Population-based reference values for IGF-I and IGF-binding protein-1: relations with metabolic and anthropometric variables

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Abstract

Population-based reference values for IGF-I and IGF-binding protein-1 (IGFBP-1) have been established. One hundred and one women and the same number of men, 20–70 years old, were randomly selected from the population registry in the community of Linköping. Participation rate was 67%. Venous blood was drawn in the fasting state. Serum IGF-I was measured by RIA after acid-ethanol extraction and IGFBP-1 was determined by ELISA. IGF-I levels did not differ between genders and the decline with age was similar in men and women (men: Y=366–3.28×age (years), r =–0.61, P<0.001; women: Y=386–3.49×age, r =–0.57, P<0.001, P=0.4 for difference in slope). There were negative correlations between IGF-I and plasma lipids and blood pressure in both genders, but none was independent of age. Serum angiotensin-converting enzyme activity correlated positively with IGF-I in men independently from age (r =0.21, P=0.01). The distribution of IGFBP-1 was positively skewed and it was higher in women than in men (5.9±4.8 μg/l and 4.0±3.3 μg/l respectively; Mann–Whitney, P=0.002). In men and in the women not taking oestrogen, IGFBP-1 correlated positively with age (Spearman rank correlation (Spearman): men: r =0.32, P=0.002; women: r =0.24, P=0.03). C-peptide correlated negatively (Spearman: men: r =–0.38, P=0.002; women: r =–0.49, P<0.001) and sex hormone binding globulin positively with IGFBP-1 (Spearman: men: r =0.50, P<0.0001; women: r =0.55, P<0.0001).

IGF-I declined with age while IGFBP-1, which is considered to modulate the free bioactive fraction of IGF-I, increased. This suggests that IGF-I activity might be even lower in elderly subjects than is accounted for by the low total IGF-I.

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Introduction

Insulin-like growth factor I (IGF-I) is mainly regulated by growth hormone (GH), insulin and nutrition (1–5). Six peptides with ability to bind IGF-I have been characterized (IGF-binding proteins (IGFBP)-1 to -6). IGF-I in plasma is mainly present in a ternary complex made up of IGFBP-3, IGF-I and a glycoprotein, the acid-labile subunit (ALS). ALS and IGFBP-3 are both GH–dependent and total IGF-I in serum correlates positively with mean 24-h GH levels (2, 6, 7).

IGFBP-1 is GH-independent and its plasma level is inversely related to insulin (1). Circulating IGFBP-1 is mainly synthesized in the liver and the rate of synthesis is regulated at the transcription level by insulin (8). IGF-I may exert a hypoglycaemic tone as infusions of IGFBP-1 to normal rats cause hyperglycaemia (9). In contrast to the other IGFBPs, serum IGFBP-1 levels fluctuate rapidly during the day, thus probably modulating the free fraction of IGF-I (10). In fact, the free unbound fraction of IGF-I in human serum has been measured after ultrafiltration by centrifugation under in vivo-like conditions, and was found to be lower after an overnight fast when IGFBP-1 levels had increased substantially (10). Another liver-produced binding protein, also regulated by insulin (11), is sex-hormone binding globulin (SHBG) which binds testosterone and thus regulates the free fraction of this anabolic hormone.

To our knowledge, there are no population-based reference materials on IGFBP-1 in adults and only one such study on IGF-I has been published (12). Our aim was to perform a population-based study of IGF-I and IGFBP-1 and to evaluate inter-relationships with anthropometric variables, SHBG, testosterone, C-peptide, blood lipids, the renin–angiotensin system as well as blood pressure (BP) in a defined population.

