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

Martin Bidlingmaier, Nele Friedrich, Rebecca T. Emeny, Joachim Spranger, Ole D. Wolthers, Josefine Roswall, Antje Koerner, Barbara Obermayer-Pietsch, Christoph Hübener, Jovanna Dahlgren, Jan Frystyk, Andreas F. H. Pfeiffer, Angela Doering, Maximilian Bielohuby, Henri Wallaschofski, and Ayman M. Arafat

Endocrine Research Laboratories (M.Bid., M.Bie.), Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, 80336 Munich, Germany; Metabolic Center (N.F., H.W.), Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Germany; Helmholtz Zentrum München-German Research Center for Environmental Health (GmbH) (R.T.E., A.D.), Institute of Epidemiology II, Neuherberg, Germany; Department of Endocrinology, Diabetes, and Nutrition (J.S., A.F.H.P., A.M.A.), Charité-University Medicine Berlin, Berlin, Germany; Experimental and Clinical Research Center (J.S.), Charité-University Medicine Berlin and Max-Delbrück Centre Berlin-Buch, Berlin, Germany; Center for Cardiovascular Research (J.S., A.M.A.), Charité-University Medicine Berlin, Berlin, Germany; Children's Clinic Randers (O.D.W.), Randers, Denmark; Göteborg Pediatric Growth Research Center (J.R.), The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; Center for Pediatric Research (A.K.), Hospital for Children and Adolescents, Department of Women's and Child Health, University of Leipzig, Leipzig, Germany; Klinische Abteilung und Labor für Endokrinologie und Stoffwechsel (B.O.-P.), Universitätsklinik für Innere Medizin, Medizinische Universität Graz, Austria; Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe-Grosshadern (C.H.), Klinikum der Universität München, Munich, Germany; Medical Research Laboratory (J.F.), Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark; Department of Endocrinology and Internal Medicine (J.F.), Aarhus University Hospital, Aarhus, Denmark; and Department of Clinical Nutrition (A.F.H.P., A.M.A.), German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

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,

doi: 10.1210/jc.2013-3059

sampling setting (community or hospital based), and rigor of exclusion criteria in our large cohort did not affect the reference intervals.

Conclusions: Using large cohorts of well-characterized subjects from different centers allowed construction of robust reference ranges for a new automated IGF-1 assay. The strict adherence to recent consensus criteria for IGF-1 assays might facilitate clinical application of the results.

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 demographical 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

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received August 5, 2013. Accepted January 24, 2014.

Abbreviations: BMI, body mass index; IGFBP, IGF binding protein.

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

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

Study Group	Lab Measured	No. of Samples for IGF-1			Sample Matrix	Age Range, y
		All	Males	Females		
Munich, Germany	Munich	146	79	67	Cord blood	0
CALIPER, Canada	San Clemente	1360	850	510	Serum	0–19
CALIPER new	Liege	588	298	290	Serum	0–20
Randers, Denmark	Aarhus	854	393	461	Serum	5–20
Halmstad, Sweden	Gothenburg	319	171	148	Serum	0.9–1.1
Leipzig, Germany	Munich	193	93	100	Serum	7–18
Athens, GA	San Clemente	737	0	737	Serum	3–19
Graz, Austria	Graz	55	0	55	Serum	3–18
MESY-BEPO	Berlin	2623	821	1802	Serum	18–87
SHIP	Greifswald	4109	2024	2085	Serum	20–81
KORA F4	Munich	2989	1446	1543	Plasma	32–81
KORA Age	Munich	1041	522	519	Serum	65–94
Total		15 014	6697	8317		

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 λ (L), μ (M), and σ (S) for each age and gender are provided. SD scores can be calculated according to the formula $z = \{[(\text{IGF-1}/M)^{L}] - 1\}/L \times S$. 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 IGF-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), mu (M) and sigma (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-

