Effects of Age, Sex and Renal Function on Urinary Insulin-Like Growth Factor I (IGF-I) Levels in Adults

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Abstract. Insulin-like growth factor I (IGF-I) levels in urine were measured in adults using specific RIA after extraction with acid-ammonium sulfate. Mean (±SD) total urine IGF-I values were 267.9±112.9 ng/day and 167.8±73.2 ng/g creatinine (Cr) in 17 normal young adults. There was a positive correlation (r=0.785, P<0.001) between IGF-I values in early morning urine and those of 24 h urine when they were corrected by urinary Cr. IGF-I values in early morning urine were ranged from 60 to 1,100 ng/gCr with a mean value of 309.6 ng/gCr in 178 normal adults aged 21–80 yr. There was a consistent trend towards higher urinary IGF-I values in males during aging and this trend did not reach statistical significance until the sixth and seventh decades. There was a positive correlation (r=0.465, P<0.005) between urinary IGF-I values and age in males but not in females. Although urinary IGF-I values were higher in females than in males of the second and third decades, no sex difference was found in older adults. Urinary IGF-I values were correlated reversely with 24 h Cr clearance (CCr) and positively with urinary β₂-microglobulin (β₂-MG) levels in patients with renal dysfunction. These findings indicate that urinary IGF-I levels are influenced by age, sex and renal function in adults.

Key words: Urinary IGF-I, Age, Sex, Renal function. (Endocrinol Japon 39: 507-515, 1992)

CIRCULATING insulin-like growth factor (IGF-I) is mainly derived from the liver, in which the synthesis and secretion of IGF-I depends on pituitary GH as well as nutritional conditions [1–3]. IGF-I is also synthesized in many other peripheral tissues including the kidneys [4]. However, it remains to be clarified whether IGF-I synthesized locally is reflected in the circulating levels of IGF-I. It has been reported that IGF-I exists in urine [5–7]. IGF-I in blood and urine is bound to specific binding proteins which may interfere the precise determination of IGF-I levels [2, 5, 6]. The determination of IGF-I extracted from urine by means of Sep-Pak C₁₈ [5], acid-ethanol [5] and acid-ammonium sulfate was reported [6]. The Sep-Pak method may extract only the free form of IGF-I in urine [5], whereas acid-ethanol and acid-ammonium sulfate methods extract both free and bound IGF-I in urine. The measurement of a free form of IGF-I in concentrated but not extracted urine by RIA was also reported [7].

It was reported that urinary IGF-I excretion was greater in infants and children than in adults [6, 7]. In infants and children, there was a positive correlation between urinary GH and IGF-I [7]. Plasma IGF-I levels were decreased with age [8, 9], possibly reflecting decreased GH secretion from the pituitary in aged subjects. We previously reported that urinary GH rather increased in aged adults [10]. There has been no report on age-related changes in urinary IGF-I in adults.

Many circulating peptides including GH are degraded in peripheral tissues and little is excreted intact in urine [11]. In the kidneys, the peptide is filtered from the glomeruli and mostly reabsorbed in the tubules [12]. Plasma IGF-I is partly excreted into urine via glomerular filtration without major modification by the tubules in adolescents with orthostatic proteinuria [13]. It
was reported that urinary GH levels are increased in patients with renal insufficiency [14]. The effect of renal dysfunction on urinary IGF-I has not been fully investigated.

In the present study, we examined the effect of age, sex and renal dysfunction on urinary IGF-I levels extracted by acid-ammonium sulfate in adults.

### Materials and Methods

#### Subjects and protocol

Urine samples were collected from 17 normal volunteers, 6 hospital controls, 178 normal subjects in a population and 32 patients with impaired renal function according to the following four different study protocols.

In the first study, 24 h storage urine was collected from 17 normal adult volunteers (10 men and 7 women, aged 19–27 yr). All subjects were in good health, not obese and taking no medication. Renal functions assessed by urinalysis, serum BUN and creatinine (Cr), and urinary β2-microglobulin (β2-MG) were normal in all subjects. A whole day’s urine was collected with a simple portable device for urine sampling (Memorette, Sumitomo Bake-lite Co., Tokyo, Japan) by which 1/50 volume of urine at each urination was accurately stored in the portable container for 24 h [15] and 10 ml out of the each timed urine sample was also collected concomitantly into the other sampling tubes at the time indicated. The timed urine collection was done at 700–800, 1000–1100, 1300–1400, 1600–1700, 1900–2000 and 2200–2300 h. The subjects were allowed to work and stay at home as usual during this study although vigorous exercise, excessive eating and drinking were prohibited.