Subjects and methods

Subjects

The aim was to evaluate approximately 100 men and 100 women aged 20 to 70 years, evenly distributed in
each 10-year age interval. To achieve this we consecutively invited 367 subjects by mail and enclosed a questionnaire to be completed. The only exclusion criteria were pregnancy and treated hypertension. The subjects were randomly obtained from the population registry of the community of Linköping. Thirty-one of the 367 invited subjects were excluded because they were taking antihypertensive medication and one woman was pregnant. Of the 335 subjects eligible for the study 223 visited our clinic: a participation rate of 67% (men: 64%; women: 69%). Non-participation was most common among men 30-39 years of age (participation rate: 55%) but in all other age categories, divided by 10-year intervals and according to gender, participation rate was at least 64%. Women 40-49 and 50-59 years of age had a participation rate of 73% and 76% respectively. The majority of non-participants were contacted by phone and the most common reason for not participating was ‘lack of time’.

In 21 of the 223 participating subjects insufficient blood was obtained to permit all analyses and thus the final study set-up presented here consisted of 101 men and 101 women. Because of technical problems data were not available for C-peptide (n = 5), plasma renin activity (PRA) (n = 1), plasma immunoreactive active renin (IRR) (n = 6), serum angiotensin-1-converting enzyme (ACE) (n = 8), and on plasma angiotensin-II (A-II) in 12 subjects.

Participants gave informed consent and the study was approved by the local ethical committee and performed according to the Declaration of Helsinki.

All participating subjects were of Caucasian origin. There were 33 current smokers (16 women and 17 men). Nine women 23–36 years of age were taking oestrogen at anticonceptional doses, a further 13 women 48–64 years of age were taking oestrogen substitution. There were 13 men and seven women with supine diastolic clinic BP (CBP) ≥90 to ≤95 mmHg. Three men had a supine diastolic CBP ≥95 to ≤100 mmHg but no subject had a diastolic BP over 100 mmHg. All subjects had normal renal and liver function as assessed by serum creatinine and urine examination by dipstick and plasma/serum aspartate aminotransferase, alanine aminotransferase, alkaline-phosphatase, conjugated and unconjugated bilirubine and lactate-dehydrogenase.

**Methods**

The subjects arrived at the clinic at 0800 h after an overnight fast from 2200 h. BP was measured at the clinic by one of two trained nurses using a mercury sphygmomanometer. They used arm cuffs of appropriate width and form depending on the shape and circumference of the subject’s upper arm. Supine BP was measured after resting for 5 min. Two to three BP recordings were made until the BP was stable and it was noted with a precision of 2 mmHg. Korotkoff sounds phase I and V were used to define systolic and diastolic BP respectively.

For non-invasive ambulatory BP (ABP) recordings we used the 24-h ABP recorder Spacelab 90202 or 90207. The Spacelab automatic edit was used and BP measurement interval was set at 20 min throughout the 24 h. ABP data were excluded from analysis if less than 70% successful BP readings during the day or night were recorded (n = 31). Daytime and night-time periods were determined from the subjects own time-notations (diary method).

A total of 160 ml venous blood was drawn in the sitting position after at least 15-min rest, for analysis of routine laboratory variables and the various peptides/hormones. Prechilled test tubes of the vacutainer type were used.

Waist circumference was measured in the standing position at the middle of the distance between the lowest rib and the iliac crest. Hip circumference, in the supine position, was measured at the widest part. Body weight was measured with the subject in light clothing and without shoes. Body mass index (BMI) was calculated according to the formula: mass (kg) divided by height (m) squared.