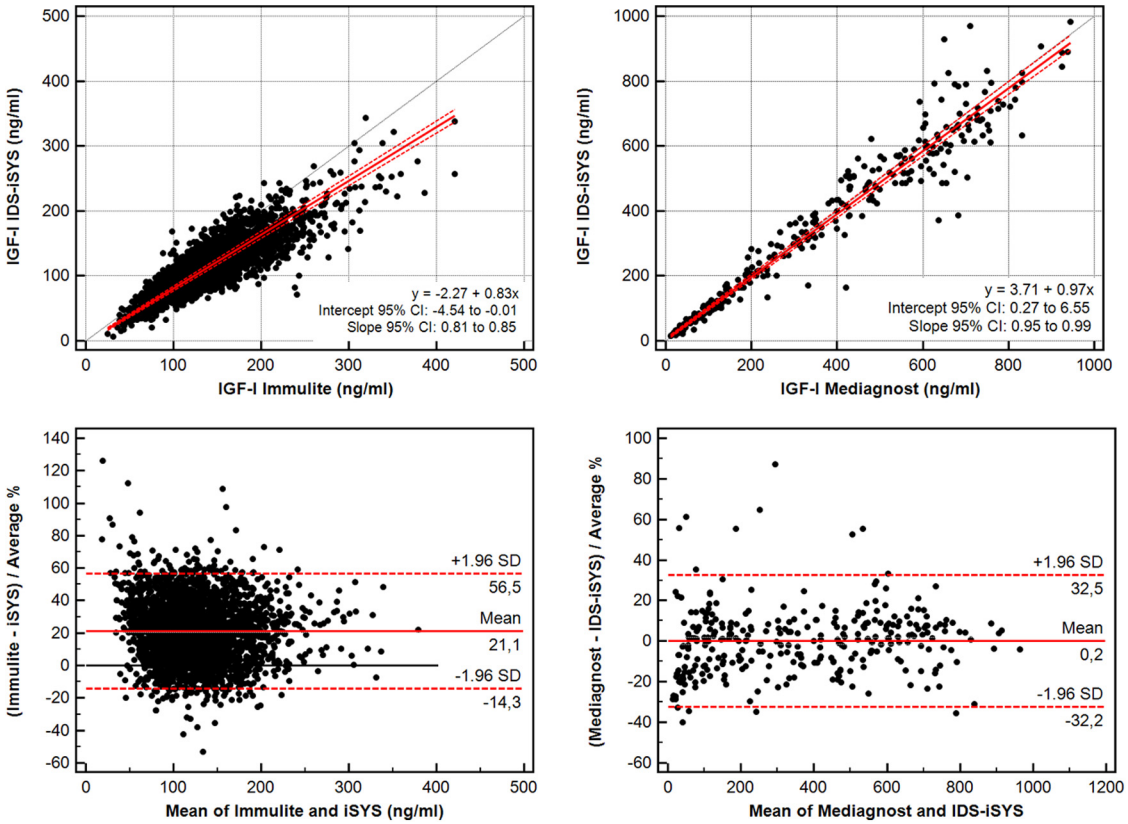

Figure 1. Comparison of the IGF-1 levels measured by Immulite and IDS-iSYS (n = 2992) on the left side and Mediagnost and IDS-iSYS (n = 298) on the right side. Passing-Bablok regression plots are displayed in the top panel: the thick solid red line represents the regression line, the dashed red lines represents 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/m^2 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-