In the second study to examine day to day variation in urinary IGF-I levels, 24 h storage urine was collected for three successive days from 6 patients (aged 43–70 yr) as hospital controls, who were non-obese and proved to have normal pituitary and renal function.

In the third study, urine samples were collected early in the morning after overnight fasting from 178 normal adults (89 men and 89 women, aged 21–80 yr), who were randomly selected from a population as a part of a continuing epidemiological study [16] including detailed history taking, physical examination, urinalysis, blood chemistry, complete blood count, ECG and ophthalmic examinations.

In the fourth study, 24 h urine samples were collected from 32 patients with impaired renal function (aged 30–81 yr), in which 24 h Cr clearance (CCr), urinary microalbumin and β2-MG were also examined.

Urine samples were supplemented with 0.005 vol of 6% sodium azide and stored at 4°C until assayed. The purpose of the study was well explained to all the subjects and informed consent was obtained.

#### Extraction of urinary IGF-I

Urinary IGF-I was extracted by a modification of the acid-ammonium sulfate method described by Yokoya et al. [6]. Four ml of each urine sample was mixed with 20 µl of 0.6% BSA (Sigma, RIA grade) and 20 µl of glacial acetic acid for 10 min, and then centrifuged at 1,600 g for 10 min. Three ml of the supernatant was mixed with 1.5 g powdered ammonium sulfate. The mixture was allowed to stand for 30 min at room temperature and centrifuged at 1,600 g for 30 min. The supernatant was discarded, and the pellets were dissolved and neutralized in 100 µl of 0.2 M Na2HPO4. Standard IGF-I was also extracted by the same way to examine the recovery rate in the extraction of IGF-I.

#### Gel filtration of urinary IGF-I

One hundred ml of fresh urine was dehydrated in dialysis tubing (Spectra/Por 6, Spectrum Med. Ind. Inc., Los Angeles, CA) against 33% polyethylene glycol at 4°C and then dialysed against 0.01 M phosphate buffer, pH 7.4, at 4°C. The concentrated urine sample was gel filtrated on a Saphadex G-50 column (0.95×54 cm), which was eluted with 0.01 M phosphate buffer, pH 7.4, containing 0.1% BSA at 4°C. A part of the urine from the same subject was mixed with glacial acetic acid and centrifuged as described above before being analyzed by gel filtration on a Sephadex G-50 column, which was eluted with 1 M acetic acid, pH 4.04, containing 0.1% BSA at 4°C. One ml fractions of the eluate were collected and the IGF-I level was measured after extraction with
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Acid-ammonium sulfate as described above.

Assays

The urinary IGF-I level was measured by specific RIA as reported previously [9]. Recombinant human IGF-I (Lot B1516YS) and rabbit anti-IGF-I serum (Lot b0166S) were obtained from Fujisawa Pharmaceutical Co. (Osaka, Japan). The purified IGF-I was iodinated with 125I (Amersham, England) by the lactoperoxidase method. One hundred µl of urine extract or standard IGF-I (0.3–40 µg/L), 100 µl of diluted antiserum (1:10,000) and 200 µl of assay buffer (0.05 M phosphate buffer, pH 7.4, containing 0.5% BSA, 0.025 M EDTA and 0.1% sodium azide) were incubated for 1 h at room temperature. One hundred µl of 125I-IGF-I (15,000 cpm) was then added. After incubation for 16 h at 4°C, 300 µl of diluted anti-rabbit IgG goat serum (1:20) and 800 µl of 25% polyethylene glycol were added. After incubation for another 30 min at 4°C, the mixture was centrifuged at 1,600 g for 30 min at 4°C. The radioactivity of the precipitate was counted by an auto gamma counter (ARC-600, Aloka, Tokyo, Japan). The minimum detectable quantity was 0.5 ng/L in the assay. The mean intra- and inter-assay coefficients of variation were 3.6% and 10.6%, respectively. There was no considerable cross-reaction of IGF-II and insulin with the anti-IGF-I serum. The urinary IGF-I value was expressed by correcting the recovery rate of extraction of standard IGF-I and in terms of the urinary Cr concentration unless specially indicated. The mean (±SD) recovery rate for standard IGF-I after extraction was 77.2±11.4%.

