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Differences in the Distribution of IGF-I Concentrations Between European and US Populations

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Abstract

Context: Method-specific reference intervals (RIs) determine utility of IGF-I as a biomarker in GH-related diseases. Differences between populations might affect applicability of RIs.

Objective: To compare population-specific RIs derived from IGF-I routine testing in laboratories in the United States and Europe using the same assay.

Design and setting: Uncensored routine IGF-I testing results generated over 5 years in 4 accredited laboratories (US, n = 778 173 males/710 752 females; Europe, n = 23 220 males/40 183 females).

Main outcome measures: Construction of RIs by indirect statistical methods designed to use routine testing data (modified Hoffmann approach). Comparison to published RIs, between the US and Europe, and between regions in the United States with lower and higher mean body mass indexes (BMIs).

Results: Lower limits (LLs) of RIs calculated from all routine data sets do not differ from the published LLs. The same is true for upper limits (ULs) calculated from European routine data. ULs derived from US routine data are significantly higher (children, 10-18 years [mean, %]: boys + 149.3 ng/mL [+34.6%]; girls + 94.9 ng/mL [+19.8%]); adults (19-95 years: males + 45 ng/mL [+20.3%]; and females + 29.7 ng/mL [+13.8%]). Average IGF-I is higher in samples from Colorado (lower mean BMI) compared with Alabama (*P* < 0.0001), although the difference is smaller than between each of them and Europe.

Conclusions: We provide evidence that in large datasets from the same population, direct sampling and the indirect Hoffmann approach provide comparable RIs. Although LLs are comparable between Europe and the United States, the UL is significantly higher in the United States. We suggest use of adapted RIs for the United States.

Key words: Hoffmann approach, growth hormone deficiency, acromegaly, nutrition

Abbreviations: BMI, body mass index; IGFBP, IGF-binding protein; LL, lower limit; RI, reference interval; UL, upper limit; ULN, upper limit of normal

IGF-I is the most widely used biomarker in the diagnosis and management of patients with GH excess (acromegaly) or GH deficiency [1-3]. Accurate reference intervals (RIs) play a crucial role for interpretation and determine its clinical utility [4]. IGF-I has a strong association with age. Concentrations are low in early childhood, followed by a steep increase with puberty, and a steady decline throughout later life. The non-Gaussian distribution of values in a healthy population in all age groups requires thorough statistical analysis for assignment of accurate RIs. Therefore, scientific societies called for appropriately sized cohorts to establish robust RIs [5]. Unfortunately, there is significant inter-method variability among IGF-I assays, mainly arising from calibration against different IGF-I preparations and different methods used to remove interference from IGF-binding proteins (IGFBPs) [6]. Thus, to allow correct patient classification and long-term monitoring of disease status, RIs must be method specific [5].

Following the consensus guidelines, we previously published a study reporting RIs for the IDS iSYS IGF-I assay (IDS-iSYS, Immunodiagnostic Systems, Boldon, UK) derived through analysis of an extensive normative data set (n = 15 000) of healthy subjects of all ages [7]. Today, clinical guidelines [8-10], numerous reports from clinical studies [11-15], and reference laboratories worldwide refer to these RIs.

In addition to age, physiological concentrations of IGF-I are influenced by sex, body mass index (BMI), nutritional status (including vitamin D status), and disease states not directly related to GH secretion [16-19]. Conflicting data currently exist as to the extent to which ethnicity influences IGF-I concentrations [20]. Some studies showed lower IGF-I in Blacks and Hispanics relative to Whites [21-23], whereas no differences were seen in Hispanics vs White males elsewhere [24]. In contrast, in females, lower IGF-I has been documented in Hispanics [24] and higher levels demonstrated in Blacks

