Reference Intervals for Insulin-like Growth Factor-1 (IGF-1) From Birth to Senescence: Results From a Multicenter Study Using a New Automated Chemiluminescence IGF-1 Immunoassay Conforming to Recent International Recommendations

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Context: Measurement of IGF-1 is a cornerstone in diagnosis and monitoring of GH-related diseases, but considerable discrepancies exist between analytical methods. A recent consensus conference defined criteria for validation of IGF-1 assays and for establishment of normative data.

Objectives: Our objectives were development and validation of a novel automated IGF-1 immunoassay (iSYS; Immunodiagnostic Systems) according to international guidelines and establishment of method-specific age- and sex-adjusted reference intervals and analysis of their robustness.

Setting and Participants: We conducted a multicenter study with samples from 12 cohorts from the United States, Canada, and Europe including 15 014 subjects (6697 males and 8317 females, 0–94 years of age).

Main Outcome Measures: We measured concentrations of IGF-1 as determined by the IDS iSYS IGF-1 assay.

Results: A new IGF-1 assay calibrated against the recommended standard (02/254) and insensitive to the 6 high-affinity IGF binding proteins was developed and rigorously validated. Age- and sex-adjusted reference intervals derived from a uniquely large cohort reflect the age-related pattern of IGF-1 secretion: a decline immediately after birth followed by an increase until a pubertal peak (at 15 years of age). Later in life, values decrease continuously. The impact of gender is small, although across the lifespan, women have lower mean IGF-1 concentrations. Geographical region,
Measurement of circulating IGF-1 is an important aid in diagnosis and monitoring of GH-related diseases (1–4). IGF-1 is also suggested as an important marker in malignant (5, 6) and metabolic (7–9) diseases. A major problem in the clinical application of IGF-1 measurements is that considerable differences exist between the results obtained from different assays (10). A recent consensus statement (11) discussed steps for improving comparability of assays as well as the quality of normative data. The recommendations encourage the use of the latest well-characterized recombinant International Standard 02/254 (12) for all assays and demand that IGF-1 assays must demonstrate insensitivity to interference from IGF binding proteins (IGFBPs). The consensus statement also requires that reference intervals must be method-specific, based on large cohorts of well-characterized individuals, and adjusted for age and, at least in certain age groups, for sex. To better reflect the pubertal development, reference intervals should also be stratified according to Tanner stages. The central 95% interval (2.5th–97.5th percentile) must be reported in mass units, and translation of concentrations into SD scores should be made possible through appropriate statistical approaches (11). The consensus asks not only for rigid validation of any IGF-1 assay but also for transparency and publication of the data.

In 2003, Brabant et al (13) published reference intervals for IGF-1 derived from a multicenter study. These reference intervals have been widely used in conjunction with an automated IGF-1 assay. Unfortunately, production of this assay was discontinued. Laboratories started using other assays requiring new reference intervals. At least 22 studies reporting normative data for IGF-1 for different assay systems have been published since 2003 (for details and references, see Supplemental Table 1, published on The Endocrine Society’s Journals Online website at http://jcem.endojournals.org). Some of the studies were limited in size, and most studies included subjects only of a certain age, gender, or ethnic background. Notably, the studies used many different, partly very simple statistical methods to define the reference intervals.

It was our aim to develop and validate a new automated monoclonal antibody-based IGF-1 assay strictly adhering to the criteria outlined in the recent consensus statement (11) and to establish appropriate method-specific reference intervals. To this purpose, we collected and analyzed more than 15 000 samples from newborns, children, adolescents, and adults. The data were analyzed statistically to provide reliable percentiles and to allow calculation of SD scores. To test the robustness of the reference intervals, we also investigated the impact of various sample collection scenarios, exclusion criteria, and demographic factors.

