Serum Insulin-Like Growth Factor I Reference Values for an Automated Chemiluminescence Immunoassay System: Results from a Multicenter Study

Georg Brabant^a Alexander von zur Mühlen^a Christian Wüster^b Michael B. Ranke^c
Jürgen Kratzsch^d Wieland Kiess^d Jean-Marie Ketelslegers^e Lars Wilhelmsen^f
Lena Hulthén^f Bernhard Saller^g Anders Mattsson^g Jürgen Wilde^h Rudolf Schemer^h
Peter Kannⁱ On behalf of the German KIMS Board

^aDepartment of Gastroenterology, Hepatology and Endocrinology, Medizinische Hochschule, Hannover, Germany;

^bDepartment of Internal Medicine I, Endocrinology and Metabolism, University Medical Clinic, Heidelberg, Germany;

^cPediatric Endocrinology Section, University Children's Hospital, Tübingen, Germany;

^dChildren's Hospital and Institute for Laboratory Medicine, University of Leipzig, Germany;

^eDepartment of Internal Medicine, School of Medicine, Catholic University of Louvain, Brussels, Belgium;

^fGöteborg University, Göteborg, Sweden; ^gPharmacia Corp., Erlangen, Germany and Stockholm, Sweden;

^hNichols Institute Diagnostica, Bad Vilbel, Germany; ⁱDepartment of Endocrinology and Diabetes, Philipps University, Marburg, Germany

Key Words

IGF-I · Chemiluminescence assay · Reference range · Growth hormone

Abstract

Background: Analysis of insulin-like growth factor I in serum (S-IGF-I) is an integral component in the diagnosis of GH-related disorders and is going to be of interest in the diagnosis and follow-up of many disorders. The objective of the present study was to develop cross-sectional reference values for S-IGF-I measured by an automated chemiluminescence immunoassay (Nichols Advantage[®]). **Methods:** The study included samples from 3,961 healthy subjects (2,201 males, 1,760 females) aged 1 month to 88 years. Six laboratories were involved in this study and the samples were analyzed by one of seven automated immunoassay systems run in these laboratories. For data analysis, polynomial age and sex-specific models were fitted after transformation of S-IGF-I

values. **Results:** The results show the well-known age dependency of S-IGF-I levels. At ages <20, higher S-IGF-I levels were seen in girls with an estimated mean peak of 410 µg/l at age 14 and an estimated mean peak of 382 µg/l at age 16 in boys. Thereafter, a rapid decrease was seen to approximately 25 years of age, followed by a slow age-dependent decrease. In adulthood, S-IGF-I in males were slightly, but significantly higher than in females. It could be shown that the mean values of some reference sample subgroups differed significantly from the total mean. However, the multicenter approach used in this study reduces the impact of systematic population, sample handling and laboratory differences on the calculated reference mean. **Conclusion:** The present study establishes age- and sex-specific reference values for a fully automated immunoassay system based on a large population of healthy subjects. The established reference values may be used for this immunoassay system in different laboratories provided that the systematic difference between systems is low.

Copyright © 2003 S. Karger AG, Basel

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2003 S. Karger AG, Basel
0301-0163/03/0602-0053\$19.50/0

Accessible online at:
www.karger.com/hre

Prof. Dr. med. Georg Brabant
Hannover Medical School
Department of Gastroenterology, Hepatology and Endocrinology
Carl-Neuberg-Strasse 1, DE-30625 Hannover (Germany)
Tel. +49 511 5326528, Fax +49 511 5323825, E-Mail brabant.georg@mh-hannover.de

Introduction

Analysis of insulin-like growth factor I in serum (S-IGF-I) has become an integral component in the diagnosis of growth hormone (GH)-related disorders in children and adults [1–3]. Moreover, S-IGF-I is used for dose adjustment of GH replacement therapy in children and adults [4], and is suggested as the most reliable component for monitoring the success of therapeutic interventions in acromegaly [5, 6]. In addition to these applications in GH-related disorders, recent studies have focused on a possible relation between S-IGF-I levels and the risk for certain malignancies as well as on a prognostic significance of S-IGF-I levels in patients with malignant diseases [7, 8]. These developments indicate that analysis and interpretation of S-IGF-I levels are no longer the mainstay of a few specialized laboratories but that S-IGF-I is going to be a component that is widely used in the diagnosis and follow-up of many disorders.