[25], whereas levels were similar in Asians and Europeans in another study [26]. In a cohort of 1085 elite athletes, a study reported [27] that IGF-I was lower in Afro-Caribbean males and females, and somewhat higher in Asian females compared with White Europeans, but no differences were observed between other ethnic groups. To our knowledge, there is no larger published study comparing levels of IGF-I in US vs European populations with inclusion of children and adults of all ages. Dietary intake is speculated to explain the differences in concentrations of IGF-I between ethnic groups, although the literature is conflicting in this field [28, 29]. The association between IGF-I and dietary intake, specifically the association of protein deprivation with decreased IGF-I, is well-documented [30-33]. Additional evidence from studies in healthy well-nourished individuals suggests that total energy consumption and, in particular, increased consumption of red meat [34, 35] or total dairy and milk [35-37] are associated with increased concentrations of IGF-I. In this context, there has been discussion as to whether IGF-I in cow's milk can be absorbed in the human gut and increase concentrations or whether a component of milk itself stimulates endogenous production [36]. Most studies so far discussing ethnicity or dietary intake in relation to IGF-I are limited by the relatively small size of the cohorts and remain silent about translation of observations to population-specific RIs. Because reports regarding systematic differences in IGF-I between populations have been vague at best, it was generally believed that its distribution might be similar worldwide, and any differences perhaps would be small in view of the overall biological variability of IGF-I, and most likely not clinically relevant. Our previously mentioned RI study [7] included an unprecedented number of samples from various geographical regions, and we also considered our RIs applicable to European and other populations when measured by the IDS-iSYS IGF-I immunoassay. However, we acknowledge that certain variables such as caloric intake and macronutrient composition of the diet were not available and thus not considered. Such variables are known to differ between Europeans and citizens of the United States [38-40], and thus might be a cause of divergence in the distribution of IGF-I concentrations and might have escaped detection in previous studies.

Systematic evaluation of subtle, but potentially significant, differences in the distribution of biochemical variables in different populations requires extremely large data sets, which are difficult to obtain from clinical studies with precise characterization of disease-free individuals. Therefore, in recent years, the concept of using large sets of data obtained during routine analysis in reference laboratories became popular in laboratory medicine. It is now generally accepted that, provided the datasets are of sufficient size, such studies can be helpful to evaluate applicability of RIs to a specific population. Moreover, statistical approaches like the Hoffmann approach have been developed to allow construction of RIs from such data sets. This is generally referred to as an "indirect method" of RI calculation, and such a technique was recently discussed and recommended for broader application in routine laboratory practices by the International Federation of Clinical Chemistry and Laboratory Medicine committee on reference intervals and decision limits [41].

Following this recommendation, in the present study, we analyzed the distribution of IGF-I levels in an extremely large number of samples (>1.4 million) submitted for routine testing to several accredited laboratories in the United States and Europe over several years, all using the IDS-iSYS assay. Extensive internal and external quality control procedures and cross-correlation studies between laboratories were implemented to ensure any findings would not have been caused by analytical variability. Our aims were to analyze the agreement between our originally published RIs and RIs derived from the indirect method for the United States and Europe, and to evaluate the potential need for adaptation of the RIs for specific populations.

Methods and Subjects

Measurement of IGF-I

All participating laboratories used the IDS-iSYS IGF-I assay to measure serum concentrations of IGF-I, as described in detail previously [7]. The functional sensitivity of the assay is 8 ng/mL and the within- and between-assay coefficients of variation are ≤ 2.9% and ≤ 7.2%, respectively. The cross-reactivity of the assay to IGF-II, insulin, and proinsulin is < 0.01%. Before starting the study, fundamental performance data were verified by all laboratories. During the study, European laboratories regularly participated in the same external quality assessment schemes to ensure comparability of analytical performance. Furthermore, a continuous cross-correlation study was performed between a European (LMU Munich) and the US laboratory with parallel assessment of sample sets every 2 to 6 months. Results of the cross-correlation study are provided as supplemental materials to this article (see Supplemental Figure 1 [42]).

Participating Laboratories and Samples Included in the Study

We retrospectively reviewed data (IGF-I, sex, age) of pediatric, adolescent, and adult males and females for whom IGF-I was requested over the past 7 years in 4 different laboratories. This included 1 nationwide reference laboratory in the United States (n = 778 173 males and n = 710 752 females), and 3 European laboratories in Germany and Belgium (n = 23 220 males and 40 183 females; see Supplemental Tables 1 and 2 for a detailed breakdown of numbers in each cohort/age group [42]). With a few theoretical exceptions (subjects from 1 geographical region were tested occasionally while travelling to the other geographical region), all samples analyzed by the European laboratories were from Europe, and those analyzed by the US laboratory were from the United States. Within the United States, the state of origin was also known, which enabled us to use a subset of US samples to compare IGF-I between regions with lower (Colorado; n = 12 136 males and 8652 females) and higher (Alabama; n = 36 490 males and 34 359 females) mean BMI. Each site's institutional review board approved the use of the anonymized, uncensored results from routine samples together with information on age and sex for the study.