Subjects and Methods

Development of the iSYS IGF-1 assay

Two mouse monoclonal antibodies raised against recombinant human IGF-1 were selected for use in an automated chemiluminescent immunoassay (IDS-iSYS; Immunodiagnostic Systems). One antibody (directed against the N-terminal fragment) is biotinylated, whereas a second antibody (raised against intact human IGF-1 linked to BSA) is coupled to an acridinium ester derivate. Following an optimized pretreatment procedure to dissociate IGF-1 from IGFBPs, samples are incubated simultaneously with both antibodies in the presence of excess IGF-2 (to prevent reaggregation with IGFBPs). After removal of unbound antibody and addition of triggers, the chemiluminescence signal is directly proportional to the amount of IGF-1 present in the sample. The new recombinant standard 02/254 is used for calibration (National Institute for Biological Standards and Control).

Methodological details about assay development, protocol, validation and characterization are given in the Supplemental Methods.

Assay validation and characterization

Following the Clinical and Laboratory Standards Institute recommendations (14, 15), limits of detection and quantification, imprecision, linearity, and recovery were determined. Agreement of results obtained with different
reagent batches on different instruments as well as the influence of different collection tubes and different storage conditions before analysis was also investigated.

To test cross-reactivity and interference, samples were spiked with increasing amounts of IGF-2 (GroPep), insulin (66/304), and proinsulin (84/611, National Institute for Biological Standards and Control), but also with high amounts of hemoglobin (Lampire), bilirubin (Merck Millipore), and triglycerides (Sigma-Aldrich). We also analyzed samples after spiking and preincubating with increasing concentrations of each of the 6 high-affinity IGFBPs (GroPep).

The new IDS-iSYS IGF-1 assay was compared with existing assays. These studies included the Immulite 2000 (Siemens) and the IGF-1 RIA-CT (Mediagnost), which were performed according to the respective manufacturers’ instructions. Comparison with the Immulite assay was performed in samples from the KORA cohort (see below). Comparison with the Mediagnost RIA was done in a set of samples (n = 298) from healthy children (Halmstad, Sweden).

Subjects included in the reference interval study

We used samples from 12 different cohorts. All studies were approved by the respective local institutional review board, and informed consent was obtained from participants or parents when appropriate. Gender distribution, age range, sample type, and the respective analytical laboratory for the cohorts are summarized in Table 1. Although ethnic backgrounds were diverse, most the participants were of Caucasian origin. Samples were collected in the nonfasted state and stored at −20°C or −80°C before analysis.

Newborns

Cord blood (serum) was obtained from healthy singleton newborns at term (n = 146; 79 males) with weight appropriate for gestational age in Munich, Germany. Not included in the calculation of reference intervals but analyzed for comparison were samples from twin (n = 73; 33 males) and triplet (n = 17; 8 males) pregnancies.

Pediatric cohorts

A total of 1360 (850 males) serum samples came from the Canadian Laboratory Initiative on Pediatric Reference Interval Database (CALIPER) conducted at the Hospital for Sick Children (Toronto, Canada). The population is ethnically diverse and deemed to be metabolically stable. Samples were surplus specimens from children attending dentistry, orthopedic, and plastic surgery clinics. Details of the cohort are provided elsewhere (16).

To investigate whether the samples from CALIPER are representative also for nonhospitalized children, we analyzed an additional set (CALIPER new) of 588 (298 males) serum samples taken from healthy children recruited in the wider community (schools, churches, and community centers) in the same multiethnic population of the greater Toronto area (17). In addition, 854 serum samples from healthy children (393 males) were collected at The Children’s Clinic in Randers, Denmark (pubertal stage was defined according to Tanner, and all children had normal height and weight); 319 serum samples from healthy infants (171 males) were collected in Halmstad, Sweden; 193 serum samples from healthy children (93 males) were collected in Leipzig, Germany in the framework of a study on growth velocity; 55 serum samples (0 males) were obtained from healthy children at the Children’s Hospital in Graz, Austria; and 737 serum samples from healthy girls