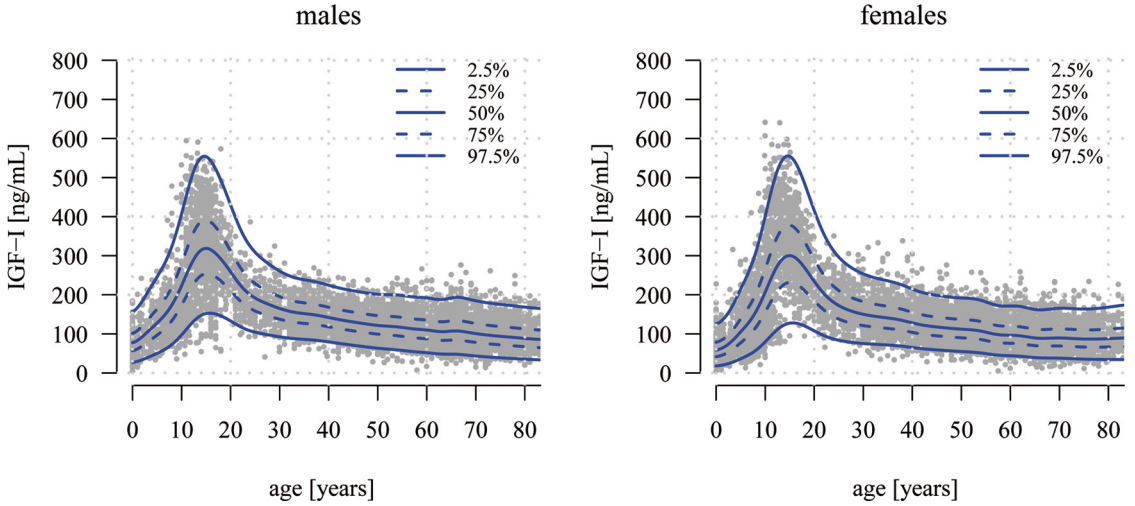

Figure 2. Individual points and fitted percentiles for males (right side) and females (left side). Displayed are the 2.5%, 25%, 50%, 75%, and 97.5% percentiles calculated by quantile regression via vector generalized additive models (LMS VGAM method).

Table 2. LMS Charts

	IGF-1 Males					IGF-1 Females				
	LMS Parameter (λ = 0.4568)		Percentiles			LMS Parameter (λ = 0.4595)		Percentiles		
Age, y	μ	σ	2.5%	50%	97.5%	μ	σ	2.5%	50%	97.5%
0	77.3625	0.4262	27.0	77.4	157.0	58.5742	0.4662	17.9	58.6	125.6
1	83.0813	0.4187	29.7	83.1	166.8	62.3128	0.4589	19.5	62.3	132.3
2	92.5995	0.4113	33.9	92.6	183.9	69.1965	0.4516	22.2	69.2	145.4
3	104.0358	0.4038	39.0	104.0	204.5	78.9225	0.4444	25.9	78.9	164.2
4	115.7263	0.3964	44.3	115.7	225.0	91.1878	0.4371	30.7	91.2	187.8
5	127.6126	0.3890	50.0	127.6	245.5	105.1525	0.4299	36.2	105.2	214.4
6	140.3556	0.3817	56.2	140.4	267.1	119.1360	0.4227	42.0	119.1	240.4
7	155.0333	0.3744	63.4	155.0	291.9	134.9677	0.4156	48.6	135.0	269.6
8	173.3780	0.3673	72.4	173.4	323.1	154.3755	0.4085	56.9	154.4	305.3
9	196.1267	0.3602	83.6	196.1	361.6	178.4732	0.4015	67.2	178.5	349.4
10	222.8130	0.3532	96.9	222.8	406.6	206.5732	0.3945	79.5	206.6	400.3
11	251.5695	0.3463	111.6	251.6	454.4	235.8698	0.3877	92.6	235.9	452.6
12	278.8827	0.3396	126.1	278.9	498.7	262.6764	0.3809	105.3	262.7	499.1
13	300.7811	0.3330	138.6	300.8	532.5	283.4969	0.3742	115.9	283.5	533.4
14	314.4302	0.3265	147.5	314.4	551.2	296.2184	0.3677	123.4	296.2	552.0
15	318.7692	0.3202	152.2	318.8	553.5	300.1497	0.3614	127.4	300.1	554.2
16	314.9775	0.3141	152.9	315.0	541.8	295.9452	0.3553	127.9	295.9	541.5
17	305.3809	0.3082	150.6	305.4	520.6	285.1932	0.3495	125.3	285.2	517.3
18	292.0343	0.3026	146.2	292.0	493.6	270.0078	0.3440	120.5	270.0	485.8
19	276.0573	0.2972	140.2	276.1	462.7	252.5661	0.3387	114.4	252.6	450.8
20	258.5452	0.2922	133.1	258.5	430.0	234.7593	0.3338	107.8	234.8	416.0
21–25	217.1600	0.2809	115.2	217.2	354.8	196.2189	0.3230	92.9	196.2	342.0
26–30	176.8353	0.2645	97.9	176.8	281.6	158.6612	0.3073	78.4	158.7	270.0
31–35	156.4434	0.2566	88.3	156.4	246.0	144.6606	0.2988	73.1	144.7	243.0
36–40	147.8521	0.2571	83.4	147.9	232.7	135.6504	0.2964	69.0	135.7	227.0
41–45	135.7125	0.2654	74.9	135.7	216.4	121.6626	0.2990	61.5	121.7	204.4
46–50	125.7431	0.2798	66.9	125.7	205.1	114.5344	0.3059	56.8	114.5	194.5
51–55	119.3813	0.2977	60.6	119.4	200.3	109.8845	0.3163	53.0	109.9	189.6
56–60	112.5445	0.3161	54.3	112.5	194.2	98.0102	0.3289	45.6	98.0	172.4
61–65	105.9676	0.3335	48.8	106.0	187.7	94.1497	0.3425	42.2	94.1	169.0
66–70	105.7704	0.3492	46.5	105.8	191.9	88.7193	0.3559	38.3	88.7	162.5
71–75	96.7081	0.3633	40.9	96.7	179.2	88.2148	0.3688	36.6	88.2	164.7
76–80	91.1025	0.3762	37.1	91.1	172.0	86.7241	0.3811	34.7	86.7	164.8
81–85	86.0783	0.3885	33.8	86.1	165.4	89.1368	0.3933	34.4	89.1	172.4
86–90	84.9649	0.4003	32.2	85.0	166.1	90.2972	0.4054	33.6	90.3	177.8