**Assay methods**

Serum IGF-I was measured with a commercial kit from Nichols Institute (CA, USA) by RIA after acid-ethanol extraction of IGF-I from its binding proteins, according to the manufacturer’s protocol. Serum IGFBP-1 was determined by an immunoenzymometric assay containing two monoclonal antibodies against IGFBP-1, using a kit from Medix Biochemica (Kainainen, Finland). Intra- and interassay coefficients of variation for serum IGF-I were 1.5% and 13% respectively, and for serum IGFBP-1 they were 2.4% and 9.7% respectively. Sera had been kept frozen at −20 °C for up to 3 years and 7 months until analysis of IGF-I and IGFBP-1. PRA was determined as generated A-I which was measured by RIA (13) and intra- and interassay coefficients of variation were 4% and 11% respectively. A-II (14) was measured by RIA and intra- and interassay coefficients of variation were 10% and 6.6% respectively. IRR was measured using a kit from the Pasteur Institute in which the second antibody is directed against the active site of the renin molecule (15). Intra- and interassay coefficients of variation for IRR were 3% and 12% respectively. Serum ACE was determined using the artificial substrate 3-(2-furyl-akryloyl)-l-phenylalanyl-glycyl-glycine from which 3-(2-furyl-akryloyl)-l-phenylalanyl is formed and its concentration was determined by spectrophotometry (16). For serum ACE intra- and interassay coefficients of variation were 3% and 7% respectively. Plasma cholesterol and plasma triglycerides were analysed by Monotest Cholesterol Chod Pap (Boehringer Mannheim, Germany) and triglycerides by GPO PAP (Boehringer Mannheim). Apolipoprotein was precipitated with magnesium chloride and phosphotungstic acid (Boehringer Mannheim), and the supernatant was
Table 1 Reference values for IGF-I in relation to age and gender. To further describe the population, BMI and C-peptide levels are also given in each age category. Values are means ± s.d.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>IGF-I (µg/l)</th>
<th>BMI (kg/m²)</th>
<th>C-peptide (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>21</td>
<td>292 ± 71</td>
<td>24.3 ± 3.3</td>
<td>0.49 ± 0.20</td>
</tr>
<tr>
<td>30–39</td>
<td>25</td>
<td>238 ± 72</td>
<td>24.6 ± 3.4</td>
<td>0.48 ± 0.22</td>
</tr>
<tr>
<td>40–49</td>
<td>18</td>
<td>211 ± 70</td>
<td>25.1 ± 3.0</td>
<td>0.44 ± 0.13</td>
</tr>
<tr>
<td>50–59</td>
<td>19</td>
<td>182 ± 42</td>
<td>26.0 ± 2.5</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td>60–69</td>
<td>18</td>
<td>163 ± 60</td>
<td>26.4 ± 3.7</td>
<td>0.58 ± 0.29</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>21</td>
<td>299 ± 76</td>
<td>22.3 ± 3.1</td>
<td>0.46 ± 0.19</td>
</tr>
<tr>
<td>30–39</td>
<td>19</td>
<td>274 ± 85</td>
<td>24.1 ± 3.0</td>
<td>0.44 ± 0.13</td>
</tr>
<tr>
<td>40–49</td>
<td>19</td>
<td>218 ± 78</td>
<td>25.0 ± 5.0</td>
<td>0.47 ± 0.17</td>
</tr>
<tr>
<td>50–59</td>
<td>20</td>
<td>175 ± 60</td>
<td>24.4 ± 2.6</td>
<td>0.38 ± 0.16</td>
</tr>
<tr>
<td>60–69</td>
<td>22</td>
<td>174 ± 64</td>
<td>26.5 ± 4.9</td>
<td>0.50 ± 0.22</td>
</tr>
</tbody>
</table>

defined as containing high-density lipoprotein (HDL) fraction. Low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald et al. (17).

Serum C-peptide was analysed by RIA (18) and intra-and interassay coefficients of variation were 7% and 8% respectively. Serum testosterone was determined by fluoroimmunoassay (19) and intra- and interassay coefficients of variation were 6% and 5% respectively. Serum SHBG was analysed by RIA (20) and intra- and interassay coefficients of variation were 4% and 5% respectively.

Statistics

Statistical calculations were made using a Macintosh personal computer and StatView 4.5 software. Comparisons within and between groups were made with Student’s paired and unpaired two-tailed t-test and correlations with Pearson’s test. Non-normal distributed variables were compared by Mann–Whitney non-parametric test and correlations calculated by Spearman’s rank correlations test. Mean ± s.d. are given unless otherwise stated. As the study was designed prospectively to compare the designated variables, no adjustment was made for multiple comparisons. Statistical significance was considered at the 5% level (P ≤ 0.05). In statistical analysis with A-II as a variable, values <2 pmol/l (level of sensitivity) were denoted as 1·0 (four cases), and as the level of sensitivity for serum testosterone was 0·5 nmol/l, values below this threshold were denoted as 0·25 nmol/l (17 cases, all women).

