny of the porcine insulin-like growth factor system. *Mol Cell Endocrinol* 93:71-80
492. **Leighton PA, Saam JR, Ingram RS, Stewart CL, Tilghman SM** 1995 An enhancer deletion affects both H19 and Igf2 expression. *Genes Dev* 9:2079-2089
493. **Fogo A, Ichikawa I** 1991 Evidence for a pathogenic linkage between glomerular hypertrophy and sclerosis. *Am J Kidney Dis* 17:666-669
494. **Ichikawa I, Ikoma M, Fogo A** 1991 Glomerular growth promoters, the common key mediator for progressive glomerular sclerosis in chronic renal diseases. *Adv Nephrol Necker Hosp* 20:127-148
495. **Fogo A, Hawkins EP, Berry PL, Glick AD, Chiang ML, MacDonell Jr RC, Ichikawa I** 1990 Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal glomerular sclerosis. *Kidney Int* 38:115-123
496. **Yoshida Y, Fogo A, Ichikawa I** 1989 Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int* 35:654-660
497. **Hirschberg R, Nast C** 1992 Glomerular hypertrophy and segmental glomerulosclerosis are not linked in subtotal nephrectomized rats. *J Am Soc Nephrol* 3:739 (Abstract)
498. **Flyvbjerg A, Orskov H, Nyborg K, Frystyk J, Marshall S, Bornfeldt K, Arnqvist H, Jorgensen K** 1991 Kidney IGF-I accumulation occurs in four different conditions with rapid initial kidney growth in rats. In: Spencer E (ed) *Modern Concepts of Insulin-Like Growth Factors*. Elsevier, New York, pp 207-217
499. **Guler HP, Zapf J, Scheiwiller E, Froesch ER** 1988 Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size in hypophysectomized rats. *Proc Natl Acad Sci USA* 85:4889-4893
500. **Marshall SM, Flyvbjerg A, Jorgensen KD, Weeke J, Orskov H** 1993 Effects of growth hormone and thyroxine on kidney insulin-like growth factor-I and renal growth in hypophysectomized rats. *J Endocrinol* 136:399-406
501. **Habener JF, Cwikel BJ, Hermann H, Hammer RE, Palmiter RD, Brinster RL** 1989 Metallothionein-vasopressin fusion gene expression in transgenic mice. Nephrogenic diabetes insipidus and brain transcripts localized to magnocellular neurons. *J Biol Chem* 264:18844-18852
502. **MacKay K, Striker LJ, Pinkert CA, Brinster RL, Striker GE** 1987 Glomerulosclerosis and renal cysts in mice transgenic for the early region of SV40. *Kidney Int* 32:827-837
503. **MacKay K, Striker LJ, Stauffer JW, Agodoa LY, Striker GE** 1990 Relationship of glomerular hypertrophy and sclerosis: studies in SV40 transgenic mice. *Kidney Int* 37:741-748
504. **Ritz E, Tönshoff B, Worgall S, Kovacs G, Mehls O** 1991 Influence of growth hormone and insulin-like growth factor-I on kidney function and kidney growth. *Pediatr Nephrol* 5:509-512
505. **Striker LJ, Doi T, Striker GE** 1991 Transgenic mice in renal research. *Adv Nephrol Necker Hosp* 20:91-108
506. **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. *Pediatr Nephrol* 5:513-521
507. **Wolf E, Kahnt E, Ehrlein J, Hermanns W, Brem G, Wanke R** 1993 Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: lessons from transgenic animal models. *Mech Ageing Dev* 68:71-87
508. **Doi T, Striker LJ, Gibson CC, Agodoa LY, Brinster RL, Striker GE** 1990 Glomerular lesions in mice transgenic for growth hormone and insulinlike growth factor-I. I. Relationship between increased glomerular size and mesangial sclerosis. *Am J Pathol* 137:541-552
509. **Ikkos D, Ljunggren H, Luft R** 1956 The relation between extracellular and intracellular water in acromegaly. *Acta Endocrinol (Copenhagen)* 21:212-218
510. **O'Shea M, Miller SB, Hammerman MR** 1993 Insulin-like growth factor I and the kidney. *Semin Nephrol* 13:96-108
511. **Mehls O, Irzyniec T, Ritz E, Eden S, Kovacs G, Klaus G, Floege J, Mall G** 1993 Effects of rhGH and rhIGF-I on renal growth and morphology. *Kidney Int* 44:1251-1258
512. **Pesce CM, Striker LJ, Peten E, Elliot SJ, Striker GE** 1991 Glomerulosclerosis at both early and late stages is associated with increased cell turnover in mice transgenic for growth hormone. *Lab Invest* 65:601-605
513. **Feld S, Hirschberg R, Artishevsky A, Nast C, Adler S** 1995 Insulin-like growth factor I induces mesangial proliferation and increases mRNA and secretion of collagen. *Kidney Int* 48:45-51
514. **Doi T, Striker LJ, Elliot SJ, Conti FG, Striker GE** 1989 Insulin-like growth factor-I is a progression factor for human mesangial cells. *Am J Pathol* 134:395-404
515. **Norman JT, Hardy D** 1994 Post-transcriptional regulation of gene expression in compensatory renal hypertrophy. *Exp Nephrol* 2:30-39
516. **Wesson L** 1989 Compensatory growth and other growth responses of the kidney. *Nephron* 51:149-184

517. Miskell C, Simpson D 1990 Hyperplasia precedes increased glomerular filtration rate in rat remnant kidney. *Kidney Int* 37:758-766
518. Humes HD, Cieslinski DA, Johnson LB, Sanchez IO 1992 Triiodothyronine enhances renal tubule cell replication by stimulating EGF receptor gene expression. *Am J Physiol* 262:F540-F545
519. Kujubu DA, Norman JT, Herschman HR, Fine LG 1991 Primary response gene expression in renal hypertrophy and hyperplasia: evidence for different growth initiation processes. *Am J Physiol* 260:F823-F827
520. Miller SB, Rogers SA, Estes CE, Hammerman MR 1992 Increased distal nephron EGF content and altered distribution of peptide in compensatory renal hypertrophy. *Am J Physiol* 262:F1032-F1038
521. Nakamura T, Ebihara I, Fukui M, Osada S, Nagaoka I, Horikoshi S, Tomino Y, Koide H 1993 Messenger RNA expression for growth factors in glomeruli from focal glomerular sclerosis. *Clin Immunol Immunopathol* 66:33-42
522. Fagin JA, Melmed S 1987 Relative increase in insulin-like growth factor I messenger ribonucleic acid levels in compensatory renal hypertrophy. *Endocrinology* 120:718-724