It is thus imperative to achieve uniform interpretations of S-IGF-I levels by improving the standardization and inter-laboratory comparability of S-IGF-I analysis procedures [9, 10]. This includes the establishment of normative data in representative populations by valid immunoassays and by considering non-GH-related factors that are known to influence S-IGF-I concentrations like age and sex.

The objective of the present study was to develop cross-sectional reference values for S-IGF-I measured by a widely used automated chemiluminescence immunoassay (Nichols Advantage®) in a normal population of children, adolescents and adults.

Subjects and Methods

Subjects

S-IGF-I has been determined in samples from 3,961 healthy subjects aged 1 month to 88 years. In a multicenter study design, six laboratories were involved, four in Germany, one in Belgium and one in Sweden. 1,469 samples were from children and adolescents (745 females and 724 males) aged up to 19.9 years, 2,492 samples were from adults (1,015 females and 1,477 males) aged 20 years or more. Table 1 gives the sex and age distribution of the subjects included.

Subjects were either selected from blood-bank sampling, study populations or clinical routine. Informed consent was obtained from the patients or the patients' parents. The study protocol had been approved by the responsible local ethics committee.

Subjects with diseases, in particular renal diseases, liver diseases, malignant disorders, diabetes mellitus and diseases of the pituitary gland, were excluded by medical history, physical examination and routine laboratory tests. In addition, adult subjects with a body mass index >30 and <18 kg/m² were excluded from the study.

All samples were collected in tubes not containing any anticoagulants and were routinely processed with a maximum time interval of 60 min between sampling and freezing. Serum was separated and stored frozen at –20 or –70 °C until analysis. Samples were assayed

Table 1. Age and sex distribution of the study population

Children and adolescents	Age, years											
	0–1	2–3	4–5	6–7	8–9	10–11	12–13	14–15	16–17	18–19		
Males	31	41	28	24	73	75	53	307	58	34		
Females	12	36	28	19	66	127	48	273	84	52		
Total	43	77	56	43	139	202	101	580	142	86		
Adults	Age, years											
	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–88
Males	119	167	182	168	176	152	121	134	128	68	40	22
Females	153	109	112	124	115	113	85	64	67	30	23	20
Total	272	276	294	292	291	265	206	198	195	98	63	42
All subjects												
Males	2,201											
Females	1,760											
Total	3,961											

within 2 months after sampling. Grossly hemolytic, lipemic or icteric samples were excluded. Prior to S-IGF-I determination the samples were allowed to come to room temperature.

Analysis of S-IGF-I

S-IGF-I concentrations were determined by a fully automated, two-site chemiluminescent immunoassay (Nichols Advantage®; Nichols Institute Diagnostics, San Clemente, Calif., USA). In this system the antibody to the C-terminal 62–70 amino acid sequence is biotinylated for capture and the antibody to the amino acid sequences of 1–23 and 42–61 is labeled with acridinium ester for detection. Subject samples are acidified to separate S-IGF-I from IGF-binding proteins. The acidified samples are incubated with the biotinylated capture antibody in the presence of excess IGF-II and acridinium ester-labeled antibody. This displacement method was found to be very accurate based on a recent comparison study with different IGF-I assays [11]. After the initial incubation period, streptavidin-coated magnetic particles are added to the reaction mixture. Free labeled antibody is then separated from the labeled antibody bound to the magnetic particles by aspiration and subsequent washing, while a strong magnetic force keeps the magnetic particles in the well. An acid hydrogen peroxide solution and a sodium hydroxide solution are added to the well to initiate the chemiluminescence reaction.