The serum and urinary Cr levels were measured by an autoanalyser using the Jaffe reaction (TBA50s, Toshiba, Tokyo, Japan). The urinary β2-MG level was measured by specific RIA with a commercial kit (Dainabot Co., Tokyo, Japan). The urinary microalbumin level was measured by RIA with a commercial kit (Diagnostic Products Co., Los Angeles, CA).

Statistics

The urine IGF-I concentration was not normally distributed. Therefore, log transformations were made before analysis of the data. Statistical differences were evaluated by analysis of variance followed by Student’s t-test. Linear regression was performed by the least squares method. A P-value less than 0.05 was considered significant.

Results

Figure 1 shows a representative standard curve of RIA for IGF-I. The displacement of 125I-IGF-I by diluted extracts of standard IGF-I and urine sample was parallel to that of standard IGF-I. When the fresh urine was gel filtered, two major peaks of immunoreactive components were obtained. About 75% of immunoreactivity was eluted coincident with 40 K mol wt. and 25% of immunoreactivity was eluted with 125I-IGF-I (Fig. 2, upper panel). On the other hand, gel filtration of extracted urine resulted in a single major peak of immunoreactivity coincidently eluted with 125I-IGF-I (Fig. 2, lower panel).

The mean (±SD) values of total urine IGF-I collected for 24 h were 267.9±112.9 ng/day and 167.8±73.2 ng/gCr in 17 normal young adults. When the IGF-I concentrations in the timed urine samples for a day were compared, the IGF-I concentrations in early morning (0700–0800 h) urine were the highest among them. However, when urinary IGF-I concentrations were corrected by urine Cr levels, there was no considerable difference among the timed urine samples. There was a positive correlation between IGF-I values for 24 h urine and those for early morning urine (r=0.785, P<0.001) as shown in Fig. 3. The mean (±SD) IGF-I value of early morning urine expressed as ng/gCr was greater in 7 young females than in 10 young males (216.9±64.3 vs. 141.4±63.9 ng/gCr, P<0.05).

When urine IGF-I values in 24 h urine were measured for three successive days in 6 hospital controls, the coefficient of variation ranged from 7.2 to 16.9% with a mean (±SD) value of 10.7±3.3%, which is smaller than the mean coefficient of variation of IGF-I values expressed as ng/day (15.4±8.2%, P<0.05).

IGF-I levels in early morning urine ranged from 60 to 1,100 ng/gCr in 178 normal subjects. In younger subjects (aged 31–40 yr), the mean urinary IGF-I value was significantly greater in females than in males (334.4 vs. 225.7 ng/gCr, P<0.05) (Table 1). In older subjects (aged 41–80 yr), there was no significant difference between
Fig. 1. Displacement of $^{125}$I-IGF-I by standard IGF-I and extracts of standard IGF-I and three different urine samples. Standard curve (○—○) was parallel to dilution curves of extracted IGF-I (●—●) and urine samples (■—■).

Fig. 2. Gel filtration pattern on Sephadex G-50 of immunoreactive IGF-I in fresh urine (left panel) and extracted urine (right panel). The arrow indicates the elution point of blue dextran (Vo), BSA (67 K), ovalbumin (43 K) and free $^{125}$I-IGF-I, respectively.
urinary IGF-I values for both sex groups. There was a consistent trend towards higher urinary IGF-I levels in males during aging and this trend did not reach statistical significance until the sixth and seventh decades (Table 1). There was a positive correlation \((r=0.465, P<0.005)\) between urinary IGF-I values and age in male subjects but not in females (Fig. 4). However, when the urinary IGF-I level was corrected by the urinary \(\beta_2\)-MG level, there was no significant correlation between urinary IGF-I and age in either sex.

A reverse correlation between urinary IGF-I values expressed as ng/gCr and 24 h Cr (r=0.651, P<0.001) was obtained in 32 patients with renal insufficiency as shown in Fig. 5 (upper panel). The urinary IGF-I level in these subjects positively correlated with the urinary \(\beta_2\)-MG level as shown in Fig. 5 (lower panel) (r=0.773, P<0.001).

There was a good correlation between urinary IGF-I values expressed as ng/gCr and those expressed as ng/day in 17 normal subjects and 32 patients with renal dysfunction as shown in Fig. 6 (r=0.929, P<0.01).