523. Stiles AD, Sosenko IR, D'Ercole AJ, Smith BT 1985 Relation of kidney tissue somatomedin-C/insulin-like growth factor I to post-nephrectomy renal growth in the rat. *Endocrinology* 117:2397-2401
524. Hise MK, Lahn JS, Shao ZM, Mantzouris NM, Fontana JA 1993 Insulin-like growth factor-I receptor and binding proteins in rat kidney after nephron loss. *J Am Soc Nephrol* 4:62-68
525. Haramati A, Lumpkin MD, Mulrone SE 1994 Early increase in pulsatile growth hormone release after unilateral nephrectomy in adult rats. *Am J Physiol* 266:F628-F632
526. Mulrone SE, Lumpkin MD, Roberts Jr CT, LeRoith D, Haramati A 1992 Effect of a growth hormone-releasing factor antagonist on compensatory renal growth, insulin-like growth factor-I (IGF-I), and IGF-I receptor gene expression after unilateral nephrectomy in immature rats. *Endocrinology* 130:2697-2702
527. Mulrone SE, Haramati A, Werner H, Bondy C, Roberts Jr CT, LeRoith D 1992 Altered expression of insulin-like growth factor-I (IGF-I) and IGF receptor genes after unilateral nephrectomy in immature rats. *Endocrinology* 130:249-256
528. Rosendahl W, Foll J, Blum W, Ranke MB 1992 Increased insulin-like growth factor-II tissue concentrations during compensatory kidney growth in infantile rats. *Pediatr Nephrol* 6:527-531
529. Mulrone SE, Haramati A, Roberts Jr CT, LeRoith D 1991 Renal IGF-I mRNA levels are enhanced following unilateral nephrectomy in immature but not adult rats. *Endocrinology* 128:2660-2662
530. Flyvbjerg A, Frystyk J, Marshall SM 1990 Additive increase in kidney insulin-like growth factor I and initial renal enlargement in uninephrectomized-diabetic rats. *Horm Metab Res* 22:516-520
531. Jennische E, Andersson G, Hansson HA 1987 Epidermal growth factor is expressed by cells in the distal tubules during postnephrectomy renal growth. *Acta Physiol Scand* 129:449-450
532. Lajara R, Rotwein P, Bortz JD, Hansen VA, Sadow JL, Betts CR, Rogers SA, Hammerman MR 1989 Dual regulation of insulin-like growth factor I expression during renal hypertrophy. *Am J Physiol* 257:F252-F261
533. Bach LA, Rechler MM 1992 Insulin-like growth factors and diabetes. *Diabetes Metab Rev* 8:229-257
534. Graubert MD, Goldstein S, Phillips LS 1991 Nutrition and somatomedin. XXVII. Total and free IGF-I and IGF binding proteins in rats with streptozocin-induced diabetes. *Diabetes* 40:959-965
535. Luo J, Murphy LJ 1992 Differential expression of the insulin-like growth factor binding proteins in spontaneously diabetic rats. *J Mol Endocrinol* 8:155-163
536. Sayed-Ahmed N, Muchaneta-Kubara EC, Besbas N, Shortland J, Cope GH, el Nahas AM 1993 Insulin-like growth factor-I and experimental diabetic kidney disease. *Exp Nephrol* 1:364-371
537. Flyvbjerg A, Bornfeldt KE, Marshall SM, Arnqvist HJ, Orskov H 1990 Kidney IGF-I mRNA in initial renal hypertrophy in experimental diabetes in rats. *Diabetologia* 33:334-8
538. Bach LA 1992 IGF-I and IGF binding proteins in diabetes-related kidney growth. *Growth Regul* 2:30-39
539. Phillip M, Werner H, Palese T, Kowarski AA, Stannard B, Bach LA, LeRoith D, Roberts Jr CT 1994 Differential accumulation of insulin-like growth factor-I in kidneys of pre- and postpubertal streptozotocin-diabetic rats. *J Mol Endocrinol* 12:215-224
540. Weiss O, Anner H, Nephesh I, Alayoff A, Bursztyn M, Raz I 1995 Insulin-like growth factor-I (IGF-I) and IGF-I receptor gene expression in the kidney of the chronically hypoinsulinemic rat and hyperinsulinemic rat. *Metabolism* 44:982-986
541. Flyvbjerg A, Orskov H 1990 Kidney tissue insulin-like growth factor I and initial renal growth in diabetic rats: relation to severity of diabetes. *Acta Endocrinol (Copenh)* 122:374-378
542. Menon RK, Stephan DA, Rao RH, Shen-Orr Z, Downs Jr LS, Roberts Jr CT, LeRoith D, Sperling MA 1994 Tissue-specific regulation of the growth hormone receptor gene in streptozocin-induced diabetes in the rat. *J Endocrinol* 142:453-462
543. Fervenza F, Tsao T, Hoffman A, Vu T, Rabkin R 1995 The intrarenal IGF-I axis in diabetic renal hypertrophy. *J Am Soc Nephrol* 6:766 (Abstract)
544. Werner H, Shen-Orr Z, Stannard B, Burguera B, Roberts Jr CT, LeRoith D 1990 Experimental diabetes increases insulin-like growth factor I and II receptor concentration and gene expression in kidney. *Diabetes* 39:1490-1497
545. Flyvbjerg A, Kessler U, Dorka B, Funk B, Orskov H, Kiess W 1992 Transient increase in renal insulin-like growth factor binding proteins during initial kidney hypertrophy in experimental diabetes in rats. *Diabetologia* 35:589-593
546. Isley WL, Underwood LE, Clemmons DR 1984 Changes in plasma somatomedin-C in response to ingestion of diets with variable protein and energy content. *JPEN* 8:407-411
547. Underwood LE, Clemmons DR, Maes M, D'Ercole AJ, Ketelslegers JM 1986 Regulation of somatomedin-C/insulin-like growth factor I by nutrients. *Horm Res* 24:166-176
548. Klahr S, Levey AS, Beck GJ, Caggiula AW, Hunsicker L, Kusek JW, Striker G 1994 The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group [see comments]. *N Engl J Med* 330:877-884
549. Lowe Jr WL, Adamo M, Werner H, Roberts Jr CT, LeRoith D 1989 Regulation by fasting of rat insulin-like growth factor I and its receptor. Effects on gene expression and binding. *J Clin Invest* 84:619-626
550. Charlton HM, Clark RG, Robinson IC, Goff AE, Cox BS, Bugnon C, Bloch BA 1988 Growth hormone-deficient dwarfism in the rat: a new mutation. *J Endocrinol* 119:51-58
