Immunoreactive insulin-like growth factor II in urine

Walter Zumkeller and Kerstin Hall

Department of Endocrinology, Karolinska Hospital and Institute, Stockholm, Sweden

Abstract. Insulin-like growth factor II and insulin-like growth factor binding protein-I were identified and quantified in the urine of 23 healthy subjects between 17 and 76 years of age. IGF-II was measured after separation by gel chromatography at low pH and compared with IGF-I levels in the same samples, whereas IGF binding protein-I was measured in dialysed urine. Urinary IGF-II was found at much higher concentrations than IGF-I (mean ±SEM: 717±69 vs 110±5 ng/mmol creatinine). The chromatographic profile indicates that pro-IGF-II may also be present. The concentrations of IGF-II appear to be less variable than the other reported parameters. The mean IGF binding protein-I concentrations in these urine samples was 414±83 ng/mmol creatinine. IGFs in the urine are in part bound to binding proteins.

Insulin-like growth factor II is a single-chain protein of 67 amino acids with a close amino acid homology to both IGF-I and insulin (1). Several variants and proforms of IGF-II have been described, indicating that IGF-II may not require processing to its mature form before release occurs (2-6). Immunoreactive IGF-II has been found in plasma (1), spinal fluid (2), seminal and follicular fluid (7), amniotic fluid (8), and saliva (9). The content of IGF-II in urine has not been reported to date, whereas that of IGF-I has been described by several laboratories (10-12). Since urinary IGF levels as determined by radioreceptor assay (RRA) were about twice as high as IGF-I measured by radioimmunoassay (RIA) (10), the presence of IGF-II in urine was proposed.

IGFs in plasma essentially are all bound to specific binding proteins, whereas about 30% of those in urine are unbound (10). Two plasma binding proteins have been identified in humans. An insulin-regulated 25 kD binding protein initially isolated from amniotic fluid (13) and a GH-regulated 150 kD complex denoted IGFBP-3 (14). In spinal fluid, the presence of a binding protein with high affinity for IGF-II has been recently proposed (15,16).

The aim of the study was to determine whether IGF-II is present in urine and if so, its level.

Material and Methods

Sample collection
Morning urine specimens were collected from 23 healthy subjects (10 males, 13 females) between 17 and 76 years with a mean age ±SEM of 39.5±2.7 years (males: 37.7±4.2, females: 40.7±3.6). From three individuals, urine specimens were collected over a period of 5 days. Of each specimen, 1- and 4 ml aliquots were frozen within 4 h of collection and kept at −20°C until analysis.

Gel Chromatography
Urine samples (1 ml for IGF-II and IGF binding protein I (IGFBP-1), 4 ml for IGF-I determinations) were lyophilized, dissolved in 250 µl 1 mol/l acetic acid (pH 4.0). The sample was applied to a Sephasil C-S-100 HR column (38 x 1 cm; Pharmacia, Uppsala) equilibrated with 1 mol/l acetic acid. Column eluate fractions of 500 µl were lyophilized, dissolved in 0.05 mol/l TRIS-HCl buffer (pH 7.4) and used in the radioimmunoassays.

Radioimmunoassay for IGF-I, IGF-II and IGFBP-1
IGF-I was quantified in a radioimmunoassay using polyclonal rabbit antibodies (gift from Prof PD Gluckman, Auckland, New Zealand) raised against recombinant IGF-I (Kabi AB, Stockholm). Truncated IGF-I (KabiGen AB)
was used as a ligand. Recombinant IGF-I (KabiGen AB) was used as standard although truncated IGF-I (17) was equally potent. Cross-reactivity with recombinant IGF-II was less than 1%. IGF-II was determined by RIA as previously described by this laboratory (18) using recombinant IGF-II (KabiGen AB) and hen yolk antibodies. The cross-reaction of IGF-I in the IGF-II RIA was less than 1%. Lyophilized samples were dissolved in TRIS-HCl buffer, incubated overnight at 4°C with [125I]IGF-II and antiserum. Microsepharos-coupled antibodies (Pharmacia, Uppsala) were used for separation of the bound from the free hormone in each assay. IGFBP-1 was determined using dialysed urine samples of 1 ml with a RIA using polyclonal antibodies raised in rabbits (19). IGF-I, IGF-II and IGFBP-1 levels were expressed as ng/mmol creatinine.

**Creatinine measurement**

Creatinine was determined by a coupled-enzyme method using Kodak Ektachem Clinical Chemistry Slide (CREA).

**Statistics**

Values are expressed as means ±SEM. Statistical significance was assessed using the Student’s t-test.

**Results**

Typical elution patterns of urinary IGF-II after gel chromatography of acidified aliquots in acetic acid are shown in Fig. 1. A majority of the IGF-II was eluted at a Kd corresponding to recombinant IGF-II, but in about 65% of the investigated material (N=23), immunoreactivity was also observed at a lower Kd suggesting the presence of higher molecular weight forms of IGF-II. The elution volume between 0.47 and 0.77 was pooled for IGF determination in order to include these larger sizes.

The mean immunoreactive IGF-II concentration for the 23 subjects in this study was 717±69 ng/mmol creatinine (Cr). The mean percentage of IGF-II eluted in fractions corresponding to the higher molecular weight form (Kd 0.47-0.57) was 17±2. No significant sex-related differences were observed in the concentration of IGF-II (males: 797±83, females: 651±104 ng/mmol Cr) or the percentage of high molecular forms of IGF-II. No correlation was found between IGF-II levels and age (Fig. 2).