**Discussion**

It was previously reported that urinary IGF-I excretion per gCr is greater in healthy premature and full term infants than in normal children [7]. Yokoya et al. [6] showed that urinary IGF-I expressed as ng/gCr was gradually decreased in children with a slight increase in early puberty and a drop in adulthood. We first investigated in the present study the age- and sex-related changes of urinary IGF-I levels in normal adults. We found that the urinary IGF-I value expressed as ng/gCr was greater in aged subjects than in young adults.

**Table 1.** Effects of age and sex on urinary IGF-I levels in normal adults

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>n</th>
<th>IGF-I (ng/gCr)</th>
<th>IGF-I (ng/mg (\beta_2)-MG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
</tr>
<tr>
<td>21–30</td>
<td>M</td>
<td>7</td>
<td>199.7 (91.0–457.9)</td>
<td>645.1 (353.2–1211.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9</td>
<td>241.8 (125.2–466.5)</td>
<td>453.6 (234.1–888.8)</td>
</tr>
<tr>
<td>31–40</td>
<td>M</td>
<td>19</td>
<td>225.7 (137.4–370.4)</td>
<td>527.1 (190.0–1461.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>18</td>
<td>334.4 (217.3–514.3)</td>
<td>577.3 (332.0–1003.0)</td>
</tr>
<tr>
<td>41–50</td>
<td>M</td>
<td>19</td>
<td>273.5 (176.8–422.9)</td>
<td>455.0 (209.1–989.9)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>18</td>
<td>329.6 (206.2–526.6)</td>
<td>439.8 (243.8–793.0)</td>
</tr>
<tr>
<td>51–60</td>
<td>M</td>
<td>23</td>
<td>294.9 (193.1–450.2)</td>
<td>356.9 (198.6–641.4)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>17</td>
<td>339.8 (186.9–617.4)</td>
<td>393.2 (177.6–870.3)</td>
</tr>
<tr>
<td>61–70</td>
<td>M</td>
<td>13</td>
<td>407.5 (235.9–703.8)</td>
<td>802.9 (327.2–1965.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>15</td>
<td>361.7 (202.2–646.9)</td>
<td>332.9 (125.4–897.8)</td>
</tr>
<tr>
<td>71–80</td>
<td>M</td>
<td>8</td>
<td>484.8 (332.8–508.4)</td>
<td>613.9 (213.5–1764.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>332.9 (185.7–596.3)</td>
<td>475.2 (190.7–1183.0)</td>
</tr>
<tr>
<td>21–80</td>
<td>M</td>
<td>89</td>
<td>291.4 (166.9–508.4)</td>
<td>506.4 (211.6–1211.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>89</td>
<td>327.7 (188.1–570.9)</td>
<td>439.9 (201.6–959.4)</td>
</tr>
</tbody>
</table>

Mean (±SD) values were obtained by transforming into logarithm.

a vs. d, P<0.05; b vs. c, P<0.05; a vs. e, P<0.025.
In the third decade, the urinary IGF-I value was greater in females than in male adults.

Expression of the results in terms of Cr excretion may have inherent problems. Cr excretion varies from day to day and is strongly influenced by muscle mass [17]. However, day to day variation in urinary IGF-I expressed as ng/gCr was smaller than that expressed as ng/day. Furthermore, there was a good correlation between the urinary IGF-I value in early morning urine and that of 24 h storage urine. Therefore, it is conceivable to evaluate urinary IGF-I values expressed as ng/gCr in early morning urine in a population.

Urinary IGF-I excretion per day correlated closely with urinary IGF-I expressed as ng/gCr. However, urinary IGF-I excretion expressed as ng/gCr could be overestimated in aged subjects, in which urinary Cr excretion is rather lowered. On the other hand, since urinary β2-MG increases with age [18], the expression of the urinary IGF-I value

**Fig. 4.** Relationship between IGF-I values in early morning urine and age in 89 normal males (left panel) and 89 normal females (right panel). Urinary IGF-I is shown as ng/gCr on a logarithmic scale. A positive correlation between urinary IGF-I and age was obtained in males but not in females (males, log y=8.1x10^{-3}x+2.05, r=0.465, P<0.005; females, log y=2.2x10^{-3}x+2.39, r=0.140).