The assay has been calibrated against the World Health Organization International Reference Reagent 87/518.

A single S-IGF-I analysis result was obtained from each subject involved. The samples were analyzed by any of the seven systems used for this study. The time period of analysis was for six of the seven systems between December 1999 and June 2001. The six systems were run by five laboratories (UCL Brussels, University of Hanover, University of Leipzig, University of Tübingen, and Nichols Diagnostica, Bad Vilbel, Germany). To ensure proper instrument performance and to allow the comparison between runs, instru-

ments and laboratories, IGF-I control samples (Nichols Institute Diagnostics, San Clemente, Calif., USA) were analyzed in each batch of reference samples. All runs with one or more controls out of given ranges (mean $\pm$ 2 SD) were recalibrated and recalculated using the 4-PL logistics of the system (Nichols Advantage® software, version 92.56) until all controls were in range.

One lot of control samples (three levels) was used in these five laboratories during the entire period December 1999 to June 2001. For control level 1 (target: 60) a mean value (SD) of 58 (3.3) μ g/l was found (n = 69); for control level 2 (target 259) 259 (16) μ g/l (n = 71), and for control level 3 (target 546) 539 (36) μ g/l (n = 74). Table 2 gives the results of the control samples for each of the six different analysis systems.

The seventh system in laboratory No. 6 did not use the same lot of control samples and the selected reference population was analyzed during April 2002. For control level 1 (target: 56) a mean value (SD) of 44 (4.0) μ g/l was found (n = 6), corresponding figures for control level 2 (target 250) were 221 (11) μ g/l (n = 6), and for control level 3 (target 488) 476 (26) μ g/l (n = 6).

Statistical Analysis

The mean and variance of population S-IGF-I-values are dependent on age and maybe on sex. Moreover, the distribution of IGF-I values is skewed (fig. 1). After inspection of the data we decided to fit polynomial models. In order to find a scale with as constant variance as possible for the observations, different transformations of the S-IGF-I values were compared (log, square root, and powers between 0.2 and 0.7 (0.5 = square root)). For a given transformation of S-IGF-I different smooth functions of age of the form

$$f(\text{age}) = b_0 + b_{11}\text{age} + b_{12}\text{age}^2 + b_{13}\text{age}^3 + b_{23}{}_i(\text{age} - x_1)^3 + b_{33}{}_i(\text{age} - x_2)^3 + b_{14}{}_i(\text{age} - x_2)^4$$

Table 2. S-IGF-I values (μ g/l) in three control samples as measured by six different instruments during the period December 1999 to June 2001

Control	Instrument No.	n	Mean	Min.	Max.	SD	CV, %
Level 1 Target 60	1	23	58.7	55.0	65.0	2.4	4.1
	2	8	56.0	52.8	60.9	2.5	4.4
	3	31	59.2	52.5	65.4	3.5	5.9
	4	1	63.0	–	–	–	–
	5	3	57.6	56.8	59.2	1.1	1.9
	6	3	54.8	52.4	56.6	1.8	3.2
Level 2 Target 259	1	23	261.5	230.1	282.9	15.0	5.7
	2	5	256.0	225.4	279.8	18.1	7.1
	3	31	257.7	229.2	286.3	14.8	5.7
	4	2	257.0	249.0	265.0	8.0	3.1
	5	5	258.5	248.6	272.5	8.3	3.2
	6	5	266.4	253.2	277.8	10.0	3.8
Level 3 Target 546	1	23	537.1	453.2	578.4	34.8	6.5
	2	8	506.3	432.0	576.4	44.8	8.8
	3	31	541.9	470.0	628.2	52.9	9.8
	4	2	592.5	556.0	629.0	36.5	6.2
	5	5	527.2	500.2	558.8	19.4	3.7
	6	5	543.5	496.9	565.5	25.6	4.7