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**Table 1.** Details of the Cohorts and Laboratories Taking Part in the Reference Interval Study

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Lab Measured</th>
<th>No. of Samples for IGF-1</th>
<th>Sample Matrix</th>
<th>Age Range, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Munich, Germany</td>
<td>Munich</td>
<td>146</td>
<td>Cord blood</td>
<td>0–19</td>
</tr>
<tr>
<td>CALIPER, Canada</td>
<td>San Clemente</td>
<td>1360</td>
<td>Serum</td>
<td>0–20</td>
</tr>
<tr>
<td>CALIPER new</td>
<td>Liege</td>
<td>588</td>
<td>Serum</td>
<td>5–20</td>
</tr>
<tr>
<td>Randers, Denmark</td>
<td>Aarhus</td>
<td>854</td>
<td>Serum</td>
<td>0.9–1.1</td>
</tr>
<tr>
<td>Halmstad, Sweden</td>
<td>Gothenburg</td>
<td>319</td>
<td>Serum</td>
<td>3–19</td>
</tr>
<tr>
<td>Leipzig, Germany</td>
<td>Munich</td>
<td>193</td>
<td>Serum</td>
<td>3–18</td>
</tr>
<tr>
<td>Athens, GA</td>
<td>San Clemente</td>
<td>737</td>
<td>Serum</td>
<td>18–87</td>
</tr>
<tr>
<td>Graz, Austria</td>
<td>Graz</td>
<td>55</td>
<td>Plasma</td>
<td>32–81</td>
</tr>
<tr>
<td>MESY-BEPO Berlin</td>
<td>Berlin</td>
<td>2623</td>
<td>Serum</td>
<td>20–81</td>
</tr>
<tr>
<td>SHIP Greifswald</td>
<td>4109</td>
<td>2024</td>
<td>Serum</td>
<td>65–94</td>
</tr>
<tr>
<td>KORA F4 Munich</td>
<td>2989</td>
<td>1446</td>
<td>Plasma</td>
<td>65–94</td>
</tr>
<tr>
<td>KORA Age Munich</td>
<td>1041</td>
<td>522</td>
<td>Serum</td>
<td>65–94</td>
</tr>
<tr>
<td>Total</td>
<td>15 014</td>
<td>6697</td>
<td>Serum</td>
<td>65–94</td>
</tr>
</tbody>
</table>

Detailed information about the exact number of subjects of each sex falling into each age group is given in Supplemental Tables 15 and 16.
were obtained in Athens, GA (the cohort was of diverse ethnic background and had normal growth velocity [longitudinal study]).

**Adult cohorts**

We used samples from 4 different population-based studies from different regions in Germany. All cohorts have been described in detail (18–21). Extensive data on demographic factors, concomitant medication, and comorbidities are available. A total of 2623 serum samples (821 males) came from the Metabolisches Syndrom Berlin Potsdam study (MESY-BEPO, Berlin, Germany), which included healthy volunteers who also underwent an oral glucose tolerance test (OGTT); 4109 serum samples (2024 males) came from the Study of Health in Pomerania (SHIP, northeastern part of Germany); 2989 EDTA plasma samples (1446 males) were obtained from the Cooperative Health Research in the Region Augsburg, Germany (KORA F4); and 1041 additional serum samples (522 males) came from the KORA Age Study, a representative sample of inhabitants of the same region aged 65 years or older.

**Measurement of IGF-1**

The 15 014 samples were analyzed on IDS iSYS instruments in 8 laboratories in 6 countries (Munich, San Clemente, Liege, Aarhus, Gothenburg, Graz, Berlin, and Greifswald) according to a standardized protocol. All analyses took place between 2010 and 2011. The same batch of reagents was used in all laboratories with the exception of the measurement of the samples from KORA F4. Between-laboratory variability was assessed using the same set of 3 quality control samples. In addition, between-instrument agreement as well as agreement between reagent batches has been formally evaluated in a separate study (see Supplemental Methods).