Results

IGF-I

Table 1 shows reference values for IGF-I and also, for comparison, BMI and C-peptide levels. The mean level of IGF-I did not differ between men and women (221 ± 78 µg/l and 227 ± 88 µg/l respectively, P = 0.6). IGF-I correlated negatively with age in both sexes and this is shown graphically in Fig. 1. The slope of the decline of IGF-I with age did not differ between genders: men: Y = 366–3.28 × age (years), r² = –0.61 and women: Y = 386–3.49 × age, r² = –0.57, P < 0.0001 for both genders and P = 0.4 for the difference in slope.

Table 2 shows simple and multiple regression analyses of IGF-I with the other variables. IGF-I correlated positively with height in men but this correlation did not persist when adjustment was made for age. IGF

Men

![Graph](image1)

Women

![Graph](image2)

Figure 1 Serum IGF-I levels in men and women in relation to age.
did not correlate with daytime ABP in either gender. In women IGF-I was positively correlated to BMI when age was adjusted for. In multiple regression analysis, however, with IGF-I as dependent and age, C-peptide and BMI as independent variables, only age (standardized regression coefficient (std. $r = -0.60$, $P < 0.0001$) and C-peptide (std. $r = 0.22$, $P = 0.03$) still showed significant correlations. All negative correlations between plasma lipids and IGF-I disappeared after age adjustment, but in women there was a positive correlation between IGF-I and C-peptide that was unaffected by age. C-peptide correlated positively with BMI in both genders (men: $r = 0.44$; women: $r = 0.61$, $P < 0.0001$) but it did not correlate with age in either gender (men: $r = 0.16$, $P = 0.1$; women: $r = 0.03$, $P = 0.98$). BMI on the other hand correlated positively with age in both genders (men: $r = 0.28$, $P = 0.005$; women: $r = 0.28$, $P = 0.005$).

Haemoglobin was positively correlated to IGF-I in men and it was unaffected by the introduction of age as second independent variable. All negative correlations between IGF-I and either CBP or ABP disappeared when age was allowed for in multiple regression analysis. There were no correlations between PRA, IRR or A-II and IGF-I but in men ACE correlated positively with this hormone both before and after adjustment for age.

Smoking, in the total material or in women and men separately, or oestrogen medication, in women, did not correlate with IGF-I in multiple regression analysis with age as second independent variable.

**IGFBP-1**

IGFBP-1 reference values are shown in Table 3. The level was higher in women than in men ($5.9 \pm 4.8 \mu g/l$ and $4.0 \pm 3.3 \mu g/l$ respectively; Mann–Whitney, $P = 0.002$). As was suspected from the high standard deviations, the distribution was positively skewed in both genders as illustrated in Fig. 2. Among women the IGFBP-1 level was higher in those 22 receiving oestrogen medication ($8.8 \pm 6.2 \mu g/l$ in women receiving oestrogen medication, $5.1 \pm 4.0 \mu g/l$ in women not receiving oestrogen...
IGFBP-1 values to their corresponding natural logarithms to accomplish normal distribution. ln(IGFBP-1), with ln(IGFBP-1) as dependent variable and C-peptide and age as independent variables, the positive correlations with age still remained statistically significant (std. \( r = 0.38, P < 0.0001 \) for age; std. \( r = -0.44, P < 0.0001 \) for C-peptide in men; std. \( r = 0.19, P = 0.05 \) for age; std. \( r = -0.54, P < 0.0001 \) for C-peptide in women not on oestrogen therapy).