Statistics

IGF-I values from the data sets were compared using MedCalc Software (version 12.3.0.0, MedCalc Software bvba, Ostend, Belgium). Comparison of means and statistical significance between Colorado/Alabama and the EU across age groups was calculated using GraphPad Prism

v8.4.1 (unpaired *t* test, *P* < 0.05 was considered statistically significant). Using a modified Hoffmann approach published previously [43], RIs adjusted for age and sex were calculated from the sets of routine data from United States and European laboratories separately. The RIs including RIs from this indirect approach were then compared with the originally published RIs (n = 6697 males, 8317 females) where adults were only of European origin, and the RIs were established using a modification of the LMS method [44]. For the purpose of calculation of RIs, age was defined in 1-year intervals up to the age of 20 years, and in 5-year intervals thereafter (X years 0 months 0 days old – X years 11 months 31 days old = X years old). Mean concentrations of IGF-I between geographical regions, age groups, or sexes were compared using unpaired *t* test, and *P* < 0.05 was considered statistically significant.

Results

Mean IGF-I values for all pediatric, adolescent, and adult male and female cohorts, including ± 2 SD for the previously published dataset and 95% CI for the new European and US cohorts, are shown in detail in Supplemental Tables 1 and 2 [42]).

Comparison of RIs from the original publication to routine data from European laboratories

Lower limits (LLs) and upper limits (ULs) of RIs calculated from the routine results from European laboratories were almost superimposable to those from the original publication (Fig. 1A and 1B), particularly in adults. Some differences were observed in young boys, but for all groups with sufficient n for statistical evaluation, comparison of the UL and LL of European data from routine analyses revealed no statistically significant differences from the originally published central 95% interval obtained by the LMS method.

Comparison of RIs from the original publication, European laboratories, and the US cohort

LLs of RIs calculated from routine results were not statistically different to LLs from the original publication for all ages and sexes, regardless of whether the IGF-I results were from Europe or the United States. However, a striking difference was observed in calculated ULs from data of European and US origin (Fig. 2A and 2B). For ages 10 through 18 years, the calculated UL was on average 149.3 ng/mL (34.6%) higher in boys and 94.9 ng/mL (19.8%) higher in girls from the United States compared with Europe. In adults (19-95 years), the
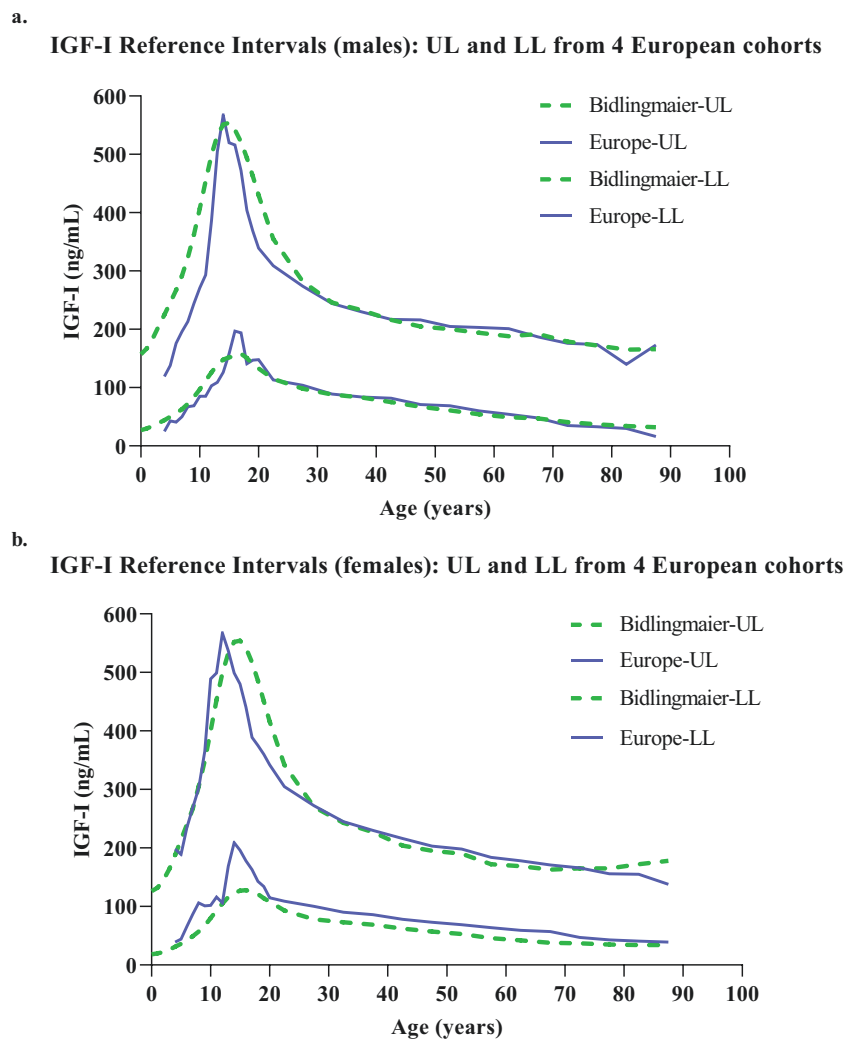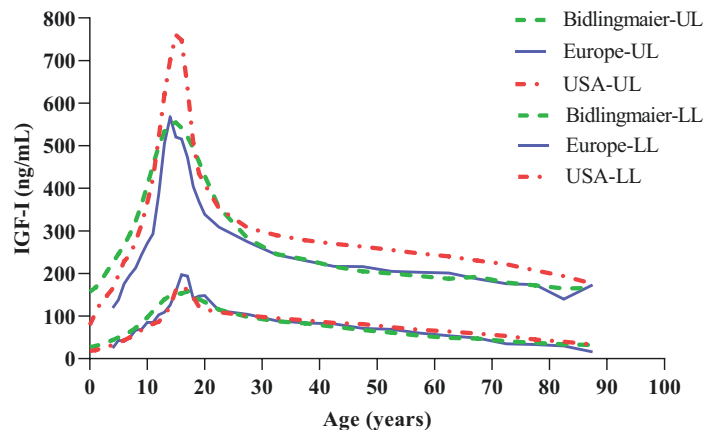
a.