**Statistics**

EP Evaluator Software (version 8.0.0114; Data Innovations Europe) was used for analysis of the data obtained during assay validation. Comparison with existing IGF-1 assays was done using MedCalc Software (version 12.3.0.0; MedCalc Software bvba), using Passing-Bablok analysis (for slope and intercept), linear regression (for R values), and Bland-Altman analysis.

Acquired results from the multicenter reference interval study were explored by several statistical models. A modification of the LMS method (22, 23) allowed the best fit and was used to construct sex- and age-adjusted reference intervals. The estimated percentiles as well as the variables \( \lambda (L) \), \( \mu (M) \), and \( \sigma (S) \) for each age and gender are provided. SD scores can be calculated according to the formula:

\[
z = \frac{|{(IGF-1/M)}^L - 1|}{L \times S}
\]

In a subset of the pediatric samples, reference intervals were also developed according to Tanner stages using the Harrell-Davis estimate of quantiles.

The influence of different exclusion criteria was examined in 3 adult cohorts by calculating reference ranges based on quantile regression (24) with restricted cubic splines with 3 predefined knots (25).

Nonparametric Mann-Whitney U test or Kruskal-Wallis one-way ANOVA was used for between-group comparisons as appropriate, and the correlation between birth weight and cord blood IGF-1 by Spearman rank analysis (Statview version 5.0; SAS Institute).

**Results**

**Assay characteristics**

Detailed results from the assay validation and characterization are provided in the Supplemental Data. The new IGF-1 assay is sensitive (limit of detection 4.4 ng/mL, limits of quantification 8.8 ng/mL) and has a broad dynamic range (10–1200 ng/mL) with excellent linearity (92%–104%) and precision (within-assay coefficient of variation 1.3%–3.7%, total coefficient of variation 3.4%–8.7%). Performance characteristics are summarized in Supplemental Table 2, and details are outlined in Supplemental Tables 3–7 and Supplemental Figure 1.

We did not observe cross-reaction of insulin, proinsulin, or IGFBP-2, and there was no interference from any of the 6 high-affinity IGFBPs even at supraphysiological concentrations (Supplemental Table 8). Other potential interferents also showed no effect (Supplemental Table 9).

**Sample material, preanalytical stability, and comparison with other assays**

Results obtained from plain serum, serum from gel separator tubes, sodium citrate, lithium heparin, sodium heparin, and potassium-EDTA plasma were not different (Supplemental Table 10). Preanalytical stability of IGF-1 was remarkable, with no significant change in measured concentrations for almost 3 days if serum was stored at 4°C and for up to 48 hours at room temperature (Supplemental Tables 11 and 12). Even in whole blood at room temperature for 4 days, on average, the change in measured IGF-1 was small (n = 25, mean bias of 3.4% [24 hours] and 9.1% [96 hours]; Supplemental Table 13). Notably, however, measured concentrations differed by more than 20% in 3 of 25 (24 hours) and 5 of 25 (96 hours) samples, respectively. Less than 10% concentration bias was seen after 5 freezing/thaw cycles (n = 6, IGF-1 55.5–394 ng/mL; Supplemental Table 14).
The new assay was compared with 2 existing IGF-1 assays (Figure 1). Results obtained by the Mediagnost RIA were in good overall agreement (IDS-iSYS = 0.97 × Mediagnost RIA + 3.71; r = 0.976; n = 305) with no significant bias. In 2 independent sets of samples (n = 2992 [Figure 1] and n = 1039 [Supplemental Figure 2]), IGF-1 concentrations were approximately 20% lower on the iSYS when compared with the Siemens Immulite assay, the latter being calibrated against the old and less pure standard.