**Fig. 5.** Correlation between urinary IGF-I values and either 24 h Cr (left panel) or urinary β2-MG levels (right panel) in 32 patients with renal dysfunction. Log-scaled urinary IGF-I values were inversely correlated with 24 h Cr (log y=-0.0095x+0.07, r=0.651, P<0.001). Urinary IGF-I values were positively correlated with urinary β2-MG (log y=0.527log x+1.46, r=0.773, P<0.001).
in terms of β2-MG excretion could be underestimated in aged subjects. In the present study, we first demonstrated that the urinary IGF-I value expressed as ng/gCr was greater in older subjects than in young adults whereas the urinary IGF-I value expressed as ng/mg β2-MG was not different among all decades of normal adults. These findings indicate, at least, that the urinary IGF-I level was in contrast to the plasma IGF-I level which consistently decreased with age in adults [9].

Quattrin et al. [7] demonstrated a strong positive correlation between urinary GH and IGF-I excretion in healthy infants and children. There has been no report on the relationship between urinary GH and IGF-I levels in adults. In our preliminary experiments, the urinary IGF-I value was well correlated with the urinary GH level expressed as ng/gCr (unpublished observation). We previously reported that urinary GH expressed as ng/gCr was rather increased with age in adults [10]. These findings also suggest that both urinary GH and IGF-I rather increased with age in adults.

As previously reported [5, 6, 18], IGF-I was bound to specific binding proteins in urine. Hizuka et al. [5] reported that 70% of IGF-I in urine was bound. It was also reported that large and small mol wt binding protein-IGF-I complexes were obtained in the urine of patients with nephrotic syndrome [19]. Very recently, Hasegawa et al. [20] clearly demonstrated the presence of two major IGF binding proteins (IGFBPs), hIGFBP-2 and hIGFBP-3, in normal urine by the use of western ligand blots, and the urinary IGFBP pattern was altered in patients with renal disease. On the other hand, Yokoya et al. [6] reported that the major component of IGF-I in urine was in a free form. Quattrin et al. [7] also reported that there was no IGF-I binding protein in urine. In the present study, we showed two components of a major peak of IGF-I-binding protein complex and a minor peak of free IGF-I. The difference between our findings and those of Yokoya et al. [6] may be explained by the extraction of each fraction before the assay. Although the same acid-ammonium sulfate was used for extraction of urinary IGF-I before gel filtration, they directly
assayed each fraction eluted with phosphate buffer, pH 7.4, and IGF-I bound to proteins in the fraction may be underestimated in the assay. Gel filtration of urinary IGF-I with acid buffer resulted in a single peak of IGF-I eluted at the position of free IGF-I as previously reported [7].

Renal handling of IGF-I has not been fully investigated. IGF-I circulates in the plasma bound to specific carrier proteins [21]. The carrier proteins were increased in the urine of patients with acute nephrotic syndrome in which the plasma IGF-I level was decreased, suggesting that the increased glomerular permeability resulted in increased urinary loss of IGF-I bound to proteins [19]. It was also reported that urinary IGF-I excretion was increased in patients with orthostatic proteinuria in which the urinary β2-MG level was normal [13]. In patients with normal serum creatinine and proteinuria due to glomerulopathy, an increase in the IGFBP-3/IGFBP-2 ratio and the absolute amount of IGFBP-3 in urine was recently reported [20].

Urinary β2-MG is a sensitive indicator of tubular dysfunction since it passes renal glomeruli freely and is reabsorbed mostly by the normally functioning tubules. Recently, Campos et al. [22] reported that IGF-I excretion was increased by i.v infusion of IGF-I but fractional excretion of IGF-I was extensively reabsorbed by the renal tubules. These findings suggest that IGF-I undergoes glomerular filtration, tubular reabsorption and possible degradation within tubular epithelial cells. Therefore, both glomerular dysfunction and tubular damage may result in increased excretion of IGF-I in renal dysfunction.

In the present study, we found a positive correlation between urinary IGF-I and β2-MG excretion. No considerable correlation was obtained between urinary IGF-I and albumin excretion. We also found that urinary IGF-I was inversely correlated with endogenous creatinine clearance as shown in urinary GH [14], suggesting that increased excretion of IGF-I is due to possible loss of size selectivity and negatively charged glycoproteins in the glomerular wall [23]. These findings indicate that increased urinary IGF-I may reflect potential dysfunction of the kidneys in aged subjects.

Acknowledgments

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References