IGF-I Reference Intervals (males): UL and LL from 4 European cohorts

b.

IGF-I Reference Intervals (females): UL and LL from 4 European cohorts

Figure 1. IGF-I reference intervals as seen in (A) males and (B) females of the original study in a population based setting (Bidlingmaier, LMS method) and the routine data from 3 European laboratories from Germany and Belgium (Europe, Hoffmann approach), showing upper (UL) and lower (LL) limits.

a.

IGF-I Reference Intervals (males): UL and LL from 4 European and 1 US cohort

b.

IGF-I Reference Intervals (females): UL and LL from 4 European and 1 US cohort
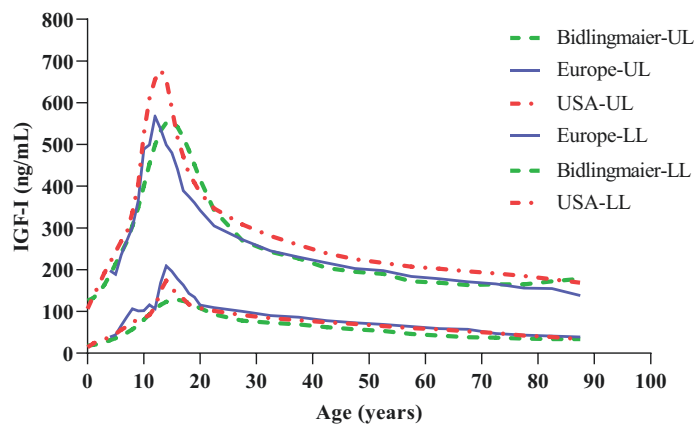

Figure 2. IGF-I reference intervals as seen in (A) males and (B) females of the original study in a population based setting (Bidlingmaier, LMS method), the routine data from 3 European laboratoriess from Germany and Belgium (Europe, Hoffmann approach), and the US laboratory (LabCorp, Hoffmann approach) showing upper (UL) and lower (LL) limits.

calculated UL was on average 45 ng/mL (20.3%) higher in males and 29.7 ng/mL (13.8%) in females from the United States. Overall, from both the EU and the US datasets, peak IGF-I values occurred earlier in girls than in boys, which might be explained by the earlier onset of puberty in females than males and as seen in other studies [16, 45].

Impact of BMI

Data regarding the height and weight of patients are not commonly available to reference laboratories. However, epidemiological studies have revealed that the mean BMI within the United States is higher in Alabama than in Colorado [46]. It is also well established that mean BMI is generally higher in the United States compared with Europe. In the large dataset from routine samples analyzed by the US laboratory, mean IGF-I was significantly higher in samples from Colorado (with lower mean BMI) than in Alabama across all age and sex groups of pediatric, adolescent, and adult male and female subjects (Fig. 3) (Supplemental Table 3 and 4 [42]). In contrast, mean IGF-I was lower in the adult cohorts from Europe (with lower mean BMI) compared with the US cohorts. The difference in IGF-I between the 2 states in the United States was smaller, however, than the difference in IGF-I between

each of them and the European cohorts. Notably, mean age was not significantly different between all cohorts. An illustration as seen in adult males and females aged 31 to 75 years is shown in Fig. 4A-D.