**Generation of reference intervals**

We first compared the IGF-1 concentrations obtained from different cohorts covering the same age range. Neither the larger pediatric cohorts nor the large adult cohorts differed significantly in age adjusted IGF-1 concentrations. Therefore, for construction of reference intervals from birth to old age we combined the data from all cohorts, and the large number of subjects across all ages allowed us to separately analyze data for males and females. After evaluation of several statistical models, we decided to use a modified LMS method (quantile regression via vector generalized additive models, LMS VGAM) which allowed the best fitting of the data. Figure 2 shows individual data points as well as the fitted percentiles for males and females. Estimated percentiles as well as the variables λ (L), μ (M) and σ (S) required for calculation of SD scores are provided in Table 2 for both sexes in increments of 1 year until the age of 20 and hereafter at 5 years increments. More detailed lists showing percentiles, standard deviations and the variables in smaller age increments are provided in Supplemental Tables 15 and 16. We also constructed reference ranges according to Tanner stages from a pediatric cohort (n = 854; Table 3).

**IGF-1 concentrations across the lifespan**

IGF-1 concentrations in cord blood samples from singleton pregnancies were significantly correlated to birth weight (P < .0001) but not significantly different between males (median [interquartile range] 68.0 [37.3] ng/mL) and females (72.3 [49.7] ng/mL; P = .13). IGF-1 concentrations in samples from twin (45.0 [31.3] ng/mL) and triplet (29.0 [30.6] ng/mL) pregnancies, which had a lower birth weight (P = .003 and P < .0001, respectively), were significantly lower (P < .0001; Supplemental Figure 5).

After birth, IGF-1 concentrations declined and remained lower than in cord blood during the first year of life (P = .03). Thereafter, concentrations increased until a pubertal peak, which occurred at 15 years in both boys and girls. Only when data are stratified according to Tan-

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**Figure 1.** Comparison of the IGF-1 levels measured by Immulite and IDS-iSYS (n = 2992) on the left side and Mediagnost and IDS-iSYS (n = 298) on the right side. Passing-Bablok regression plots are displayed in the top panel: the thick solid red line represents the regression line, the dashed red lines represent the 95% confidence interval (CI) for the fit. Bland-Altman plots are displayed in the lower panel.
ner stages (Table 3) do peak IGF-1 concentrations (50% percentile) tend to occur slightly earlier in girls. Notably, the 95% central interval for IGF-1 concentrations around puberty is broader for girls than for boys when data are analyzed according to chronological age, but not if analyzed according to Tanner stages. In both sexes, peak values for the 2.5th percentile based on chronological age are lower compared with the peak values based on Tanner stage IV (males, 152 vs 245 ng/mL; females, 128 vs 258 ng/mL; Tables 2 and 3). Later in life, although the difference is small, mean IGF-1 concentrations are significantly higher in males than females (males, 111.7 [39.0] ng/mL; females, 103.4 [37.0] ng/mL; $P < .0001$). This sex difference was also detectable when data were analyzed separately for each decade (fifth through eighth).

**Validation of the reference ranges**

We compared IGF-1 concentrations in samples from children with normal growth collected in a community-based setting outside the hospital (CALIPER new) with concentrations seen in the original CALIPER cohort, where samples were collected from metabolically healthy children who were attending a hospital for various reasons (elective minor surgery or dentistry). No differences were observed between the 2 groups in age-adjusted mean IGF-1, and concentrations seen in samples from the community-based setting all were within the age-specific reference range constructed from the other studies (Supplemental Figures 3 and 4). Also, IGF-1 concentrations measured in the 5 other pediatric cohorts from different countries did not differ systematically.

We also compared estimated percentiles for adults by comparing reference intervals established based on cohorts from different geographic regions separately (KORA, SHIP, and MESY-BEPO), but no significant differences were seen. Applying reference intervals constructed from the other studies to interpret IGF-1 concentrations measured in KORA F4 revealed that 2.5% and 2.8% of the 1148 males (2.4% and 2.3% of the 1544 females) were found below and above the 2.5th and 97.5th centile, respectively.