551. Caverzasio J, Shigematsu T, Rizzoli R, Bonjour J 1995 Potential role for IGF-I in parathyroid hormone-related renal growth induced by high protein diet in uninephrectomized rats. *Kidney Int* 48:33-38
552. Coxam V, Davicco MJ, Durand D, Bauchart D, Barlet JP 1990 Parathyroid hormone and calcitonin may modulate hepatic IGF-I production in calves. *Acta Endocrinol (Copenh)* 123:471-475
553. Coxam V, Davicco MJ, Durand D, Bauchart D, Lefaiivre J, Barlet JP 1992 The influence of parathyroid hormone-related protein on hepatic IGF-1 production. *Acta Endocrinol (Copenh)* 126:430-433
554. Flyvbjerg A, Marshall SM, Frystyk J, Rasch R, Bornfeldt KE, Arnqvist H, Jensen PK, Pallesen G, Orskov H 1992 Insulin-like growth factor I in initial renal hypertrophy in potassium-depleted rats. *Am J Physiol* 262:F1023-F1031
555. Frokiaer J, Flyvbjerg A, Knudsen L 1992 Obstructive nephropathy in the pig. Possible roles for insulin-like growth factor I. *Urol Res* 20:335-339
556. Marshall SM, Flyvbjerg A, Frokiaer J, Orskov H 1991 Insulin-like growth factor 1 and renal growth following ureteral obstruction in the rat. *Nephron* 58:219-224
557. Kobayashi S, Clemmons DR, Nogami H, Roy AK, Venkatachalam MA 1995 Tubular hypertrophy due to work load induced by furosemide is associated with increases of IGF-1 and IGFBP-1. *Kidney Int* 47:818-828
558. Nyengaard JR, Flyvbjerg A, Rasch R 1993 The impact of renal growth, regression and regrowth in experimental diabetes mellitus on number and size of proximal and distal tubular cells in the rat kidney. *Diabetologia* 36:1126-1131
559. Flyvbjerg A 1994 The growth hormone/insulin-like growth factor axis in the kidney: aspects in relation to chronic renal failure. *J Pediatr Endocrinol* 7:85-92
560. Allen DB, Fogo A, el-Hayek R, Langhough R, Friedman AL 1992

- Effects of prolonged growth hormone administration in rats with chronic renal insufficiency. *Pediatr Res* 31:406-410
561. Goya RG, Castelletto L, Sosa YE 1991 Plasma levels of growth hormone correlate with the severity of pathologic changes in the renal structure of aging rats. *Lab Invest* 64:29-34
 562. Trachtman H, Futterweit S, Schwob N, Maesaka J, Valderrama E 1993 Recombinant human growth hormone exacerbates chronic puromycin aminonucleoside nephropathy in rats. *Kidney Int* 44:1281-1288
 563. Doi T, Striker LJ, Kimata K, Peten EP, Yamada Y, Striker GE 1991 Glomerulosclerosis in mice transgenic for growth hormone. Increased mesangial extracellular matrix is correlated with kidney mRNA levels. *J Exp Med* 173:1287-1290
 564. Yang CW, Striker LJ, Pesce C, Chen WY, Peten EP, Elliot S, Doi T, Kopchick JJ, Striker GE 1993 Glomerulosclerosis and body growth are mediated by different portions of bovine growth hormone. *Studies in transgenic mice. Lab Invest* 68:62-70
 565. Yang CW, Striker LJ, Kopchick JJ, Chen WY, Pesce CM, Peten EP, Striker GE 1993 Glomerulosclerosis in mice transgenic for native or mutated bovine growth hormone gene. *Kidney Int Suppl* 39: S90-S94
 566. Stewart TA, Cliff S, Pitts-Meek S, Martin L, Terrell TG, Liggitt D, Oakley H 1992 An evaluation of the functions of the 22-kilodalton (kDa), the 20-kDa, and the N-terminal polypeptide forms of human growth hormone using transgenic mice. *Endocrinology* 130:405-414
 567. Chen NY, Chen WY, Bellush L, Yang CW, Striker LJ, Striker GE, Kopchick JJ 1995 Effects of streptozotocin treatment in growth hormone (GH) and GH antagonist transgenic mice. *Endocrinology* 136:660-667
 568. Haylor J, Johnson T, El Nahas M 1995 Renal matrix protein mRNA elevated by insulin-like growth factor-I. *J Am Soc Nephrol* 6:896 (Abstract)
 569. Peten E, Striker L, Garcia-Perez A, Striker G 1993 Studies by competitive PCR of glomerulosclerosis in growth hormone transgenic mice. *Kidney Int* 43:S55-S58
 570. Lombet JR, Adler SG, Anderson PS, Nast CC, Olsen DR, Glassock RJ 1989 Sex vulnerability in the subtotal nephrectomy model of glomerulosclerosis in the rat. *J Lab Clin Med* 114:66-74
 571. Wang M, Vyhmeister I, Kopple JD, Swendseid ME 1976 Effect of protein intake on weight gain and plasma amino acid levels in uremic rats. *Am J Physiol* 230:1455-1459
 572. Garber AJ 1978 Skeletal muscle protein and amino acid metabolism in experimental chronic uremia in the rat: accelerated alanine and glutamine formation and release. *J Clin Invest* 62:623-632
 573. Kopple JD, Brunori G, Leiserowitz M, Mattimore C, Hirschberg R 1991 Growth hormone treatment for patients with renal failure. *Nippon Jinzo Gakkai Shi* 33:468-474
 574. Kopple JD 1992 The rationale for the use of growth hormone or insulin-like growth factor I in adult patients with renal failure. *Miner Electrolyte Metab* 18:269-275
 575. Martin AA, Tomas FM, Ballard FJ, Read LC 1992 Insulin-like growth factor I and its variant, des(1-3)IGF-I, improve nitrogen balance and food utilization in rats with renal failure. *Miner Electrolyte Metab* 18:264-268
 576. May RC, Kelly RA, Mitch WE 1987 Mechanisms for defects in muscle protein metabolism in rats with chronic uremia. Influence of metabolic acidosis. *J Clin Invest* 79:1099-1103
 577. Slomowitz LA, Monteon FJ, Grosvenor M, Laidlaw SA, Kopple JD 1989 Effect of energy intake on nutritional status in maintenance hemodialysis patients. *Kidney Int* 35:704-711
 578. Mitch WE, Medina R, Griebler S, May RC, England BK, Price SR, Bailey JL, Goldberg AL 1994 Metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes. *J Clin Invest* 93:2127-2133
 579. Rannels SR, Jefferson LS 1980 Effects of glucocorticoids on muscle protein turnover in perfused rat hemi-corpus. *Am J Physiol* 238: E564-E572