Fig. 1. S-IGF-I as a function of age. The figure gives the individual values of the 3,961 healthy subjects (2,201 males, 1,760 females) as well as the curves for the fitted mean, ± 1 SD and ± 2 SD functions given by $[f(\text{age}) \pm k\text{SD}]^{2.5}$ $k = 0, 1, 2$. (**a** all subjects; **b** males; **c** females).

where $i(\text{age} - x_j)$ is 0 if $\text{age} < x_j$ and $(\text{age} - x_j)$ if $\text{age} \geq x_j$ ($j = 1, 2$, and $x_1 < x_2$) were compared. Different values on x_j were compared for adjusting location of peak and curvature. The model that reached the highest explanation in terms of coefficient of determination, R^2 , and where the transformed S-IGF-I observations 'best' followed a normal distribution around the fitted function of age was chosen [12]. In the final model, gender was incorporated and its contribution tested. Gender was coded 1 if female and 0 if male. Potential interaction between age and gender was tested by gender times $i(\text{age} < 20)$, where $i(\text{age} < 20) = 1$ if $\text{age} < 20$ years, otherwise 0. Reference curves for S-IGF-I were approximated by $[f(\text{age}) \pm kSD]^{1/P}$, where P is the power of transformation of S-IGF-I and k number of model based standard deviations (SD). For the logarithmic transformation $[f(\text{age}) \pm kSD]$ was exponentiated.

In an additional analysis, a random intercept model was fitted assuming the polynomial structure $f(\text{age}, \text{gender})$ but at different levels for each lab. The random intercept model is structured so that each lab has its own intercept. These intercepts are formulated as deviations from the 'population mean'. In graphical terms it means that the polynomial structure is represented at different levels according to [population mean at a given age + lab-specific intercept deviation] $^{1/P}$. In hypothesis testing the deviations are assumed to follow a normal distribution with mean $\mu = 0$ and variance σ^2 .

PROC REG and PROC MIXED in SAS software were used for the statistical analyses (Statistical Analysis System, version 8.2, SAS Institute, Cary, N.C., USA).

Results

Figure 1 shows plots of S-IGF-I values in 3,961 healthy subjects versus age of the subjects. Scattergrams are given for the whole study population (fig. 1a), as well as for males (fig. 1b) and females (fig. 1c) separately. Females appear to have higher values than males at ages < 20 . The peak also occurs 1–2 years earlier (13–14 years among girls and 15–16 years among boys). After 20 years of age, females have slightly lower values.

The transformation that best conformed these S-IGF-I data to the normal distribution was the power 0.4. Mean value, SD and reference limits have been calculated on the IGF-I $^{0.4}$ scale and then back transformed, i.e. raised to the power of 2.5. The IGF-I $^{0.4}$ scale should also be used when standard deviation score (SDS) is calculated: $\text{IGF-I SDS} = (\text{IGF-I}^{0.4} - f(\text{age}))/\text{SD}$.

The age-specific model fitted to all data shows – after a plateau in the first years of life – a rapid increase of S-IGF-I up to a peak at around 14–16 years of age. After the peak, S-IGF-I decreases rapidly to approximately 25 years of age. From there a slow decrease begins persisting up to

Table 3. Parameter estimates with standard errors, SD and R^2 for three fitted polynomial models (see details in Methods and Results sections)

