SHORT COMMUNICATION

Quantification of urinary insulin-like growth factors (IGFs) and IGF binding protein 3 in healthy volunteers before and after stimulation with recombinant human growth hormone

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We examined excretion of urinary insulin-like growth factors I and II (IGF-I and IGF-II) and their major binding protein IGFBP-3 in comparison to their respective serum concentration in nine healthy female volunteers (median age 25 years, range 22–27) under baseline conditions and after stimulation with recombinant human growth hormone (rhGH), 4.5 IU twice daily subcutaneously for a period of 3 days. The IGFs were measured in unconcentrated urine by use of recently developed, highly sensitive radioimmunoassays. The IGFBP-3 was measured by a specific radioimmunoassay. The mean (±sd) urinary concentrations of IGF-I (0.08 ± 0.07 μg/l), IGF-II (1.02 ± 0.47 μg/l) and IGFBP-3 (19.1 ± 6.9 μg/l) were two to three orders of magnitude lower than in serum. The ratio of IGF-II over IGF-I concentration in urine (13:1) was five times higher than in serum (2.5:1), and the ratio of IGFBP-3 over the sum of IGF-I and IGF-II in urine (17:1) was four times higher than in serum (4:1). Urinary excretion was 63.3 ± 46.6 ng·m⁻²·24 h⁻¹ for IGF-I, 1002 ± 598 ng·m⁻²·24 h⁻¹ for IGF-II and 18039 ± 4983 ng·m⁻²·24 h⁻¹ for IGFBP-3. Using fast protein liquid exclusion chromatography, only immunoreactive IGFBP-3 components of less than 60 kD were detected in urine, with a major peak at 20 kD. Urinary IGFBP-3 excretion correlated with serum IGFBP-3 (r = 0.61, p < 0.01) and the glomerular filtration rate (r = 0.56, p < 0.05) measured by steady-state insulin infusion clearances. Administration of rhGH stimulated significantly (p < 0.005) the serum IGF-I concentration by 50%, but not the urinary IGF-I excretion. In conclusion: the considerably higher ratio of IGF-II to IGF-I in urine compared to serum indicates that urinary IGF excretion does not represent only filtered IGFs, urinary IGF-I is a less sensitive indicator of GH activity than serum IGF-I, and as urinary IGFBP-3 excretion is in proportion to the glomerular filtration rate and serum IGFBP-3, it presumably reflects renal filtration of small immunoreactive IGFBP-3 fragments from the circulation.

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Insulin-like growth factors I and II (IGF-I and IGF-II) are bound to specific IGF binding proteins (IGFBP). To date, six different human IGFBPs have been identified on the basis of protein and DNA sequence analysis (1). IGFBP-3 is the predominant circulating IGFBP in postnatal life. Because of their GH dependency, serum IGF-I and. even more accurately, serum IGFBP-3 determinations serve as tools for the assessment of GH secretory status (2). Immunoassayable IGF-I and IGF-II also have been found in other human organ fuids, including urine (3–6). Quantification of urinary IGF-I appeared as a tempting approach for evaluating GH status, because it is accessible readily and may reflect integrated IGF-I production over time. However, these reports have yielded conflicting results, which may be due both to methodological problems and to biological variability.

The aim of the present study was to evaluate the relation of urinary IGFs and IGFBP-3 to their respective serum concentrations, and the GH dependency of these variables under controlled conditions in healthy volunteers. For measurements, highly sensitive radioimmunoassays (RIAs) in unconcentrated urine were used to bypass possible problems of urine sample processing. As no information is available on the dependency of urinary IGFs and IGFBP-3 on renal function, the glomerular filtration rate (GFR) was monitored concomitantly by use of steady-state insulin infusion clearances.