 580. Caufriez A, Abramowicz D, Vanherweghem JL, Copinschi G 1993 Insulin-like growth factor I values in patients on maintenance hemodialysis: relationship to growth hormone and albumin levels. *J Endocrinol Invest* 16:691-696
 581. Hondo MM, Vanderschueren-Lodeweyckx M, De Meyts P, Eggermont E 1990 Serum growth hormone levels measured by radioimmunoassay and radioreceptor assay: a useful diagnostic tool in children with growth disorders? *J Clin Endocrinol Metab* 70:1445-1451
 582. Mehls O, Tönshoff B, Haffner D, Wuhl E, Schaefer F 1994 The use of recombinant human growth hormone in short children with chronic renal failure. *J Pediatr Endocrinol* 7:107-113
 583. Yang H, Deng S, Huang S 1991 An observation on growth hormone levels in patients with chronic renal failure. *Hua Hsi I Ko Ta Hsueh Hsueh Pao* 22:100-102
 584. Tönshoff B, Veldhuis JD, Heinrich U, Mehls O 1995 Deconvolution analysis of spontaneous nocturnal growth hormone secretion in prepubertal children with preterminal chronic renal failure and with end-stage renal disease. *Pediatr Res* 37:86-93
 585. Tönshoff B, Eden S, Weiser E, Carlsson B, Robinson IC, Blum WF, Mehls O 1994 Reduced hepatic growth hormone (GH) receptor gene expression and increased plasma GH binding protein in experimental uremia. *Kidney Int* 45:1085-1092
 586. Blum WF 1991 Insulin-like growth factors (IGFs) and IGF binding proteins in chronic renal failure: evidence for reduced secretion of IGFs. *Acta Paediatr Scand Suppl* 379:24-32
 587. Fouque D 1995 Insulin-like growth factor 1 resistance in chronic renal failure. *Miner Electrolyte Metab* 22:133-137
 588. Fouque D, Le Bouc Y, Laville M, Combarnous F, Joly M 1995 Insulin-like growth factor-1 and its binding proteins during a low protein diet in chronic renal failure. *J Am Soc Nephrol* 6:1427-1433
 589. Hodson EM, Brown AS, Roy LP, Rosenberg AR 1992 Insulin-like growth factor-1, growth hormone-dependent insulin-like growth factor-binding protein and growth in children with chronic renal failure. *Pediatr Nephrol* 6:433-438
 590. Powell D, Rosenfeld R, Sperry J, Baker B, Hintz R 1987 Serum concentrations of insulin-like growth factor (IGF)-1, IGF-2 and unsaturated somatomedin carrier proteins in children with chronic renal failure. *Am J Kidney Dis* 4:287-292
 591. Powell DR, Rosenfeld RG, Baker BK, Liu F, Hintz RL 1986 Serum somatomedin levels in adults with chronic renal failure: the importance of measuring insulin-like growth factor I (IGF-I) and IGF-II in acid-chromatographed uremic serum. *J Clin Endocrinol Metab* 63:1186-1192
 592. Coen G, Mazzaferro S, Ballanti P, Bonucci E, Bondatti F, Pasquali M, Perruzza I, Manni M, Sardella D 1991 Plasma insulin-like growth factor-1 and bone formation parameters in predialysis chronic renal failure. *Miner Electrolyte Metab* 17:153-159
 593. Phillips LS, Kopple JD 1981 Circulating somatomedin activity and sulfate levels in adults with normal and impaired kidney function. *Metabolism* 30:1091-1095
 594. Fouque D, Peng SC, Kopple JD 1995 Impaired metabolic response to recombinant insulin-like growth factor-1 in dialysis patients. *Kidney Int* 47:876-883
 595. Chan W, Valerie KC, Chan JC 1993 Expression of insulin-like growth factor-1 in uremic rats: growth hormone resistance and nutritional intake. *Kidney Int* 43:790-795
 596. Lee P, Hintz R, Sperry J, Baxter R, Powell D 1989 IGF binding proteins in growth-retarded children with chronic renal failure. *Pediatr Nephrol* 5:539-544
 597. Suikkari AM, Baxter RC 1992 Insulin-like growth factor-binding protein-3 is functionally normal in pregnancy serum. *J Clin Endocrinol Metab* 74:177-183
 598. Langford K, Blum W, Nicolaidis K, Jones J, McGregor A, Miell J 1994 The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by undernutrition? *Eur J Clin Invest* 24:851-856
 599. Langford KS, Nicolaidis KH, Jones J, Abbas A, McGregor AM, Miell JP 1995 Serum insulin-like growth factor-binding protein-3 (IGFBP-3) levels and IGFBP-3 protease activity in normal, abnormal, and multiple human pregnancy. *J Clin Endocrinol Metab* 80:21-27
 600. Bang P, Brismar K, Rosenfeld RG 1994 Increased proteolysis of insulin-like growth factor-binding protein-3 (IGFBP-3) in noninsulin-dependent diabetes mellitus serum, with elevation of a 29-kilodalton (kDa) glycosylated IGFBP-3 fragment contained in the

- approximately 130- to 150-kDa ternary complex. *J Clin Endocrinol Metab* 78:1119-1127
601. Davenport ML, Isley WL, Pucilowska JB, Pemberton LB, Lyman B, Underwood LE, Clemmons DR 1992 Insulin-like growth factor-binding protein-3 proteolysis is induced after elective surgery. *J Clin Endocrinol Metab* 75:590-595
 602. Davenport ML, Pucilowska J, Clemmons DR, Lundblad R, Spencer JA, Underwood LE 1992 Tissue-specific expression of insulin-like growth factor binding protein-3 protease activity during rat pregnancy. *Endocrinology* 130:2505-2512
 603. Fielder PJ, Thordarson G, Talamantes F, Rosenfeld RG 1990 Characterization of insulin-like growth factor binding proteins (IGFBPs) during gestation in mice: effects of hypophysectomy and an IGFBP-specific serum protease activity. *Endocrinology* 127:2270-2280
 604. Nonoshita LD, Wathen NC, Dsupin BA, Chard T, Giudice LC 1994 Insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and proteolyzed IGFBP-3 in embryonic cavities in early human pregnancy: their potential relevance to maternal-embryonic and fetal interactions. *J Clin Endocrinol Metab* 79:1249-1255
 605. Lamson G, Giudice LC, Rosenfeld RG 1991 A simple assay for proteolysis of IGFBP-3. *J Clin Endocrinol Metab* 72:1391-1393
 606. Lamson G, Giudice LC, Cohen P, Liu F, Gargosky S, Muller HL, Oh Y, Wilson KF, Hintz RL, Rosenfeld RG 1993 Proteolysis of IGFBP-3 may be a common regulatory mechanism of IGF action *in vivo*. *Growth Regul* 3:91-95