	Model 1a: Males + females			Model 1b: Males			Model 1b: Females		
	effect estimate: b	se(b)	p value	effect estimate: b	se(b)	p value	Effect estimate: b	se(b)	p value
f(age)									
intercept	5.44721	0.21	<0.0001	5.24323	0.23	<0.0001	5.79754	0.36	<0.0001
age	−0.43311	0.08	<0.0001	−0.41428	0.09	<0.0001	−0.45049	0.14	0.0015
age ²	0.13725	0.01	<0.0001	0.12330	0.01	<0.0001	0.14574	0.015	<0.0001
age ³	−0.005612	0.0003	<0.0001	−0.0047353	0.0003	<0.0001	−0.00618	0.0005	<0.0001
i(age-15) ³	0.027046	0.001	<0.0001						
i(age-17) ³	−0.021702	0.001	<0.0001						
i(age-17) ⁴	0.0000018566	2.31E-7	<0.0001						
i(age-16) ³				0.024105	0.001	<0.0001			
i(age-18) ³				−0.019613	0.001	<0.0001			
i(age-18) ⁴				0.0000016876	2.79E-7	<0.0001			
i(age-15) ³							0.055932	0.003	<0.0001
i(age-16) ³							−0.050105	0.003	<0.0001
i(age-16) ⁴							0.0000025107	4.05E-7	<0.0001
	SD = 1.17866 R ² = 64.1%			SD = 1.10048 R ² = 65.6%			SD = 1.2348 R ² = 64.2%		
f(age, gender)	Model 2: Interaction, age terms not shown								
Gender (f vs. m)	−0.26	0.05	<0.0001						
Gender*i(age <20)	0.63	0.07	<0.0001						
	R ² = 64.7%								
Mean IGF-I = [f(age)] ^{2.5} ; reference curves = [f(age) + kSD] ^{2.5} where k = 0,1,2.									

at least 80 years of age (fig. 1a; table 3: model 1a, no adjustment for gender).

When incorporating an interaction term between gender and age into the model, the data show a statistically significant difference in mean levels between males and females. The p value for both gender and the interaction term gender*_i(age <20) was <0.0001. R² for the model was 64.7% (table 3: model 1b). Due to this significant effect of gender and different sizes of the standard deviations, we decided to fit two separate models, one for each

gender. Both showed similar R², 65.6% for males and 64.2% for females (table 3: model 1b). The implications of these models are shown in figure 1b, c. Table 4 shows read values from figure 1 for ages 1 year up to 75–80 for males and females as well as for the whole population. The model for males had an estimated mean peak of S-IGF-I at 382 µg/l around 16 years of age. The mean peak among girls was estimated to 410 µg/l at 14 years of age. The difference between fitted gender-specific means was around 20 the first 3 years of life. The difference was greatest

Table 4. S-IGF-I (µg/l) reference ranges for the Nichols Advantage^c at ages 1–19 years (**A**) and 20 to 75–80 years (**B**). Values were calculated from the fitted polynomial models (see Results section, fig. 1 and table 3)

A	Age, years																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Total (fig. 1a)																				
+2 SD	154	151	159	176	203	239	284	336	395	457	518	572	614	638	639	615	581	548	519	
+1 SD	101	98	104	118	139	168	204	248	297	349	401	448	485	506	506	486	456	427	402	
Mean	60	58	63	73	89	111	140	175	215	259	302	342	373	391	391	374	348	324	303	
−1 SD	31	30	33	40	51	68	90	117	149	184	220	252	278	294	294	279	258	238	220	
−2 SD	13	12	14	18	26	37	52	72	96	124	152	179	200	212	213	201	183	167	153	
Males (fig. 1b)																				
+2 SD	136	133	138	152	173	203	240	285	336	392	449	504	552	588	608	608	586	554	524	
+1 SD	90	87	91	102	119	143	173	210	253	300	349	396	438	470	487	487	468	440	414	
Mean	54	53	55	63	76	95	119	149	184	223	264	304	340	367	382	382	365	341	319	
−1 SD	29	28	30	35	44	58	76	99	127	159	193	226	256	279	292	292	278	257	239	
−2 SD	13	12	13	16	22	31	44	62	83	107	134	162	186	205	216	216	204	187	172	
Females (fig. 1c)																				
+2 SD	178	175	184	205	235	276	327	386	450	515	577	629	665	678	663	622	582	546	515	
+1 SD	117	115	122	137	162	195	236	285	339	394	447	492	523	534	521	486	451	421	394	
Mean	70	69	74	86	104	130	163	202	246	292	336	374	401	410	399	369	340	314	292	
−1 SD	37	36	40	48	61	80	105	135	170	208	244	276	297	305	296	271	247	226	208	
−2 SD	16	15	17	22	31	44	62	84	111	140	169	194	212	219	211	191	171	154	140	
B	Age in years																			
	20	25	30	35	40	45	50	55	60	65	70	75–80								
Total (fig. 1a)																				
+2 SD	493	402	354	329	315	304	292	276	257	237	220	209								
+1 SD	380	303	263	242	230	221	211	198	183	167	153	144								
Mean	284	220	187	170	161	153	145	135	123	110	99	92								
−1 SD	205	153	127	113	106	100	94	86	77	67	59	54								
−2 SD	140	100	79	69	64	60	55	49	43	36	31	27								
Males (fig. 1b)																				
+2 SD	497	402	350	323	307	296	285	271	255	238	223	213								
+1 SD	391	309	265	242	228	219	210	199	186	172	159	151								
Mean	299	230	194	174	163	156	148	139	129	118	108	101								
−1 SD	222	165	135	120	111	105	99	92	84	75	68	63								
−2 SD	158	112	89	77	70	66	61	56	50	44	38	35								
Females (fig. 1c)																				
+2 SD	488	397	352	330	318	307	292	272	248	223	204	199								
+1 SD	371	294	257	239	229	220	208	192	172	152	137	133								
Mean	273	209	179	165	157	150	140	127	112	97	85	82								
−1 SD	192	141	117	106	100	95	88	78	67	56	48	45								
−2 SD	128	89	71	63	58	54	49	42	35	27	22	21								