The variables μ and σ as well as the estimated percentiles (2.5%, 50%, and 97.5%) are provided. Note that an extended version of this table providing the data split by smaller increments in age and also providing the exact number of subjects of each sex falling into each age group is given in Supplemental Tables 15 and 16.

ratory Medicine, and it defines specific criteria for validation of IGF-1 assays. We strictly followed these criteria by using the international reference standard 02/254 (12), demonstrating recovery of added highly purified IGF-1 (10), and by rigorously testing the potential interference of all 6 high-affinity IGFBPs. As requested by the consensus statement, also the results of validation of antibody specificity, preanalytical conditions, matrix requirements, and freeze/thaw stability are made available in detail in this article (see also Supplemental Data).

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

Table 3. Percentiles for IGF-1 According to Tanner Stages Based on the Danish Cohort (n = 854)

Tanner	Age Range, y	IGF-1, ng/mL				
		2.5%	25%	50%	75%	97.5%
Males						
I	6.1–12.9	81.3	132.5	160.0	187.9	255.3
II	8.1–14.8	106.2	212.4	276.9	331.8	432.3
III	10.9–16.0	244.9	341.2	407.2	449.0	511.4
IV	12.4–17.1	222.6	364.5	439.0	492.4	577.7
V	13.5–20.0	227.4	308.6	355.7	412.3	517.8
Females						
I	5.8–12.1	85.9	152.6	187.7	235.3	323.0
II	9.3–14.1	117.5	190.0	247.3	323.2	451.3
III	9.3–15.1	258.3	335.5	382.8	430.8	528.5
IV	11.8–16.6	224.2	339.8	378.3	437.5	585.8
V	12.5–19.9	188.2	277.4	339.1	394.9	511.6

Estimated percentiles (2.5%, 25%, 50%, 75%, and 97.5%) derived by Harrell-Davis estimate of quantiles are provided.

to analyze a potential bias introduced in reference intervals by the inclusion of samples from metabolically healthy children collected inside or outside the hospital. After adjustment for sex and age, IGF-1 levels were not significantly different in samples from children attending a hospital for minor elective surgery compared with those measured in samples from healthy children in the same geographic region but collected outside the hospital. This confirms the validity of our reference intervals and also supports the validity of the approach undertaken by CALIPER. We also investigated samples from of healthy children characterized by experienced pediatricians in several other centers in different countries. Overall, the comparability of IGF-1 concentrations across all 7 pediatric cohorts from different countries confirms that, at least in industrialized countries with unlimited availability of food, age and to a lesser extent sex are the only major determinants of circulating IGF-1 concentrations.