Subjects and methods

We examined nine healthy women of median age 25 (range 22–27) years. Their heights were in the normal range of the population. Subjects with a history of renal disease, high blood pressure or endocrine disease were excluded. The subjects were on no medication apart
from oral contraceptives in all but one of the women. Pregnancy at the time of investigation was ruled out by measurement of β-hCG in urine. The subjects abstained from nicotine, caffeine and alcohol during the study period. The volunteers were advised to refrain from sexual activity in order to avoid artificial contamination of urine with seminal IGF binding proteins (7). All participants gave written informed consent. The protocol had been approved by the Ethics Committee of the University of Heidelberg. The study was carried out under outpatient conditions. The subjects were advised by a dietitian to consume a diet containing 150 mmol/day sodium chloride and 1 g protein/kg body weight per day. Compliance was verified by determination of urea and sodium in the 24-h urine. The maximal deviation of sodium excretion and calculated protein ingestion from the intended intake was 30 mmol sodium and 0.2 g/kg body weight per day protein, respectively. Each participant was investigated during one period of 5 days. To avoid complications with premenstrual fluid retention, studies were restricted to the 14 days following the end of a normal menstrual period. For baseline investigation, the subjects were studied in the fasted state at 08.00 h. Blood was taken for baseline measurements after a 30-min rest in supine position. All blood samples were drawn on ice and centrifuged immediately. Serum and urine samples were stored at −20°C until analysis. Inulin clearances (C\text{in}), inulin and creatinine (Cr) were measured as described previously (8). Subsequently, the participants administered by self injection 4.5 IU of recombinant human (rh) GH (Genotropin, Kabi Pharmacia, Stockholm, Sweden) twice daily from day 2 to day 4. Urine was collected during 24 h on the day before the first GH injection and during the day of the third GH injection. On the morning of the 5th day, 12 h after the last injection of rhGH, the subjects were re-examined as described above.

IGF-I was measured by a novel IGFBP-blocked RIA utilizing a specific high-affinity antiserum (kind gift of Drs Breier and Gluckman, Auckland, New Zealand) (9), as published previously (10). For measurement of urinary IGF-I, 300 µl of untreated urine samples was acidified by adding 30 µl of 0.5 mol/l phosphoric acid. Serum and urinary IGF-II was measured as described previously (11). Residual IGFBP was blocked by 25 ng of rhGH per tube. IGFBP-3 was measured by a specific RIA (12). Serum samples were diluted 1 : 600, and unprocessed urine samples were diluted 1 : 5 with assay buffer before measurement. All samples were assayed in duplicate. For determination of the molecular size of IGFBP-3, 50 µl of serum or concentrated urine samples (about 1 : 100 using an Amicon ultrafiltration cell at a molecular weight cut-off of 2000) was chromatographed at room temperature over a Superox 12 column using a fast protein liquid chromatography (FPLC) system (Pharmacia, Freiburg, Germany). Fractions of 0.5 ml were collected at a rate of 1 ml/min. The elution was performed with 0.05 mol/l sodium phosphate buffer (pH 7.4) containing 0.1 mol/l NaCl, 0.1% BSA (w/v) and 0.02% NaN\textsubscript{3} (w/v) and assayed for IGFBP-3 by RIA after 1 : 10 dilution with assay buffer.

Data are given as the mean ± s.d. The results were analyzed using Student’s t-test for paired differences. Associations between variables were calculated by linear regression analysis. P < 0.05 was accepted for statistical significance.

Results

Serum IGF-I, IGF-II and IGFBP-3 levels were in the normal age-related range for all subjects (Table 1). The concentration of urinary IGFs and IGFBP-3 was markedly lower than the corresponding serum concentration, with the urinary concentration of IGF-I lower by about three orders of magnitude and the concentration of IGF-II and IGFBP-3 lower by two orders of magnitude compared to circulating concentrations (Table 1). The ratio of IGF-II over IGF-I in urine (13 : 1) was five times higher than in serum (2.5 : 1), and the difference in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum concentration (µg/l)</th>
<th>Urinary concentration (µg/l)</th>
<th>Urinary excretion (ng/m^2·24 h⁻¹)</th>
<th>(ng/mmol Cr)</th>
<th>(mg/mg Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I, baseline</td>
<td>252 ± 59</td>
<td>0.08 ± 0.07</td>
<td>63.3 ± 46.6</td>
<td>11.1 ± 8.3</td>
<td>0.10 ± 0.07</td>
</tr>
<tr>
<td>rhGH</td>
<td>387 ± 100</td>
<td>0.11 ± 0.07</td>
<td>93.0 ± 60.1</td>
<td>15.4 ± 8.9</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>IGF-II, baseline</td>
<td>632 ± 93</td>
<td>1.02 ± 0.47</td>
<td>1002 ± 598</td>
<td>175 ± 96</td>
<td>1.55 ± 0.85</td>
</tr>
<tr>
<td>rhGH</td>
<td>593 ± 200</td>
<td>1.05 ± 0.58</td>
<td>777 ± 333</td>
<td>132 ± 57</td>
<td>1.16 ± 0.50</td>
</tr>
<tr>
<td>IGFBP-3, baseline</td>
<td>3792 ± 897</td>
<td>19.1 ± 6.9</td>
<td>18039 ± 4983</td>
<td>3174 ± 740</td>
<td>28.1 ± 6.6</td>
</tr>
<tr>
<td>rhGH</td>
<td>4054 ± 1043</td>
<td>28.3 ± 10.2b</td>
<td>21236 ± 5711b</td>
<td>3523 ± 940b</td>
<td>31.2 ± 8.3b</td>
</tr>
</tbody>
</table>