between 11 and 13 years when girls had fitted means that were around 70 µg/l higher. At ages above 20, males had 6–26 µg/l higher mean values. The smallest between-gender differences were seen between 35 and 50 years of age. Moreover, the reference range was a bit broader for females compared to males, for a given age (tables 3, 4). Reference values outside the age range 0.5–80 years should be judged cautiously for all models.

The fitted curves in figure 1 represent mean $\pm$ kSD, calculated as $[f(\text{age}) \pm \text{kSD}]^{2.5}$, $k = 0, 1$ and 2 . The distribution of observed S-IGF-I values on reference SD intervals closely followed what is expected from the normal distribution (data not shown). In an additional analysis, the laboratory and population was incorporated into the age- and gender-adjusted model and showed a statistically significant effect ($p < 0.0001$). R^2 increased to about 68%.

In the fitted random intercepts age- and gender-adjusted model for laboratories/populations, laboratories/populations 3 and 5a showed significantly higher mean S-IGF-I measurements, whereas laboratories/populations 5b and 6a were significantly lower. The other 4 laboratories/populations did not significantly deviate from the overall mean (table 5). The laboratories/populations with high values (3 and 5a) had mean values that were 20–60 µg/l higher at population mean levels from 60 to 400 µg/l. Laboratories 5 (subpopulation b) and 6 (subpopulation a) showed mean values at population mean levels of 100, 150, 200 µg/l that were around 27, 35, 42 µg/l lower.

Table 5. Estimates and t tests of laboratory- and population-specific deviations from the age- and gender-dependent population mean using a random intercept model

Lab	Deviation estimate	SE	t value	Pr > t
1	0.008	0.201	0.04	0.969
2	0.023	0.198	0.12	0.908
3	0.569	0.204	2.79	0.005
4	0.244	0.203	1.20	0.231
5a	0.699	0.256	2.73	0.006
5b	−0.744	0.205	−3.63	0.0003
6a	−0.783	0.207	−3.78	0.0002
6b	−0.015	0.209	−0.07	0.943

H_0 hypothesis is that laboratory/population deviations is normally distributed ($0, \sigma^2$) around the population mean. The numbers 1–6 indicate different laboratories, a and b indicate different populations measured in one laboratory.

A reference range was also calculated excluding the subpopulations a and b in laboratory 6. Compared to the reference range based on the total population, the mean decreased by 2–6 µg/l depending on age but the SD on the IGF-I^{0.4} scale was practically unchanged (increased from 1.17866 to 1.18046).