Discussion

In the present study, we provide evidence of a significant difference in the UL of IGF-I RIs established in US and European cohorts, whereas the LL is not different. It was possible to observe the discrepancy between the RIs in large part because of the extensive datasets used (>1.4 million subjects). We also provide evidence that the indirect Hoffmann algorithms we applied to the statistical analysis in the same background population provide comparable RI limits and can accurately depict the distribution of IGF-I values in disease-free populations.

Measurements of IGF-I are known to be fraught with pitfalls [5], and comparability of results between laboratories [47] or across assay batches [48] can be poor. For our study, all laboratories used the same, thoroughly validated IGF-I assay [7]. In addition, we implemented a continuous cross-correlation study among a European and the US laboratory to

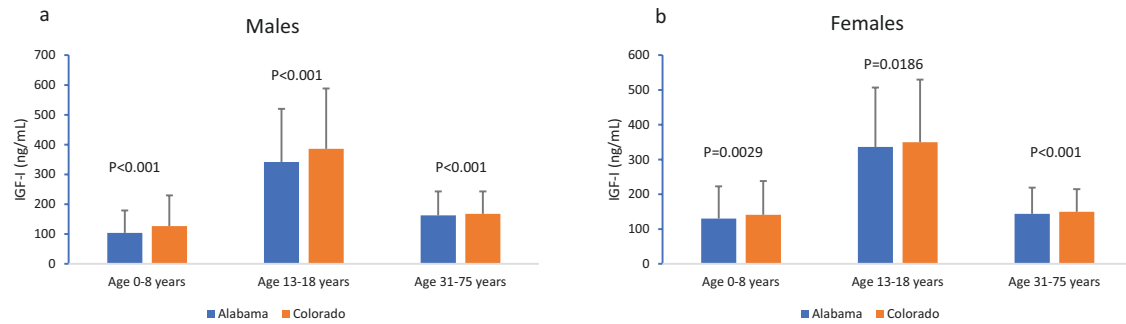IGF-I in a low (Colorado) vs. high (Alabama) BMI region

Figure 3. Mean IGF-I concentrations as seen in 2 regions of the United States, 1 state with lower (Colorado) and 1 with higher (Alabama) mean body mass index (BMI) in pediatric, adolescent, and adult males (A) and females (B).