 607. Davies SC, Wass JA, Ross RJ, Cotterill AM, Buchanan CR, Coulson VJ, Holly JM 1991 The induction of a specific protease for insulin-like growth factor binding protein-3 in the circulation during severe illness. *J Endocrinol* 130:469-473
 608. Buyan N, Cinaz P, Hasanoglu E, Gokcora N, Bircan Z 1995 Changes in serum levels of IGF-I and IGFBP-3 in children with chronic renal failure. *Endocr J* 42:429-433
 609. Powell DR, Liu F, Baker B, Lee PD, Belsha CW, Brewer ED, Hintz RL 1993 Characterization of insulin-like growth factor binding protein-3 in chronic renal failure serum. *Pediatr Res* 33:136-143
 610. Lee DY, Park SK, Yorgin PD, Cohen P, Oh Y, Rosenfeld RG 1994 Alteration in insulin-like growth factor-binding proteins (IGFBPs) and IGFBP-3 protease activity in serum and urine from acute and chronic renal failure. *J Clin Endocrinol Metab* 79:1376-1382
 611. Lee Y, Rechler M 1996 Proteolysis of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in 150-kilodalton IGFBP complexes by a cation-dependent protease activity in adult rat serum promotes the release of bound IGF-I. *Endocrinology* 137:2051-2058
 612. Baxter RC, Martin JL 1986 Radioimmunoassay of growth hormone-dependent insulin-like growth factor binding protein in human plasma. *J Clin Invest* 78:1504-1512
 613. Blat C, Villaudy J, Binoux M 1994 *In vivo* proteolysis of serum insulin-like growth factor (IGF) binding protein-3 results in increased availability of IGF to target cells. *J Clin Invest* 93:2286-2290
 614. Gargosky SE, Owens PC, Walton PE, Owens JA, Robinson JS, Wallace JC, Ballard FJ 1991 Most of the circulating insulin-like growth factors-I and -II are present in the 150 kDa complex during human pregnancy. *J Endocrinol* 131:491-497
 615. Blum W, Ranke M, Kietzmann K, Tönshoff B, Mehls O 1989 Excess of IGF-binding protein in chronic renal failure: Evidence for relative growth hormone resistance and inhibition of somatomedin activity. In: Drop S, Hintz R (eds) *Insulin-Like Growth Factor Binding Proteins*. Elsevier Science Publishers, Basel, pp 93-98
 616. Valentini RP, Mudge NA, Bunchman TE 1994 Dialysis modality comparison of insulin-like growth factor binding protein-3 removal in children: could this have a potential growth benefit? *Adv Perit Dial* 10:327-330
 617. Blum W, Ranke M, Kietzmann K, Tönshoff B, Mehls O 1991 Growth hormone resistance and inhibition of somatomedin activity by excess of insulin-like growth factor binding protein in uremia. *Pediatr Nephrol* 5:539-544
 618. Blum WF, Horn N, Kratzsch J, Jorgensen JO, Juul A, Teale D, Mohnike K, Ranke MB 1993 Clinical studies of IGFBP-2 by radioimmunoassay. *Growth Regul* 3:100-104
 619. O'Shea M, Miller SB, Hammerman MR 1993 Effects of IGF-I on renal function in patients with chronic renal failure. *Am J Physiol* 264:F917-F922
 620. Miller SB, Moulton M, O'Shea M, Hammerman MR 1994 Effects of IGF-I on renal function in end-stage chronic renal failure. *Kidney Int* 46:201-207
 621. Hammerman MR, Miller SB 1994 Therapeutic use of growth factors in renal failure (editorial). *J Am Soc Nephrol* 5:1-11
 622. Daughaday WH, Trivedi B 1992 Heterogeneity of serum peptides with immunoactivity detected by a radioimmunoassay for proinsulin-like growth factor-II E domain: description of a free E domain peptide in serum. *J Clin Endocrinol Metab* 75:641-645
 623. Hokken-Koelega AC, Stijnen T, De Jong MC, Donckerwolcke RA, De Muinck Keizer-Schrama SM, Blum WF, Drop SL 1994 Double blind trial comparing the effects of two doses of growth hormone in prepubertal patients with chronic renal insufficiency. *J Clin Endocrinol Metab* 79:1185-1190
 624. Laron Z, Klingler B 1994 Laron syndrome: Clinical features, molecular pathology and treatment. *Horm Res* 42:198-202
 625. Ding H, Gao X, Hirschberg R, Vadgama J, Kopple J 1996 Impaired actions of insulin-like growth factor 1 on protein synthesis and degradation in skeletal muscle of rats with chronic renal failure: evidence for a post-receptor defect. *J Clin Invest* 97:1064-1075
 626. Mehls O, Blum WF, Schaefer F, Tönshoff B, Scharer K 1992 Growth failure in renal disease. *Baillieres Clin Endocrinol Metab* 6:665-685
 627. Mehls O, Ritz E, Hunziker E, Egli P, Heinrich U, Zapf J 1988 Improvement of growth and food utilization by human recombinant growth hormone in uremia. *Kidney Int* 33:45-52
 628. Nakano M, Kainer G, Foreman J, Ko D, Chan J 1989 The effects of exogenous rat growth hormone therapy on growth of uremic rats fed an 8% protein diet. *Pediatr Res* 26:204-207
 629. Fine RN 1991 Growth hormone and the kidney: the use of recombinant human growth hormone (rhGH) in growth-retarded children with chronic renal insufficiency. *J Am Soc Nephrol* 1:1136-1145
 630. Tönshoff B, Mehls O, Heinrich U, Blum WF, Ranke MB, Schauer A 1990 Growth-stimulating effects of recombinant human growth hormone in children with end-stage renal disease. *J Pediatr* 116:561-566
 631. Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Wit JM, Wolff ED, de Jong MC, Donckerwolcke RA, Abbad NC, Bot A, Blum WF, Drop S 1991 Placebo-controlled, double-blind, cross-over trial of growth hormone treatment in prepubertal children with chronic renal failure. *Lancet* 338:585-590
 632. Fine RN 1994 Pathophysiology of growth retardation in children with chronic renal failure. *J Pediatr Endocrinol* 7:79-83
 633. Fine RN, Kohaut EC, Brown D, Perlman AJ 1994 Growth after recombinant human growth hormone treatment in children with chronic renal failure: report of a multicenter randomized double-blind placebo-controlled study. *Genentech Cooperative Study Group. J Pediatr* 124:374-382