Discussion

In the present study, reference values for S-IGF-I have been established based on a large number of healthy subjects between 1 and >80 years of age. The objective of the study was to generate data for S-IGF-I in a large set of clinically well-characterized healthy subjects under conditions close to the normal routine situation, i.e. with inclusion of serum samples from different populations run in different laboratories over a long period of time. To achieve this, a multicenter approach including IGF-I measurements in six different laboratories over a period of more than 2 years was used. The polynomial fitting approach makes the reference values well suited for long-term monitoring of S-IGF-I levels by Nichols Advantage® system.

The long-term reproducibility, including five laboratories and six instruments, estimated as coefficient of variation (CV) was clearly <7%. Based on published data on intra-individual S-IGF-I variations in healthy subjects, indicating a biological variation of CV about 14% [13, 14], an analytical system offering a total reproducibility of CV <7% seems well suited for long-term monitoring of S-IGF-I [15].

By having a number of laboratories with a common quality control procedure involved in the work to establish reference ranges for an analyte, it is possible to detect and eliminate systematic analytical errors in any of the laboratories before the reference samples are analyzed.

In children and adolescents, the present study confirms previous studies showing an age-related increase in S-IGF-I levels during the prepubertal and early pubertal stages followed by a decrease in late puberty [16–19]. Peak S-IGF-I levels during puberty occurred at 14 years of age in girls and at 16 years of age in boys with slightly higher peak values in girls.

In adult subjects, our results show a steady decrease of S-IGF-I levels with age, which has already been demonstrated by other authors [20, 21]. In addition to this well-known age-dependent S-IGF-I decrease, this study strongly indicates a gender difference in the adult reference ranges with males having 6–26 µg/l higher mean S-IGF-I

values. The gender difference were smallest between 35 and 50 years of age. Moreover, the reference range for females were generally a bit broader compared to males. A possible explanation for these gender differences may be the effect of estrogen use in females which is well known to influence S-IGF-I levels [22, 23]. Nevertheless, these gender difference is small and, in consequence, the use of a common adult S-IGF-I reference range seems to be reasonable for clinical practice.

Another important finding of this study is that the mean values of some reference sample subgroups differed significantly from the total mean. This result obviously reflects the influence of several factors that could affect S-IGF-I levels, i.e. differences between populations with respect to genetic background or nutritional status, pre-analytical influences like sample collection and storage, as well as differences between laboratories. The finding underlines the fact that selection of a representative refer-

ence population is a delicate task and a big sample size, collected from different sources, reduce the risk of a non-desirable impact from a single or few subpopulations.

In conclusion, the present study establishes age- and sex-specific S-IGF-I reference values for a fully automated immunoassay system based on a large population of healthy subjects. It also demonstrates that the reference values may be used for Nichols Advantage systems in different laboratories provided that the bias between systems is low. To achieve this, the same calibrators should be used in the different systems and an efficient intra- and inter-laboratory control procedure should be established.

Acknowledgements

The authors thank Prof. Berdel and Dr. Fössing, Department of Pediatrics, Marienhospital Wesel, Germany, for providing serum samples from healthy children.