Using the detailed information on comorbidities and anthropometric measures available in 3 adult cohorts, we analyzed whether different inclusion/exclusion criteria to define the reference population influence the resulting reference intervals. Table 4 shows reference intervals (2.5th to 97.5th centiles) constructed with and without exclusion of subjects with diabetes, history of cancer, various degrees of reduction in creatinine clearance, liver disease, diseases of the pituitary or body mass index (BMI) $< 18$ or $> 30$ kg/m² for males and females aged 30 and 70 years, respectively. Subjects with very low and very high BMI or liver disease had slightly, but significantly, lower IGF-1 concentrations compared with the rest of the cohort (data not shown), but the overall impact of applying stricter exclusion criteria was negligible.

**Discussion**

We report the development and validation of a new automated IGF-1 assay calibrated against the new recombinant standard. We also report the establishment of method-specific reference intervals for IGF-1 derived from a very large number of healthy subjects of all ages from different populations.

The recent consensus statement on GH and IGF-1 assays (11) was formulated by representatives of the Growth Hormone Research Society, the IGF Society, and the International Federation for Clinical Chemistry and Labo-
We also followed the recommendations of the consensus statement regarding the requirements for the development of appropriate age- and sex-specific reference intervals for IGF-1. In our study, we used samples from a large number of subjects of all ages coming from different studies, all with sufficient clinical data being available for adequate characterization. To our knowledge, this is the largest cohort of well-characterized individuals covering the whole age range ever investigated for IGF-1 concentrations.

Due to obvious ethical restrictions, studies to establish reference ranges in children are difficult to perform and often limited in size. By combining several studies, we were able to include 4252 samples from newborns, children, and adolescents from several geographical regions. The dataset is of sufficient size to calculate and validate reference intervals adjusted for age and sex. An important contribution came from samples collected for the Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER), a study organized to overcome the existing gaps in the availability of reliable reference intervals for children (16). We made use of 2 subcohorts of this study.
Our observation that in both sexes, the 2.5th percentile calculated based only on chronological age is lower compared with that calculated based on Tanner stages supports the notion that reference intervals adjusted for pubertal development can be useful. Especially if IGF-1 is low, the impact of pubertal development seems not appropriately reflected by reference intervals purely based on chronological age. After puberty, a gradual decrease in IGF-1 concentrations is seen in both sexes, which is accelerated in females between the fifth and sixth decade, possibly due to an influence of postmenopausal decline in sex steroids. In accordance with the findings from others (13), IGF-1 concentrations between the ages of 50 and 80 remain significantly higher in males.

We used data from 4 population-based studies to investigate the impact of potentially confounding factors on the reference intervals. As expected, IGF-1 was lower in subjects with very low or very high BMI. Clinically, however, the effect is far too small to justify weight-based adjustment of reference intervals. IGF-1 was also lower in subjects with comorbidities like diabetes, liver disease, or moderate reduction of kidney function. However, neither the restriction to a certain BMI range nor the exclusion of subjects with certain comorbidities had any significant impact on the estimated percentiles (Table 4), further confirming the robustness of the reference intervals.

Our study was not specifically designed to investigate the potential impact of the ethnic background, because the cohorts consisted mainly of Caucasians. The pediatric cohorts from Canada and the United States better reflected a mixed ethnic background but were not different from the European pediatric cohorts. Some studies indicate a potential effect of ethnicity on circulating IGF-1 (31), but others suggest it is of minor importance (11, 32). Because IGFBP-3 concentrations are more obviously influenced by ethnic background (33, 34), differences in the efficacy of the IGF-1 assays used to remove IGFBP3 interference could potentially contribute to the divergent findings.

The modified LMS approach (35) we have chosen to calculate percentiles and variables for SD score calculation has been widely used to calculate reference intervals for several anthropometric and biochemical variables (36–41) where a strong age dependency can be observed. The model also has the advantage that, despite the skewed distribution of the IGF-1 values within each age-group, it allows a very simple calculation of dimensionless SD scores from individual IGF-1 concentrations. Currently more common in pediatric endocrinology, SD scores directly indicate the relative position of an individual’s IGF-1 concentration compared with the distribution of IGF-1 in the reference population and thereby also facilitate the comparison of concentrations across ages.