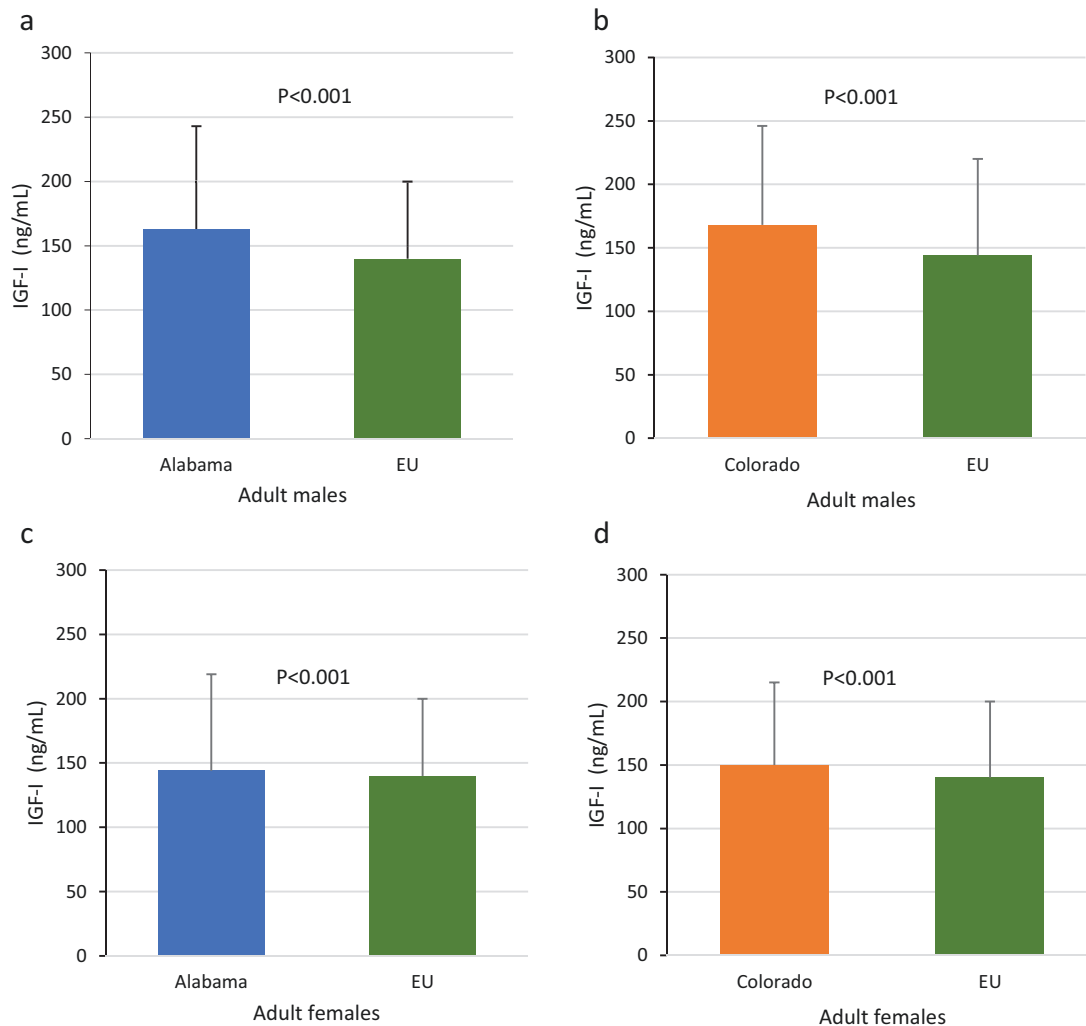IGF-I Colorado and Alabama vs. the EU

Figure 4. Mean IGF-I concentrations as seen in the 2 states of Alabama and Colorado compared with European adults of identical age. Data for males (A, B) and females (C, D) are shown.

document comparability of results obtained across laboratories and across assay batches (see supplemental materials [42]). This stringent cross-correlation studies and continuous monitoring performed over the years when the measurements took place revealed extremely robust performance (Passing-Bablok analysis between laboratories: y = 1.921 + 0.9886 × x,

$r^2 = 0.991$) and allows us to rule out our finding were significantly affected by technical pitfalls associated with the assay.

In this study, routine data from three European laboratories in Germany and Belgium were used to confirm appropriateness of the IGF-I RIs, both at the upper and lower limits, identified in our original publication [7]. The adult population

used to establish the originally published reference intervals almost exclusively consisted of European subjects; thus, we were unable previously to compare RIs between adult populations from Europe and the United States. A major strength of this study is our ability to compare RIs from large datasets of populations of adults and children from different regions of the globe. Our dataset included > 800 000 male and 750 000 female samples in total, making it not only unique in size, but also the only recent study to directly compare IGF-I RIs between the United States and Europe. Although the LL of the RI of IGF-I was consistent across populations and with our previous study [7], there was a striking and potentially clinically relevant difference between Europe and the United States at the UL of the reference intervals.

The difference in the UL of IGF-I between European and US populations is intriguing, and there are several potential explanations that could be explored. An inherent limitation of studies using results from routine analyses is the lack of detailed clinical and anthropometric information. Although this is largely irrelevant for the calculation of RIs in sufficiently large cohorts, it makes it challenging if not impossible to establish a clear cause of the observed difference in the ULs of the normal distribution of IGF-I between European and US populations. Nevertheless, possible explanations for the observed data include BMI/nutritional status, time of peak puberty, environmental factors, and differing ethnicities of the populations.

Nutritional factors could also play a role in the differences in IGF-I RIs observed between US and European populations. For example, increased vitamin D levels are associated with elevated IGF-I levels. The prevalence rates of severe vitamin D deficiency are lower in the United States, potentially explaining the higher UL of the IGF-I RIs compared with European populations [40, 49]. In addition, the link between IGF-I and BMI and obesity is complex, but important [50]. Although some studies have revealed that IGF-I levels are high [51] or are unchanged [52] in obesity, several population-based studies have suggested an inverse relationship between BMI and IGF-I, whereby IGF-I tends to decrease with increasing BMI and IGF-I levels decrease with obesity [18, 53]. Close analysis of the data suggest that BMI does not explain the difference between the UL of the RI between EU and the United States because, although BMI is lower in Europe than in the United States [54], the UL of the RI also is lower. In addition, that we had generalized demographic information for the otherwise anonymized samples tested in the US cohort allowed us to perform a subanalysis by state. Thus, we attempted to indirectly evaluate the potential impact of BMI on the UL of RIs by comparing IGF-I results from states with known differences in BMI: Colorado (with the lowest mean BMI in the United States) and Alabama (with the one of the highest mean BMIs) [46]. Comparison of the data from these states revealed an inverse relationship of BMI to the calculated UL of the RI for IGF-I, whereby higher mean levels were observed in Colorado compared with Alabama. Although this finding is consistent with findings indicating IGF-I tends to decrease with increasing BMI [18, 53], the overall impact of BMI seems to be small because the difference in mean IGF-I between Colorado and Alabama was smaller than the difference between European and the US cohorts. We speculate that a more likely explanation is differences in protein intake or another nutritional factor such as processing or additives; however, because of the nature of our study and the data available, this cannot be investigated.