IGF-1 concentrations were not different between sexes in cord blood. Concentrations were significantly lower in cord blood from twins and triplets in line with the lower birth weight in these babies. One might also speculate about the impact of nutrient availability, but a potential biological relevance of these findings for later life health remains to be clarified (26–28). Differences between circulating IGF-1 levels in boys and girls around puberty have been described in some studies (13, 29) but not in others (30). In our cohorts, the peak in mean IGF-1 tended to occur only slightly earlier in girls when the data were stratified according to Tanner stages (Tanner III–IV vs IV), whereas the peak was similar when data were stratified to chronological age. However, during adolescence, the reference interval (2.5th to 97.5th percentile) was broader in girls, indicating a higher variability of normal IGF concentrations. 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 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)

Exclusion Criteria	Reference Limits (2.5%–97.5%) for IGF-1, ng/mL			
	Men		Women	
	30 y	70 y	30 y	70 y
No exclusion (3992 males; 4147 females)	89.1–270.6	40.7–188.4	82.1–263.5	39.6–172.2
Exclusion of subjects with diabetes, history of cancer, creatinine clearance <60 ml/min/1.73 m^2, liver disease, disease of the pituitary gland	91.1–272.8	44.8–187.2	81.2–262.0	39.8–172.5
Exclusion of subjects with diabetes, history of cancer, creatinine clearance <60 ml/min/1.73 m^2, liver disease, disease of the pituitary gland, BMI <18 or >30 kg/m^2	90.8–272.9	47.6–186.8	81.7–260.4	43.1–176.2
Exclusion of subjects with diabetes, history of cancer, creatinine clearance <30 ml/min/1.73 m^2, liver disease, disease of the pituitary gland	91.2–270.2	44.1–187.7	81.7–262.4	41.2–172.2
Exclusion of subjects with diabetes, history of cancer, creatinine clearance <30 ml/min/1.73 m^2, liver disease, disease of the pituitary gland, BMI <18 or >30 kg/m^2	90.7–271.8	48.0–185.0	81.9–260.9	43.5–174.5

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.

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.

Acknowledgments

The support from Dr Carsten Heuck, Mrs Anne Karina Kjaer, study nurse, and Mrs Charlotte Gradman, study nurse, for taking the blood samples as well as from headmasters, teachers and students at Randers Realskole, Hadsundvejens Skole, Hobrovejens Skole and Randers Statsskole, Randers, for providing fine working conditions is gratefully acknowledged. The Randers study was supported by grants from Clinical Institute, Health, Aarhus University, Johannes M. Klein og Hustrus Mindelegat, Forskningsfonden Laegekredsforeningen for Aarhus Amt, Kong Christian den Tiendes Fond, Overlaege Johan Boserup og Lise Boserups Legat, Koventualinde Emilie De Lancy´s Fond.

Address all correspondence and requests for reprints to: Martin Bidlingmaier, MD, Endocrine Research Laboratories, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Ziemssenstr. 1, 80336 Munich, Germany, Tel.: +49–89–5160–2277, Fax: +49–89–5160–4457, E-mail: martin.bidlingmaier@med.uni-muenchen.de.

This study was in part supported by a grant from the German Federal Ministry of Education and Research (BMBF) to M.Bid. (FKZ 01ET1003D).

This work is part of the research project Greifswald Approach to Individualized Medicine (GANI_MED). The GANI_MED consortium is funded by the Federal Ministry of Education and Research and the Ministry of Cultural Affairs of the Federal State of Mecklenburg-West Pomerania (03IS2061A). Data collection was supported by the Community Medicine Research Network (CMR) of the University of Greifswald, Germany, and is funded by the Federal Ministry of Education and Research, the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania. The CMR encompasses several research projects that share data from the population-based Study of Health in Pomerania (SHIP; http://www.community-medicine.de).

The MONICA/KORA Augsburg studies were initiated and financed by the GSF-National Research Center for Environment and Health, Neuherberg, Germany (now Helmholtz Zentrum München, German Research Center for Environmental Health) and the German Federal Ministry of Education, Science, Research, and Technology and by the State of Bavaria.

The MeSy-BePo study is supported by a grant from the German Federal Ministry of Education and Research to A.F.H.P. (BMBF 0313042). A.M.A. is supported by the graduate school (GK1208) of the German Research Foundation (DFG).

This work was supported by BioPersMed (COMET K-project 825329), which is funded by the Austrian Federal Ministry of Transport, Innovation, and Technology (BMVIT) and the Austrian Federal Ministry of Economics and Labor/the Federal Ministry of Economy, Family, and Youth and the Styrian Business Promotion Agency.

The Research and Development Board, Halland County Council, and the Halland Regional Development Council have supported the study in Sweden.