SHBG was positively correlated with IGFBP-1 in both genders (Spearman: men: \( r = 0.50, P < 0.0001 \); women: \( r = 0.55, P < 0.0001 \)) and it correlated negatively with C-peptide (men: std. \( r = -0.28, P = 0.007 \); women: std. \( r = -0.33, P = 0.0009 \)). The positive correlations between ln(IGFBP-1) and SHBG are shown in Fig. 3. SHBG was higher in women on oestrogen therapy than in those without this medication (7.1 ± 4 and 4.3 ± 2 mg/l respectively, \( P < 0.0001 \)).

### Discussion

In agreement with previous studies we found IGF-I to be negatively correlated with age (6, 12, 21, 22). C-peptide levels, reflecting insulin secretion, did not correlate significantly with age in this study, making it unlikely that the drop of IGF-I with age was caused by a lack of insulin stimulation of IGF-I production. Age-related differences in nutrition as a cause of the negative correlation between age and IGF-I were not assessed in this study; however, as IGF-I is GH-regulated, the most likely explanation for the negative correlation between IGF-I and age is the well-known parallel drop in GH

**Table 3** Reference values for IGFBP-1 (\( \mu g/l \)) in relation to age and gender.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>10th percentile</th>
<th>50th percentile</th>
<th>90th percentile</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>21</td>
<td>0.60</td>
<td>2.2</td>
<td>5.6</td>
<td>0.50–19</td>
</tr>
<tr>
<td>30–39</td>
<td>25</td>
<td>1.1</td>
<td>2.6</td>
<td>7.0</td>
<td>0.30–8.3</td>
</tr>
<tr>
<td>40–49</td>
<td>18</td>
<td>1.0</td>
<td>2.5</td>
<td>7.6</td>
<td>0.90–8.7</td>
</tr>
<tr>
<td>50–59</td>
<td>19</td>
<td>1.4</td>
<td>4.6</td>
<td>10.0</td>
<td>0.40–13</td>
</tr>
<tr>
<td>60–69</td>
<td>18</td>
<td>1.5</td>
<td>4.5</td>
<td>12.0</td>
<td>0.80–16</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>21</td>
<td>1.8</td>
<td>3.8</td>
<td>13.0</td>
<td>1.1–26</td>
</tr>
<tr>
<td>30–39</td>
<td>19</td>
<td>1.4</td>
<td>4.4</td>
<td>14.0</td>
<td>1.1–17</td>
</tr>
<tr>
<td>40–49</td>
<td>19</td>
<td>1.1</td>
<td>3.3</td>
<td>9.6</td>
<td>0.70–15</td>
</tr>
<tr>
<td>50–59</td>
<td>20</td>
<td>2.8</td>
<td>6.6</td>
<td>16.0</td>
<td>1.3–20</td>
</tr>
<tr>
<td>60–69</td>
<td>22</td>
<td>1.4</td>
<td>6.1</td>
<td>11.0</td>
<td>0.50–17</td>
</tr>
</tbody>
</table>

*Figure 2* The distribution of serum IGFBP-1 was positively skewed in both men (solid bars) and women (shaded bars).
levels (2). In contrast to another Swedish reference material by Landin-Wilhelmsen et al. (12), the slope of the regression line did not differ between genders. The reason for this discrepancy is unclear and the same method for IGF-I analysis was used in both studies. The population presented in this paper had a somewhat wider age range and would thus be suited for studying gender differences. We do not believe that the greater size of the population studied by Landin-Wilhelmsen et al. (12) \((n = 392)\) was the reason for finding a gender difference in the slope of the regression line, as there was no trend towards such a difference in our study.

In men there was a positive correlation between height and IGF-I which disappeared when age was allowed for in the multiple regression analysis. This is agreement with earlier studies (12). We also found a positive correlation between haemoglobin concentration and IGF-I in men which was independent of age. To our knowledge this has not been shown before in adult subjects, but Anttila et al. (23) have found similar results in boys.