References

- 1 Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: Summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency. *J Clin Endocrinol Metab* 1998;83:379–381.
- 2 Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: Summary statement of the GH Research Society. *GH Research Society. J Clin Endocrinol Metab* 2000;85:3990–3993.
- 3 Hilding A, Hall K, Wivall-Helleryd IL, Saaf M, Melin AL, Thoren M: Serum levels of insulin-like growth factor I in 152 patients with growth hormone deficiency, aged 19–82 years, in relation to those in healthy subjects. *J Clin Endocrinol Metab* 1999;84:2013–2019.
- 4 Drake WM, Howell SJ, Monson JP, Shalet SM: Optimizing GH therapy in adults and children. *Endocr Rev* 2001;22:425–450.
- 5 Peacey SR, Shalet SM: Insulin-like growth factor I measurement in diagnosis and management of acromegaly. *Ann Clin Biochem* 2001;38:297–303.
- 6 Leung KC, Ho KK: Measurement of growth hormone, insulin-like growth factor I and their binding proteins: The clinical aspects. *Clin Chim Acta* 2001;313:119–123.
- 7 Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M: Plasma insulin-like growth factor-I and prostate cancer risk: A prospective study. *Science* 1998;279:563–566.
- 8 Burroughs KD, Dunn SE, Barrett JC, Taylor JA: Insulin-like growth factor-I: A key regulator of human cancer risk? *J Natl Cancer Inst* 1999;91:579–581.
- 9 Bang P: Valid measurements of total IGF concentrations in biological fluids: Recommendations from the 3rd International Symposium on Insulin-Like Growth Factors. *Eur J Endocrinol* 1995;132:338–339.
- 10 Ranke MB, Feldt-Rasmussen U, Bang P, Baxter RC, Camacho-Hubner C, Clemmons DR, Juul A, Orskov H, Strasburger CJ: How should insulin-like growth factor I be measured? A consensus statement. *Horm Res* 2001;55:106–109.
- 11 Chestnut RE, Quarmby V: Evaluation of total IGF-I assay methods using samples from Type I and Type II diabetic patients. *J Immunol Methods* 2002;259:11–24.
- 12 Armitage P, Berry G, Matthews JNS: *Statistical Methods in Medical Research*, ed 4. Oxford, Blackwell Science, 2002.
- 13 De Boer H, van der Veen E: Guidelines for optimizing growth hormone replacement therapy in adults. *Horm Res* 1997;48:21–30.
- 14 Gelerander L, Blum WF, Larsson L, Rosberg S, Albertsson-Wikland K: Monthly measurements of insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in healthy prepubertal children: Characterization and relationship with growth. The 1-year growth study. *Pediatr Res* 1999;45:377–383.
- 15 Fraser CG: General strategies to set quality specifications for reliability performance characteristics. *Scand J Clin Lab Invest* 1999;59:487–490.
- 16 Juul A, Holm K, Kastrup KW, Pedersen SA, Michaelsen KF, Scheike T, Rasmussen S, Muller J, Skakkebaek NE: Free insulin-like growth factor I serum levels in 1,430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. *J Clin Endocrinol Metab* 1997;82:2497–2502.
- 17 Andrade Olivie MA, Garcia-Mayor RV, Gonzalez Leston D, Rodriguez Sousa T, Segura Dominguez A, Alvarez-Novoa R, Antelo Corti-
- 18 Lofqvist C, Andersson E, Gelerander L, Rosberg S, Blum WF, Albertsson-Wikland K: Reference values for IGF-I throughout childhood and adolescence: A model that accounts simultaneously for the effect of gender, age and puberty. *J Clin Endocrinol Metab* 2001;86:5870–5876.
- 19 Silbergeld A, Litwin A, Bruchis S, Varsano I, Laron Z: Insulin-like growth factor I in healthy children, adolescents and adults as determined by a radioimmunoassay specific for the synthetic 53–70 peptide region. *Clin Endocrinol (Oxf)* 1986;25:67–74.
- 20 Strasburger CJ, Bidlingmaier M, Wu Z, Morrison KM: Normal values of insulin-like growth factor I and their clinical utility in adults. *Horm Res* 2001;55:100–105.
- 21 Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, Cappa M, Bartolotta E, Dammacco F, Camanni F: Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. *J Clin Endocrinol Metab* 1996;81:3323–3327.
- 22 Anderson SM, Wideman L, Patrie JT, Weltman A, Bowers CY, Veldhuis JD: E₂ supplementation selectively relieves GH's autonegative feedback on GH-releasing peptide-2-stimulated GH secretion. *J Clin Endocrinol Metab* 2001;86:5904–5911.
- 23 O'Sullivan AJ, Crampton LJ, Freund J, Ho KK: The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. *J Clin Invest* 1998;102:1035–1040.