In conclusion, we developed and validated a new au-

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Age Range, y</th>
<th>IGF-1, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td>2.5%</td>
</tr>
<tr>
<td>I</td>
<td>6.1–12.9</td>
<td>81.3</td>
</tr>
<tr>
<td>II</td>
<td>8.1–14.8</td>
<td>106.2</td>
</tr>
<tr>
<td>III</td>
<td>10.9–16.0</td>
<td>244.9</td>
</tr>
<tr>
<td>IV</td>
<td>12.4–17.1</td>
<td>222.6</td>
</tr>
<tr>
<td>V</td>
<td>13.5–20.0</td>
<td>277.4</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td>2.5%</td>
</tr>
<tr>
<td>I</td>
<td>5.8–12.1</td>
<td>85.9</td>
</tr>
<tr>
<td>II</td>
<td>9.3–14.1</td>
<td>117.5</td>
</tr>
<tr>
<td>III</td>
<td>9.3–15.1</td>
<td>258.3</td>
</tr>
<tr>
<td>IV</td>
<td>11.8–16.6</td>
<td>242.4</td>
</tr>
<tr>
<td>V</td>
<td>12.5–19.9</td>
<td>188.2</td>
</tr>
</tbody>
</table>

Estimated percentiles (2.5%, 25%, 50%, 75%, and 97.5%) derived by Harrell-Davis estimate of quantiles are provided.
tomated immunoassay to measure IGF-1 concentrations. The method is calibrated against the new recombinant IGF-1 reference preparation, involves only monoclonal antibodies, has demonstrated stability across batches and laboratories, and is insensitive to interference from IGFBPs. In combination with the very robust age- and sex-specific reference intervals derived from an international multicenter study, the new assay can be a useful tool in diagnosis and monitoring of GH-related diseases.

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**Table 4. Impact of Different Exclusion Criteria Upon the Reference Limits Calculated From the Results Obtained in 3 Large Adult Cohorts (KORA F4, KORA Age, and SHIP)**

<table>
<thead>
<tr>
<th>Exclusion Criteria</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>No exclusion (3992 males; 4147 females)</td>
<td>89.1–270.6</td>
<td>82.1–263.5</td>
</tr>
<tr>
<td>Exclusion of subjects with diabetes, history of cancer, creatinine clearance &lt; 60 ml/min/1.73 m², liver disease, disease of the pituitary gland</td>
<td>91.1–272.8</td>
<td>81.2–262.0</td>
</tr>
<tr>
<td>Exclusion of subjects with diabetes, history of cancer, creatinine clearance &lt; 60 ml/min/1.73 m², liver disease, disease of the pituitary gland, BMI &lt; 18 or &gt; 30 kg/m²</td>
<td>90.8–272.9</td>
<td>81.7–260.4</td>
</tr>
<tr>
<td>Exclusion of subjects with diabetes, history of cancer, creatinine clearance &lt; 30 ml/min/1.73 m², liver disease, disease of the pituitary gland</td>
<td>91.2–270.2</td>
<td>81.7–262.4</td>
</tr>
<tr>
<td>Exclusion of subjects with diabetes, history of cancer, creatinine clearance &lt; 30 ml/min/1.73 m², liver disease, disease of the pituitary gland, BMI &lt; 18 or &gt; 30 kg/m²</td>
<td>90.7–271.8</td>
<td>81.9–260.9</td>
</tr>
</tbody>
</table>

Reference limits (2.5th and 97.5th centiles) were calculated from all subjects (no exclusions) and from the smaller number of subjects remaining after application of different exclusion criteria. As an example, reference limits are shown for 30- and 70-year-old males and females, respectively.

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