 634. Fine RN, Yadin O, Moulton L, Nelson PA, Boechar MI, Lippe BM 1994 Five year experience with recombinant human growth hormone treatment of children with chronic renal failure. *J Pediatr Endocrinol* 7:1-12
 635. Chan JC, Boineau FG, Ruley EJ, Lum GM, Weiss RA, Waldo FB, Pomrantz A, Hellerstein S, Fine RN 1990 Descriptions of the participating centers and patient population in the Growth Failure in Children with Renal Diseases Study. *J Pediatr* 116:S24-S27
 636. Fine R, Kohaut E, Brown D, Kuntze J, Attie K 1995 Long term (5-year) treatment of growth retarded children with chronic renal insufficiency with recombinant human growth hormone. *J Am Soc Nephrol* 6:386 (Abstract)
 637. Nivet H, Andre J, Berard E 1995 Growth hormone (rhGH) treatment in children with renal failure. A multicenter study. *J Am Soc Nephrol* 6:1025 (Abstract)
 638. Watkins S, Blifield C, Klee K, Wicklund L, Striegel J, Avner E 1994 Intraperitoneal recombinant human growth hormone to improve the short stature associated with chronic renal failure in pediatric peritoneal dialysis patients. *J Am Soc Nephrol* 5:344 (Abstract)
 639. Tönshoff B, Haffner D, Mehls O, Dietz M, Ruder H, Blum W, Heinrich U, Strover B 1993 Efficacy and safety of growth hormone treatment in short children with renal allografts: three year experience. Member of the German study group for growth hormone treatment in children with renal allografts. *Kidney Int* 44:199-207
 640. Wuhl E, Haffner D, Tönshoff B, Mehls O 1993 Predictors of

- growth response to rhGH in short children before and after renal transplantation. German study group for growth hormone treatment in chronic renal failure. *Kidney Int* 43:S76-S82
641. **Furlanetto R, Lawson Wilkins Pediatric Endocrine Society** 1995 Guidelines for the use of growth hormone in children with short stature. *J Pediatr* 127:857-867
642. **Bartosh S, Wagner-Weiner L, Spencer C** 1995 Renal deterioration in children with lupus following treatment with growth hormone. *J Am Soc Nephrol* 6:412 (Abstract)
643. **Tönshoff B, Tönshoff C, Mehls O, Pinkowski J, Blum WF, Heinrich U, Stover B, Gretz N** 1992 Growth hormone treatment in children with preterminal chronic renal failure: no adverse effect on glomerular filtration rate. *Eur J Pediatr* 151:601-607
644. **Tönshoff B, Dietz M, Haffner D, Tönshoff C, Stover B, Mehls O** 1991 Effects of two years of growth hormone treatment in short children with renal disease. The German Study Group for Growth Hormone Treatment in Chronic Renal Failure. *Acta Paediatr Scand Suppl* 379:33-41
645. **Tönshoff B, Heinrich U, Mehls O** 1991 How safe is the treatment of uraemic children with recombinant human growth hormone? *Pediatr Nephrol* 5:454-460
646. **Riedl M, Haas M, Oberbauer R, Gisinger J, Luger A, Mayer G** 1995 The effects of prolonged substitution of recombinant human growth hormone on renal functional reserve in growth hormone-deficient adults. *J Am Soc Nephrol* 6:1434-1438
647. **Maxwell H, Nair DR, Dalton RN, Rigden SP, Rees L** 1995 Differential effects of recombinant human growth hormone on glomerular filtration rate and renal plasma flow in chronic renal failure. *Pediatr Nephrol* 9:458-463
648. **Baker D, Kees U, Price P, Willoghby M** 1993 Late leukemic relapse 10 years from diagnosis in a child on recombinant human growth hormone. *Pediatr Hematol Oncol* 10:55-62
649. **Stahnke N** 1992 Leukemia in growth-hormone-treated patients: an update, 1992. *Horm Res* 38:56-62
650. **Fradkin JE, Mills JL, Schonberger LB, Wysowski DK, Thomson R, Durako SJ, Robison LL** 1993 Risk of leukemia after treatment with pituitary growth hormone. *JAMA* 270:2829-2832
651. **Kopple J, Massry S** 1988 Is there an association between neoplasia and primary or secondary hyperparathyroidism? *Am J Nephrol* 8:437-448
652. **Rapaport R, Oberfield S, Robison L, Salisbury S, David R, Rao J, Rdmond G** 1995 Relationship of growth hormone deficiency and leukemia. *J Pediatr* 126:759-761
653. **Boose A, Pieters R, Delmarre-van de Waal H, Veerman A** 1992 Growth hormone therapy and leukemia. *Tijdschr Kindergeneesk* 60:1-6
654. **Ogilvy-Stuart A, Shalet S** 1992 Tumour occurrence and recurrence. *Horm Res* 38:50-55
655. **Ron E, Gridley G, Hrubec Z, Page W, Arora S, Fraumeni J** 1991 Acromegaly and gastrointestinal cancer. *Cancer* 68:1673-1677
656. **Ron E, Gridley G, Hrubec Z, Page W, Arora S, Fraumeni J** 1992 Acromegaly and gastrointestinal cancer. *Cancer* 69:549
657. **Buchanan C, Preece M, Milner R** 1991 Mortality, neoplasia, and Creutzfeldt-Jacob disease in patients treated with human pituitary growth hormone in the united kingdom. *Br Med J* 302: 824-828
658. **Nelson H** 1985 Growth hormone may be tied to three deaths. *Los Angeles Times*, Los Angeles, CA, 1-3 & 1-18
659. **Guevara-Aguirre J, Vasconez O, Martinez V, Martinez AL, Rosenbloom AL, Diamond Jr FB, Gargosky SE, Nonoshita L, Rosenfeld RG** 1995 A randomized, double blind, placebo-controlled trial on safety and efficacy of recombinant human insulin-like growth factor-I in children with growth hormone receptor deficiency. *J Clin Endocrinol Metab* 80:1393-1398
660. **Hakim R, Levin N** 1993 Malnutrition in hemodialysis patients. *Am J Kidney Dis* 21:125-137
661. **Bergström J** 1985 Protein catabolic factors in patients on renal replacement therapy. In-depth review. *Blood Purif* 3:215-236
662. **Bergström J** 1995 Why are dialysis patients malnourished? *Am J Kidney Dis* 26:229-241