* p < 0.005 vs baseline.

b p < 0.05 vs baseline.
ratio of IGFBP-3 over IGF-I in urine (244:1) versus serum (15:1) was even more pronounced. Urinary IGF-I excretion before rhGH tended to correlate with serum IGF-I \( (r = 0.61, p = 0.11) \). Urinary IGF-II did not correlate with serum IGF-II \( (r = 0.2, p = 0.6) \). Neither urinary IGF-I nor IGF-II were related to the GFR measured by inulin clearances.

Treatment with rhGH over a period of 3 days significantly increased serum IGF-I concentration by 50%, whereas serum IGF-II tended to decrease and IGFBP-3 to increase without statistical significance (Table 1). Urinary IGF-I increased slightly during rhGH \( (p = 0.37) \). Urinary IGFBP-3 excretion increased significantly during rhGH by approximately 10% (Table 1).

In order to compare the molecular weight of serum and urinary immunoreactive IGFBP-3 under baseline conditions, serum and urine samples of four individuals were subjected to IFLC. In serum, IGFBP-3 was present as a high-molecular-weight complex of about 150 kD. Almost identical results were obtained for the four individuals (Fig. 1). In urine, only immunoreactive IGFBP-3 components of less than 60 kD were detected, with a major peak at ~20 kD (Fig. 1). To examine whether urinary IGFBP-3 excretion reflects renal filtration of serum IGFBP-3, urinary IGFBP-3 excretion was compared to serum IGFBP-3 and the GFR was measured by inulin clearances. There was a significant correlation between urinary and serum IGFBP-3 \( (r = 0.61, p < 0.01) \) and urinary IGFBP-3 and the GFR \( (r = 0.56, p < 0.05) \).

Discussion

The present study reports, for the first time, direct measurements of immunoreactive IGF-I and IGF-II in unconcentrated urine of normal subjects by use of recently developed highly sensitive RIAs \( (11) \). Urine samples in our study were not dialyzed, concentrated or lyophilized, treatments that may have altered the IGF profile in other studies. The urinary IGF-I excretion in our study was comparable to values reported by some authors \( (3, 4, 6) \) but not all \( (5) \). The urinary IGF-II excretion in the present study was approximately 10 times higher than that reported by Gargorsky et al. \( (6) \), and four times lower than the measurements of Zunmeller et al. \( (5) \). The latter study also found a higher urinary IGF-II to IGF-I excretion ratio \( (6.5:1) \), which, however, was less pronounced than the ratio in our study \( (16:1) \). Possible explanations for these discrepancies may involve differences in processing of the urine samples.

The cellular source of urinary IGFs is unknown. The considerably higher ratio of IGF-II to IGF-I in urine compared to serum indicates that urinary IGF excretion represents not only filtered IGFs but also IGFs produced in renal tissue. Human mature kidney under baseline conditions contains undetectable amounts of IGF-I mRNA via in situ hybridization, but abundant amounts of IGF-II mRNA in afferent arterioles and in vascular and interstitial stromal cells of the renal medulla \( (13) \). Provided that gene expression reflects local peptide production, then the human kidney produces more IGF-II than IGF-I under baseline conditions, which may explain the markedly higher urinary IGF-II vs IGF-I excretion in healthy adults.