Another possible explanation for the differences between European and US IGF-I levels is puberty. It is well-established that the onset of puberty is generally earlier in the United States compared with Europe [55], and our data indicate an earlier and higher peak IGF-I from the age of likely puberty onwards in both males and females. Although we are able to analyze the data by age, we are unable to compare with pubertal stage because Tanner staging is not available for the subjects in our cohort.

Our study also provides evidence that in sufficiently large datasets from the same population background, both direct sampling (as in [7]) and the indirect Hoffmann algorithms used here provide statistically comparable RI limits and may be considered accurate representation of results distribution in disease-free populations [43, 56, 57]. Although direct sampling is frequently used to generate RIs, the indirect approach performs analysis of results generated as part of routine pathology testing followed by appropriate statistical techniques to determine RIs. Indirect approaches are faster, cheaper, and do not involve patient inconvenience, discomfort, or the risks associated with generating new patient health information. In addition, they use the same preanalytical and analytical techniques used for patient management and can provide very large numbers for assessment. In view of these advantages, a working group of the International Federation of Clinical Chemistry Committee on Reference Intervals and Decision Limits recently published a statement encouraging the use of indirect methods to establish and verify reference intervals [41].

Although our observations have important clinical implications, there are some limitations that must be acknowledged. Most importantly, we are unable to pinpoint the cause of the discrepancy in IGF-I level because of the absence of demographic information and data regarding the height, BMI, and underlying disease states of the individual participants. Although we also acknowledge that theoretical limitations of the indirect method include possible effects of diseased subpopulations on the derived interval, the similarity of the original RIs from the direct method to those obtained by the indirect method suggest that such effects might be small. This most likely reflects the rarity of GH-related diseases in samples submitted to routine testing.

In terms of clinical implications, our data indicate that the main difference between the US population and a European population regarding the distribution of IGF-I concentrations is the higher UL of the RI in the United States. In clinical practice, this is mainly important for the diagnosis and management of patients with acromegaly. Although the difference of 15% to 20% might be too small to complicate the diagnosis of active acromegaly, where IGF-I is significantly elevated in most cases, it can have important implications for the definition of control of the disease with treatment. As an example, an IGF-I concentration of 260 ng/mL in a 50-year-old male is more than 1.2 times the UL of normal based on the originally published and European RIs from this study but would be considered normal (<1.0× ULN) if the US RIs from this study were applied. It is noteworthy that clinical studies and guidelines are highly heterogeneous with respect to criteria used to define biochemical control of the disease, and cut-offs used for IGF-I range from 1.0× ULN to < 1.5× ULN

[58]. In part, this might reflect the uncertainty associated with applicability of RIs to different populations. Our data suggest subtle, though significant differences in the ULN between European and US populations. Adjustment of our previously published RIs to those derived by the Hoffmann approach in this study might better reflect the normal distribution of IGF-I in the United States.

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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request. Ethical and legal restrictions might apply, such as their containing information that could compromise the privacy of research participants.

References

1. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM, 2007 ISS Consensus Workshop participants. 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. *The Journal of clinical endocrinology and metabolism*. 2008;93(11):4210-7. 10.1210/jc.2008-0509

2. Melmed S, Bronstein MD, Chanson P, et al. A Consensus Statement on acromegaly therapeutic outcomes. *Nat Rev Endocrinol*. 2018;14(9):552-561. doi:10.1038/s41574-018-0058-5.

3. Ho KK; 2007 GH Deficiency Consensus Workshop Participants. 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(6):695-700. doi:10.1530/EJE-07-0631.

4. Frystyk J, Freda P, Clemmons DR. The current status of IGF-I assays – A 2009 update. *Growth Hormone & IGF Research* 2010;20(1):8-18.

5. Clemmons DR. Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. *Clin Chem*. 2011;57(4):555-559. doi:10.1373/clinchem.2010.150631.

6. Chanson P, Arnoux A, Mavromati M, et al. Reference values for IGF-I serum concentrations: comparison of six immunoassays. *J Clin Endocrinol Metab*. 2016;101(9):3450-3458.