In this study we aimed to evaluate correlations between IGF-I and BP and to the renin–angiotensin system, thus subjects on antihypertensive medication were excluded. The main reason for this design was ethical, since we did not find it appropriate to discontinue antihypertensive treatment for at least a month to eliminate the effects of such medication on the renin–angiotensin system and BP. It is conceivable that the fluid-retaining effect of IGF-I (24) under basal conditions would suppress renin or aldosterone, but we found no correlations supporting this.

There was a positive correlation between ACE and IGF-I in men. Left ventricular hypertrophy has been reported to be positively correlated to IGF-I in humans (25) and in animal experiments IGF-I has been linked to local growth of vascular smooth muscle (26). Plasma ACE has also been shown to correlate positively with carotid wall thickening in humans (27). The growth-promoting properties of IGF-I could possibly be accentuated by the association with higher ACE levels in men.

In women there were positive correlations between IGF-I and body weight, BMI and C-peptide when age was taken into account. To our knowledge this has not been shown before. As the correlation between BMI and IGF-I disappeared when C-peptide was introduced as third independent variable, it was possibly the confounding effect of the positive correlation between C-peptide and IGF-I that caused the correlation between IGF-I and BMI in women. The same explanation could be possible for the positive correlation between weight and IGF-I in women as it also disappeared after similar multiple regression analysis together with C-peptide and age as independent variables (data not shown). The production of IGF-I has been shown to be stimulated by insulin secretion in the rat (3, 5) and in humans in vivo (1) which is in accordance with the positive correlation between insulin, measured as C-peptide, and IGF-I in our study.

In men we found negative correlations between IGF-I and BMI and waist/hip ratio (WHR), but these correlations disappeared after age adjustment.

The distribution of fasting IGFBP-1 levels was positively skewed in both genders. We found negative correlations between C-peptide and IGFBP-1 in both genders. On the other hand, IGFBP-1 was higher in women than in men and the level was even higher among women taking oestrogen medication. The gender difference in IGFBP-1 levels has been shown earlier (28, 29). We also found a positive correlation between IGFBP-1 and SHBG in both genders. Pekonen et al. (30)
have shown this relationship before in premenopausal women and Lönnning et al. (31) found similar results in postmenopausal women with breast cancer. This is probably a parallel phenomenon as IGFBP-1 and SHBG are influenced similarly by insulin which lowers their production in the liver (8, 11), and oestrogen, which raises both SHBG and IGFBP-1 levels (28, 32). Accordingly, in this study we found negative correlations between C-peptide and SHBG, and in women on oestrogen therapy SHBG levels were higher than in women without this medication.

We also found that IGFBP-1 levels were positively correlated with age which has been shown before (33). This could have been caused by higher production rates or slower clearance from plasma but it was probably not caused by any change of insulin secretion with age as the correlation remained after adjustment for C-peptide levels. The positive correlation between IGFBP-1 and age could imply that the decline of free IGF-I with age is even more pronounced than is the fall of total serum IGF-I shown in Fig. 1. However, these findings must be interpreted with some caution as IGFBP-1 levels fluctuate widely (8) and the results in our study were calculated from a single fasting value. In a study by Hilding et al. (34) however, fasting IGFBP-1 was shown to correlate strongly with mean 24-h IGFBP-1 values.

IGF-I correlated negatively to IGFBP-1 in both genders independently of age. Lönnning et al. (31) and Holly et al. (35) have shown this relationship before. In women a negative association between IGF-I and SHBG became apparent after adjustment for age. This is also a confirmation of earlier results (31). Possibly high GH levels, positively correlated with high IGF-I, make an elevation of insulin levels necessary to avoid hyperglycaemia. The high insulin levels act on the liver to suppress SHBG and IGFBP-1.

In conclusion, in this population-based study we found IGF-I to be inversely related to age. The distribution of IGFBP-1 was positively skewed in both genders and IGFBP-1 levels were higher in women than in men. We suggest that bioactive IGF-I might be even lower in elderly subjects than is accounted for by low total IGF-I, as binding protein IGFBP-1, which is considered to regulate the free fraction of IGF-I, was positively correlated with age.

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References


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