Our data allow a rough estimation of the renal handling of IGF-I. It can be assumed that free IGFs and a fraction of IGFs bound to smaller IGFBPs are removed from the circulation by renal filtration and tubular metabolism. Indeed, a previous study identified the kidney as a site of IGF-I degradation \( (14) \). No experimental data about the filtered load of IGFs

 Fig. 1. Elution profiles of immunoreactive insulin-like growth factor binding protein 3 (IGFBP-3) in normal serum \( (N = 4) \) (closed symbols) and normal urine \( (N = 4) \) (open symbols). Nearly identical results were obtained for serum IGFBP-3 in the four individuals tested \( (r = 0.998) \). The standard error was too small to be shown.
and their glomerular sieving coefficient (GSC) are available. However, because the molecular weight and structure of IGF-I are similar to those of insulin, one can estimate the GSC of IGF-I to be close to that of insulin, i.e. 0.89 (15). As free IGF-I represents about 1% of the total IGF-I, the filtered load of IGF-I can be approximated as GFR × (serum concentration of free IGF-I) × GSC = 1001·m⁻²·24 h⁻¹ × 2.5 μg/l × 0.89 = 223 μg·m⁻²·24 h⁻¹. Because we found a urinary excretion of IGF-I of only 0.063 μg·m⁻²·24 h⁻¹, we conclude that filtered IGF-I can account easily for the amount of IGF-I excreted in the urine, but that filtered IGF-I is metabolized mostly (>99.9%) by the kidney. The filtered amount of IGF-I per day, as calculated above, is more than one order of magnitude lower than the daily IGF-I production rate (3–5 mg·m⁻²·24 h⁻¹), as estimated by infusion experiments with rhIGF-I (16). This indicates that the major part (>90%) of IGF-I produced daily is not filtered and metabolized within the kidneys, but leaves the circulation presumably by transcapillary movement for binding to its receptor on target cells.

Administration of rhGH over a period of 3 days in our study lead to a marked increase in serum IGF-I, but not a significant increase in urinary IGF-I, indicating that urinary IGF-I is less GH-sensitive than serum IGF-I. This is not surprising in view of the fact that tubular metabolism of proteins is not saturated over a wide range of filtered load (15). Therefore, an increase in serum IGF-I concentration might well lead to an increase of filtered IGF-I, but not necessarily to urinary IGF-I, because by far the greater part of polypeptide hormones in the ultrafiltrate is metabolized in the proximal tubule (15). A certain dependency of urinary IGF-I was observed in adult patients with excessive or deficient GH secretion (3, 4), but the overlap of urinary IGF-I with the normal range in both patient groups in these studies supports the concept that urinary IGF-I is a less-sensitive indicator of GH activity than serum IGF-I.

In contrast to IGF-I, serum IGFBP-3 levels increased only slightly after 3 days of rhGH administration, although IGFBP-3 is known to be GH-dependent (2, 11). This is in line with the concept that the dynamics of IGF-I levels certainly are faster than IGFBP-3 in terms of the response to exogenous administration of GH (17). The increase of urinary IGFBP-3 after rhGH administration indicates that, at least in normal volunteers, urinary IGFBP-3 also is under GH control, in contrast to findings in GH-deficient patients (7). The absolute amount of urinary immunoreactive IGFBP-3 excretion in our study was comparable to those published by Gargosky et al. (6) in a group of subjects of similar age. Using FPLC to determine the molecular size of immunoreactive IGFBP-3, we found a major peak at ~20 kD with little intact IGFBP-3 in the 40–50 kD range. This finding is in contrast to a previous report (6), which by Western ligand blot and immunoblot analysis detected urinary IGFBP-3 at 40–50 kD. This discrepancy may be due to different processing of the samples or recognition of different fragments. Similar to urinary IGFs, the cellular source of urinary IGFBP-3 is unknown. In adult human kidney tissue, IGFBP-3 mRNA was not detectable by Northern analysis (18). This indicates that renal IGFBP-3 productions in adult humans under baseline conditions is low or absent. Therefore, urinary immunoreactive IGFBP-3 presumably reflects mainly renal filtration of small IGFBP-3 fragments from the circulation. Consistent with this hypothesis is the linear relationship between urinary and serum IGFBP-3 and between urinary IGFBP-3 and GFR in the present study.

In summary, the present study found a considerably higher ratio of IGF-II to IGF-I in urine compared to serum, indicating that urinary IGF excretion does not represent only filtered IGFs. Filtered IGF-I can account for urinary IGF-I, but by far the greater part of the filtered load of IGF-I appears to be metabolized by the kidney. Urinary IGF-I is a less sensitive indicator of GH activity than serum IGF-I. Urinary IGFBP-3 excretion was in proportion to serum IGFBP-3 concentration and GFR consistent with the concept of removal of small immunoreactive IGFBP-3 fragments from the circulation by renal filtration.

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References


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