Disclosure Summary: M.Bid. received research support and/or lecture fees from IDS, Siemens, Novartis, Pfizer, Chiasma, and Prolor and consults for IDS, Chiasma, and Prolor. J.D., J.F., H.W., and A.M.A. received research support from IDS. N.F., R.T.E., J.S., O.D.W., J.R., A.K., B.O.-P., C.H., A.F.H.P., A.D., and M.Bie have nothing to declare.

References

1. **Melmed S, Colao A, Barkan A, et al.** Guidelines for acromegaly management: an update. *J Clin Endocrinol Metab*. 2009;94:1509–1517.
2. **Cohen P, Rogol AD, Deal CL, et al.** Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab*. 2008;93:4210–4217.
3. **Ho KK.** Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *Eur J Endocrinol*. 2007;157:695–700.
4. **Growth Hormone Research Society, Pituitary Society** Biochemical assessment and long-term monitoring in patients with acromegaly. statement from a joint consensus conference of the Growth Hormone Research Society and the Pituitary Society. *J Clin Endocrinol Metab* 2004;89:3099–3102.
5. **Endogenous Hormones and Breast Cancer Collaborative Group; Key TJ, Appleby PN, Reeves GK, Roddam AW.** Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *Lancet Oncol*. 2010;11:530–542.
6. **Khosravi J, Diamandi A, Mistry J, Scorilas A.** Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in benign prostatic hyperplasia and prostate cancer. *J Clin Endocrinol Metab*. 2001;86:694–699.
7. **Stöckl D, Döring A, Peters A, et al.** Age at menarche is associated with prediabetes and diabetes in women (aged 32–81 years) from the general population: the KORA F4 Study. *Diabetologia*. 2012;55:681–688.
8. **Friedrich N, Thuesen B, Jørgensen T, et al.** The association between IGF-I and insulin resistance: a general population study in Danish adults. *Diabetes Care*. 2012;35:768–773.
9. **Rosskopf D, Schwahn C, Neumann F, et al.** The growth hormone–IGF-I axis as a mediator for the association between FTO variants and body mass index: results of the Study of Health in Pomerania. *Int J Obes (Lond)*. 2011;35:364–372.
10. **Frystyk J, Freda P, Clemmons DR.** The current status of IGF-I assays–a 2009 update. *Growth Horm IGF Res*. 2010;20:8–18.
11. **Clemmons DR.** Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. *Clin Chem*. 2011;57:555–559.
12. **Burns C, Rigsby P, Moore M, Rafferty B.** The First International Standard For Insulin-like Growth Factor-1 (IGF-1) for immunoassay: preparation and calibration in an international collaborative study. *Growth Horm IGF Res*. 2009;19:457–462.
13. **Brabant G, von zur Mühlen A, Wüster C, et al.** Serum insulin-like growth factor I reference values for an automated chemiluminescence immunoassay system: results from a multicenter study. *Horm Res*. 2003;60:53–60.
14. **NCCLS.** *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*. 2nd ed. NCCLS Document EP5-A2. Wayne, PA: NCCLS; 2004
15. **NCCLS.** *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. NCCLS Document EP17-A. Wayne, PA: NCCLS; 2004.
16. **Adeli K.** Closing the gaps in pediatric reference intervals: the CALIPER initiative. *Clin Biochem*. 2011;44:480–482.
17. **Colantonio DA, Kyriakopoulou L, Chan MK, et al.** Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. *Clin Chem*. 2012;58:854–868.
18. **Völzke H, Alte D, Schmidt CO, et al.** Cohort profile: the study of health in Pomerania. *Int J Epidemiol*. 2011;40:294–307.
19. **Lacruz ME, Emeny RT, Haefner S, et al.** Relation between depressed mood, somatic comorbidities and health service utilisation in older adults: results from the KORA-Age study. *Age Ageing*. 2012;41:183–190
20. **Kaplan RC, Petersen AK, Chen MH, et al.** A genome-wide association study identifies novel loci associated with circulating IGF-I and IGFBP-3. *Hum Mol Genet*. 2011;