663. **USRDS Annual Data Report**. 1993 The National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, pp D26-D43
664. **Marckmann P** 1989 Nutritional status and mortality of patients in regular dialysis therapy. *J Intern Med* 226:429-432
665. **Ziegler T, Leader I** 1994 Adjunctive human growth hormone therapy in nutrition support: potential to limit septic complications in intensive care unit patients. *Semin Respir Infect* 9:240-247
666. **Ziegler TR, Lazarus JM, Young LS, Hakim R, Wilmore DW** 1991 Effects of recombinant human growth hormone in adults receiving maintenance hemodialysis. *J Am Soc Nephrol* 2:1130-1135
667. **Ikizler TA, Wingard RL, Breyer JA, Schulman G, Parker RA, Hakim RM** 1994 Short-term effects of recombinant human growth hormone in CAPD patients. *Kidney Int* 46:1178-1183
668. **Ikizler TA, Wingard RL, Hakim RM** 1995 Interventions to treat malnutrition in dialysis patients: the role of the dose of dialysis, intradialytic parenteral nutrition, and growth hormone. *Am J Kidney Dis* 26:256-265
669. **Schulman G, Wingard R, Hutchinson R, Lawrence P, Hakim R** 1993 The effects of recombinant human growth hormone and intradialytic parenteral nutrition in malnourished hemodialysis patients. *Am J Kidney Dis* 21:527-534
670. **Kopple J, Brunori G, Leiserowitz M, Mattimore C, Hirschberg R** 1991 Growth hormone treatment for patients with chronic renal failure. *Nippon Jinzo Gakkai Shi* 33:468-474
671. **Kopple J** 1992 The rationale for the use of growth hormone or insulin-like growth factor I in adult patients with renal failure. *Miner Electrolyte Metab* 18:269-275
672. **Kupfer SR, Underwood LE, Baxter RC, Clemmons DR** 1993 Enhancement of the anabolic effects of growth hormone and insulin-like growth factor I by use of both agents simultaneously. *J Clin Invest* 91:391-396
673. **Young S, Underwood L, Celniker A, Clemmons D** 1992 Effects of recombinant insulin-like growth factor-I (IGF-I) and growth hormone on serum IGF-binding proteins in calorically restricted adults. *J Clin Endocrinol Metab* 75:603-608
674. **Clemmons D** 1992 Role of insulin-like growth factor-1 in reversing catabolism. *J Clin Endocrinol Metab* 75:1183-1185
675. **Clemmons D, Smith-Banks A, Underwood L** 1992 Reversal of diet-induced catabolism by infusion of recombinant insulin-like growth factor-I in humans. *J Clin Endocrinol Metab* 75:234-238
676. **Kopple JD, Ding H, Gao XL** 1995 Altered physiology and action of insulin-like growth factor 1 in skeletal muscle in chronic renal failure. *Am J Kidney Dis* 26:248-255
677. **Shamir E, Peng S, Fouque D, Kopple J** 1995 Effect of insulin-like growth factor 1 on nitrogen balance in malnourished CAPD patients. *J Am Soc Nephrol* 6:587 (Abstract)
678. **De Marchi S, Pirisi M, Ferraccioli GF** 1993 Erythropoietin and the anemia of chronic diseases. *Clin Exp Rheumatol* 11:429-444
679. **Urena P, Bonnardeaux A, Eckardt KU, Kurtz A, Druke TB** 1992 Insulin-like growth factor I: a modulator of erythropoiesis in uraemic patients? *Nephrol Dial Transplant* 7:40-44
680. **Miller SB, Hansen VA, Hammerman MR** 1990 Effects of growth hormone and IGF-I on renal function in rats with normal and reduced renal mass. *Am J Physiol* 259:F747-F751
681. **Franklin S, Moulton M, Hammerman M, Miller S** 1995 Sustained improvement of renal function and amelioration of symptoms in patients with chronic renal failure (CRF) treated with insulin-like growth factor I (IGF I). *J Am Soc Nephrol* 6:387 (Abstract)
682. **Bonventre J** 1993 Mechanisms of ischemic acute renal failure. *Kidney Int* 43:1160-1178
683. **Jennische E, Andersson G** 1986 Selective damage to S3-segments in post-ischemic kidney as demonstrated by a simple histochemical method. *Acta Pathol Microbiol Immunol Scand [A]* 94:167-168
684. **Barnes JL, Osgood RW, Reineck HJ, Stein JH** 1981 Glomerular alterations in an ischemic model of acute renal failure. *Lab Invest* 45:378-386
685. **Yagil Y, Myers BD, Jamison RL** 1988 Course and pathogenesis of postischemic acute renal failure in the rat. *Am J Physiol* 255:F257-F2564
686. **Schumer M, Colombel M, Sawczuk I, Gobe G, Connor J, O'Toole K, Olsson C, Wise G, Buttyan R** 1992 Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. *Am J Pathol* 140:831-838
687. **Beeri C, Symon Z, Brezis M, Ben-Sasson S, Baehr P, Rosen S,**

- Zager R 1995 Rapid DNA fragmentation from hypoxia along the thick ascending limb of rat kidneys. *Kidney Int* 47:1806–1810
688. Zager R, Fuerstenberg S, Baehr P, Myerson D, Torok-Starb B 1994 An evaluation of antioxidant effects on recovery from postischemic acute renal failure. *J Am Soc Nephrol* 4:1588–1597
689. Savill J 1995 Apoptosis and renal injury. *Curr Op Nephrol Hypertens* 4:263–269
690. Nath K, Dvergsten J, Correa-Rotter R, Hostetter T, Manivel J, Rosenberg M 1994 Induction of clusterin in acute and chronic oxidative renal disease in the rat and its dissociation from cell injury. *Lab Invest* 71:209–218
691. Silnkens J, Skubitz K, Rosenberg M 1994 Clusterin: a novel renal epithelial cell aggregation and adhesion molecule. *J Am Soc Nephrol* 5:819 (Abstract)
692. Kellerman P, Clark R, Hoilien C, Linas S, Molitoris B 1990 Role of microfilaments in maintenance of proximal tubule structural and functional integrity. *Am J Physiol* 259:F279–F285
693. Molitoris B 1992 New insights into the cell biology of ischemic acute renal failure. *J Am Soc Nephrol* 1:1263–1270
694. Ding H, Qing P-Y, Gao X-L, Hirschberg R, Kopple J 1995 Time dependent changes in the expression of insulin-like growth factor 1 (IGF-1) and IGF-1 receptor (IGF-1 R) in tissues of rats with acute renal failure (ARF). *J Am Soc Nephrol* 6:461 (Abstract)