The mean level of IGF-I determined in 12 of the 23 subjects was 110±5 ng/mmol Cr (compared with 662±76 ng/mmol Cr of IGF-II). There was no significant correlation between concentrations of IGF-I and IGF-II immunoreactivity.

**Discussion**

Immunoreactive IGF-II was found in the urine
Fig. 2.
Urinary IGF-II expressed as ng/mmol creatinine (Cr) in healthy males (●) and females (○) in relation to age.

from healthy adults at a mean concentration (717±69 ng/mmol creatinine) which was several fold higher than the IGF-I concentration. The elution profile of IGF-II on gel chromatography suggests the presence of pro-IGF-II in addition to the IGF-II polypeptide. As we did not purify this high molecular form of IGF-II to homogeneity, it was impossible to determine its potency in the IGF-II radioimmunoassay and its concentration in the urine. However, recombinant IGF-II E (with a C-terminal prolongation of 21 amino acids) has been shown to be equipotent to IGF-II in the assay. Higher molecular weight forms of IGF-II have previously been found in human plasma and spinal fluid (2,3,20,21).

The IGF-II concentration was sixfold higher than that of IGF-I. The IGF-I presented here is 5-10 fold higher than those of earlier reports (10-12). This discrepancy is unlikely to be related to differences in standards used, since all groups used recombinant IGF-I. The use of different antibodies and separation techniques may explain the different results. In our assay, truncated IGF-I (17) is equipotent to intact IGF-I, whereas the question of measurement of this peptide is not addressed by previous reports. In addition, Hizuka et al. (10) who found 8±1 ng IGF-I per mmol creatinine in the urine of healthy subjects used octadecyl silica cartridges (Sep-Pak C_{18}) as the separation step. They also reported that free IGF-I is about 30% of total urinary IGF-I. Yokoya et al. (12) found mean values of about 22±41 ng/mmol creatinine in adults between 25-45 years of age using an ammonium sulphate precipitation method prior to the availability of a RIA.

The sources of IGF-I and IGF-II in urine are unknown. The higher ratio of IGF-II to IGF-I in urine in comparison to that in plasma suggests that it is unlikely to represent filtered IGFs. IGFs in the circulation are bound to binding proteins which can prevent renal filtration of the complex. Any dissociated IGF-I and IGF-II would be expected to appear in the urine in proportion to the total plasma concentration. Furthermore, the concentrations of IGF-II are less variable compared with those of IGF-I and IGFBP-1 when measured at several occasions. During development, an inverse relationship was observed between plasma and urinary IGF-I with higher urinary excretion occurring
during childhood (12), and the urinary IGF-I was proposed to reflect IGF-I production in the kidney. The higher IGF-I concentration in the urine in acromegaly than in normal adults, and the increase observed within 12 hours of growth hormone administration to GH-deficient children may reflect renal IGFs (11).

IGF-I is expressed in the kidney (22-24). Human mesangial cells as well as the rat collecting ducts express IGF-I mRNA transcript and secrete IGF-I (24,25). Bortz et al. (25) reported 10-fold higher IGF-I mRNA in isolated collecting ducts compared with whole kidney, 12-fold more than the amount in isolated glomeruli, and 7-fold more than in isolated proximal tubules. Furthermore, they estimated that the level of IGF-I mRNA in the collecting ducts is about 25% of that in the liver. Dot blot hybridizations revealed a 200-fold higher concentration of IGF-II than IGF-I mRNA in the fetal kidney (23). By use of in situ hybridization both IGF-I and IGF-II mRNA were localized in the capsule, calyces and the interstitium but not in the glomerulus and tubulus (22). According to Hirvonen et al. (26), IGF-II mRNA was located predominantly in the stromal and blastemal cells with a relative lack of hybridization over the epithelial structures, suggesting that IGF-II may represent a paracrine stimulus for the growth and differentiation of the kidney epithelium. Most IGF-II immunoreactivity was found in the renal tubular cells of the human fetus rather than in mesenchymal or connective tissues (22). IGF-II mRNA is also found in the adult kidney although at much lower concentrations than observed in Wilms' tumour and in the fetal kidney (20,27). However, in Wilms' tumour the increase in IGF-II mRNA is not reflected by increased IGF-II in the tumour extract (20).

As pointed out by Hizuka et al. (10), IGF-I in the urine is partly (70%) bound to IGFBPs. Only a small portion of total IGF-I can be bound to IGFBP-1, since its molar concentration in the urine was only 15% of that of IGFs. Urinary IGFBPs are not yet characterized and their sources are unknown. However, Ballard et al. (28) has shown that culture medium conditioned by the bovine kidney cell lineMDBK contains IGF-binding proteins. IGFBP-2 mRNA has been detected in fetal rat kidney (29) as well as in an epithelial-like clone of normal rat kidney cells (30). Northern blot analysis using tissues of 6-week-old rats has demonstrated IGFBP-3 mRNA in the kidney (31).

In conclusion, we suggest that both urinary IGF-II and IGFBP-1 may be secreted by the kidney and that determinations of their excretion may contribute to the understanding of their role both in developmental and pathologic processes of the kidney.

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References

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