21. **Isken F, Abraham U, Weickert MO, Keyhani-Nejad F, Arafat AM, Spranger J, Pfeiffer AF, Mohlig M.** Annual change in insulin sensitivity. *Horm Metab Res*. 2011;43:720–722.
22. **Cole TJ, Green PJ.** Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med*. 1992;11:1305–1319.
23. **Yee TW.** Quantile regression via vector generalized additive models. *Stat Med*. 2004;23:2295–2315.
24. **Koenker R.** *Quantile Regression*. New York, NY: Cambridge University Press; 2005.
25. **Stone C, Koo CY.** *Additive Splines in Statistics*. Washington, DC: American Statistical Association; 1985.
26. **Wu Z, Bidlingmaier M, Friess SC, et al.** A new nonisotopic, highly sensitive assay for the measurement of human placental growth hormone: development and clinical implications. *J Clin Endocrinol Metab*. 2003;88:804–811.
27. **Javaid MK, Godfrey KM, Taylor P, et al.** Umbilical venous IGF-1 concentration, neonatal bone mass, and body composition. *J Bone Miner Res*. 2004;19:56–63.
28. **Gohlke BC, Schreiner F, Fimmers R, Bartmann P, Woelfle J.** Insulin-like growth factor-I in cord blood is predictive of catch-up growth in monozygotic twins with discordant growth. *J Clin Endocrinol Metab*. 2010;95:5375–5381.
29. **Juul A.** Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Horm IGF Res*. 2003;13:113–170.
30. **Elmlinger MW, Kühnel W, Weber MM, Ranke MB.** Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). *Clin Chem Lab Med*. 2004;42:654–664.
31. **Berrigan D, Potischman N, Dodd KW, et al.** Race/ethnic variation in serum levels of IGF-I and IGFBP-3 in US adults. *Growth Horm IGF Res*. 2009;19:146–155.
32. **Nelson AE, Howe CJ, Nguyen TV, et al.** Influence of demographic factors and sport type on growth hormone-responsive markers in elite athletes. *J Clin Endocrinol Metab*. 2006;91:4424–4432.
33. **Hopkins KD, Lehmann ED, Jones RL, et al.** Ethnicity affects IGFBP-3 and IGF-II in normal healthy young adult subjects. *Clin Endocrinol (Oxf)*. 1996;45:327–331.
34. **Casazza K, Higgins PB, Fernández JR, Goran MI, Gower BA.** Longitudinal analysis of the insulin-like growth factor system in African-American and European American children and adolescents. *J Clin Endocrinol Metab*. 2008;93:4917–4923.
35. **Cole TJ.** The LMS method for constructing normalized growth standards. *Eur J Clin Nutr*. 1990;44:45–60.
36. **Fischer DC, Mischek A, Wolf S, et al.** Paediatric reference values for the C-terminal fragment of fibroblast-growth factor-23, sclerostin, bone-specific alkaline phosphatase and isoform 5b of tartrate-resistant acid phosphatase. *Ann Clin Biochem*. 2012;49:546–553.
37. **La Marca A, Spada E, Grisendi V, et al.** Normal serum anti-Müllerian hormone levels in the general female population and the re-

lationship with reproductive history. *Eur J Obstet Gynecol Reprod Biol*. 2012;163:180–184.
38. **Garagorri JM, Rodríguez G, Lario-Elboj AJ, Olivares JL, Lario-Muñoz A, Orden I.** Reference levels for 17-hydroxyprogesterone, 11-desoxycortisol, cortisol, testosterone, dehydroepiandrosterone sulfate and androstenedione in infants from birth to six months of age. *Eur J Pediatr*. 2008;167:647–653.
39. **Kong AP, Wong GW, Choi KC, et al.** Reference values for serum levels of insulin-like growth factor (IGF-1) and IGF-binding protein 3 (IGFBP-3) and their ratio in Chinese adolescents. *Clin Biochem*. 2007;40:1093–1099.
40. **Jackson LV, Thalange NK, Cole TJ.** Blood pressure centiles for Great Britain. *Arch Dis Child*. 2007;92:298–303.
41. **Fredriks AM, van Buuren S, van Heel WJ, Dijkman-Neerincx RH, Verloove-Vanhorick SP, Wit JM.** Nationwide age references for sitting height, leg length, and sitting height/height ratio, and their diagnostic value for disproportionate growth disorders. *Arch Dis Child*. 2005;90:807–812.