695. Lin JJ, Cybulsky AV, Goodyer PR, Fine RN, Kaskel FJ 1995 Insulin-like growth factor-1 enhances epidermal growth factor receptor activation and renal tubular cell regeneration in postischemic acute renal failure [see comments]. *J Lab Clin Med* 125:724–733
696. Hammerman MR, Miller SB 1995 Growth factor gene expression in tubular epithelial injury. *Curr Opin Nephrol Hypertens* 4:258–262
697. Noji S, Harada K, Itano Y, Hirakawa M 1992 *In situ* hybridization histochemistry for the kidney diseases. *Nippon Rinsho* 50:2855–2860
698. Tsao T, Wang J, Fervenza FC, Vu TH, Jin IH, Hoffman AR, Rabkin R 1995 Renal growth hormone—insulin-like growth factor-I system in acute renal failure. *Kidney Int* 47:1658–1668
699. Clark R, Mortensen D, Rabkin R 1994 Recovery from acute ischaemic renal failure is accelerated by des-(1–3)-insulin-like growth factor-1. *Clin Sci* 86:709–714
700. Ding H, Kopple JD, Cohen A, Hirschberg R 1993 Recombinant human insulin-like growth factor-I accelerates recovery and reduces catabolism in rats with ischemic acute renal failure. *J Clin Invest* 91:2281–2287
701. Miller SB, Martin DR, Kissane J, Hammerman MR 1992 Insulin-like growth factor I accelerates recovery from ischemic acute tubular necrosis in the rat. *Proc Natl Acad Sci USA* 89:11876–11880
702. Hirschberg R 1993 Insulin-like growth factor 1 and recovery from experimental acute renal failure [editorial; comment]. *Nutrition* 9:562–563
703. Rabkin R 1995 Insulin-like growth factor-I treatment of acute renal failure [editorial; comment]. *J Lab Clin Med* 125:684–685
704. Noguchi S, Kashihara Y, Ikegami Y, Morimoto K, Miyamoto M, Nakao K 1993 Insulin-like growth factor-I ameliorates transient ischemia-induced acute renal failure in rats. *J Pharmacol Exp Ther* 267:919–926
705. Martin AA, Gillespie CM, Moore L, Ballard FJ, Read LC 1994 Effects of insulin-like growth factor-I peptides in rats with acute renal failure. *J Endocrinol* 140:23–32
706. Miller SB, Martin DR, Kissane J, Hammerman MR 1994 Rat models for clinical use of insulin-like growth factor I in acute renal failure. *Am J Physiol* 266:F949–F956
707. Schaudies RP, Nonclercq D, Nelson L, Toubeau G, Zanen J, Heuson-Stiennon JA, Laurent G 1993 Endogenous EGF as a potential renotropic factor in ischemia-induced acute renal failure. *Am J Physiol* 265:F425–F434
708. Kawaida K, Matsumoto K, Shimazu H, Nakamura T 1994 Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice. *Proc Natl Acad Sci USA* 91:4357–4361
709. Igawa T, Matsumoto K, Kanda S, Saito Y, Nakamura T 1993 Hepatocyte growth factor may function as a renotropic factor for regeneration in rats with acute renal injury. *Am J Physiol* 265:F61–F69
710. Coimbra TM, Cieslinski DA, Humes HD 1990 Epidermal growth factor accelerates renal repair in mercuric chloride nephrotoxicity. *Am J Physiol* 259:F438–F443
711. Sell C, Baserga R, Rubin R 1995 Insulin-like growth factor-I (IGF-I) and the IGF-I receptor prevent etoposide-induced apoptosis. *Cancer Res* 55:303–306
712. Woo D 1995 Apoptosis and loss of renal tissue in polycystic kidney diseases. *N Engl J Med* 333:18–25
713. Padanilam B, Martin D, Hammerman M 1996 Insulin-like growth factor I-enhanced renal expression of osteopontin after acute ischemic injury in rats. *Endocrinology* 137:2133–2140
714. Humes HD, Beals TF, Cieslinski DA, Sanchez IO, Page TP 1991 Effects of transforming growth factor-beta, transforming growth factor-alpha, and other growth factors on renal proximal tubule cells. *Lab Invest* 64:538–545
715. Cowley BD, Gudapaty S 1995 Temporal alterations in regional gene expression after nephrotoxic renal injury. *J Lab Clin Med* 125:187–199
716. Iizuka K 1993 Immunohistochemical localization of epidermal growth factor in proximal tubules of rats with acute renal failure. *Nippon Jinzo Gakkai Shi* 35:1015–1021
717. Price PM, Megyesi J, Saggi S, Safirstein RL 1995 Regulation of transcription by the rat EGF gene promoter in normal and ischemic murine kidney cells. *Am J Physiol* 268:F664–F670
718. Taira T, Yoshimura A, Inui K, Oshiden K, Ideura T, Koshikawa S, Solez K 1994 Immunohistochemical study of epidermal growth factor in rats with mercuric chloride-induced acute renal failure. *Nephron* 67:88–93
719. Liu ZH 1992 Epidermal growth factor and its receptor in the renal tissue of patients with acute renal failure and normal persons. *Chung Hua I Hsueh Tsa Chih* 72:593–595; 638–639
720. Valentin J, Qiu C, Muldowney W, Ying W, Gardner D, Humphreys M 1992 Cellular basis for blunted volume expansion natriuresis in experimental nephrotic syndrome. *J Clin Invest* 90:1302–1312
721. Hirschberg R 1994 Evidence for biologic activity of tubule fluid IGF-I in rats with the nephrotic syndrome. *J Am Soc Nephrol* 5:944 (Abstract)
722. Vadgama J, Franklin K, Ding H, Chang K, Hirschberg R, Kopple J 1995 Serum and skeletal muscle IGF-I and IGF-binding protein expression in rats with chronic renal failure. *J Am Soc Nephrol* 6:1033 (Abstract)
723. Tönshoff B, Powell D, Zhao D, Domene H, Blum W, Moor L, Kaskel F 1995 Decreased hepatic insulin-like growth factor (IGF)-1 and increased IGF binding protein (IGFBP)-1 and -2 gene expression in experimental uremia. *J Am Soc Nephrol* 6:1032
724. Fine RN, Yadin O, Moulten L, Nelson PA, Boechat MI, Lippe BH 1992 Extended recombinant human growth hormone treatment after renal transplantation in children. *J Am Soc Nephrol* 2:S274–S283
725. Jabs K, Van Dop C, Harmon WE 1993 Growth hormone treatment of growth failure among children with renal transplants. *Kidney Int Suppl* 43:S71–S75