7. Bidlingmaier M, Friedrich N, Emeny RT, et al. Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *J Clin Endocrinol Metab*. 2014;99(5):1712-1721. doi:10.1210/jc.2013-3059.

8. Collett-Solberg PF, Ambler G, Backeljauw PF, et al. Diagnosis, genetics, and therapy of short stature in children: a Growth Hormone Research Society International Perspective. *Hormone research in paediatrics* 2019;92(1):1-14. doi:10.1159/000502231.

9. Sklar CA, Antal Z, Chemaitilly W, et al. Hypothalamic-pituitary and growth disorders in survivors of childhood cancer: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2018;103(8):2761-2784. doi:10.1210/jc.2018-01175.

10. Katznelson L, Laws ER Jr., Melmed S, et al. Acromegaly: an Endocrine Society Clinical practice guideline. *J Clin Endocrinol Metab*. 2014;99(11):3933-3951.

11. Tumati S, Burger H, Martens S, van der Schouw YT, Aleman A. Association between cognition and serum insulin-like growth factor-1 in middle-aged & older men: an 8 year follow-up study. *PLoS One*. 2016;11(4):e0154450-e0154450. doi:10.1371/journal.pone.0154450.

12. Strasburger CJ, Vanuga P, Payer J, et al. MOD-4023, a long-acting carboxy-terminal peptide-modified human growth hormone: results of a Phase 2 study in growth hormone-deficient adults. *Eur J Endocrinol*. 2017;176(3):283-294.

13. Johannsson G, Feldt-Rasmussen U, Håkonsson IH, et al. Safety and convenience of once-weekly somapacitan in adult GH deficiency: a 26-week randomized, controlled trial. *Eur J Endocrinol*. 2018;178(5):491-499.

14. Sävendahl L, Battelino T, Brod M, et al. Once-weekly somapacitan vs daily GH in children with GH deficiency: results from a randomized phase 2 trial. *J Clin Endocrinol Metab*. 2020;105(4):e1847-e1861.

15. Rasmussen MH, Janukonyté J, Klose M, et al. Reversible albumin-binding GH possesses a potential once-weekly treatment profile in adult growth hormone deficiency. *J Clin Endocrinol Metab*. 2016;101(3):988-998. doi:10.1210/jc.2015-1991.

16. Brabant G, Wallaschofski H. Normal levels of serum IGF-I: determinants and validity of current reference ranges. *Pituitary* 2007;10(2):129-133. doi:10.1007/s11102-007-0035-9.

17. Nguyen TV, Nelson AE, Howe CJ, et al. Within-subject variability and analytic imprecision of insulinlike growth factor axis and collagen markers: implications for clinical diagnosis and doping tests. *Clin Chem*. 2008;54(8):1268-1276. doi:10.1373/clinchem.2008.105726.

18. Hawkes CP, Grimberg A. Insulin-like growth factor-i is a marker for the nutritional state. *Pediatric endocrinology reviews: PER* 2015;13(2):499-511.

19. Kord-Varkaneh H, Rinaldi G, Hekmatdoost A, et al. The influence of vitamin D supplementation on IGF-1 levels in humans: a systematic review and meta-analysis. *Ageing Res Rev*. 2020;57:100996. doi:10.1016/j.arr.2019.100996.

20. Bagg W, Aoina J, Cross PA, et al. Serum IGF-I levels are similar in Samoan, Maori and European populations despite differences in body composition. *Growth Horm. IGF Res*. 2006;16(1):57-60.

21. Gapstur SM, Kopp P, Chiu BC, Gann PH, Colangelo LA, Liu K. Longitudinal associations of age, anthropometric and lifestyle factors with serum total insulin-like growth factor-I and IGF binding protein-3 levels in Black and White men: the CARDIA Male Hormone Study. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2004;13(12):2208-2216.

22. Platz EA, Pollak MN, Rimm EB, et al. Racial variation in insulin-like growth factor-1 and binding protein-3 concentrations in middle-aged men. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 1999;8(12):1107-1110.

23. McGreevy K, Hoel B, Lipsitz S, Bissada N, Hoel D. Racial and anthropometric differences in plasma levels of insulin-like growth factor I and insulin-like growth factor binding protein-3. *Urology* 2005;66(3):587-592. doi:10.1016/j.urology.2005.03.070.

24. DeLellis K, Rinaldi S, Kaaks RJ, Kolonel LN, Henderson B, Le Marchand L. Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): The Multiethnic Cohort. *Cancer Epidemiol. Biomark. Prev.* 2004;13(9):1444-1451.

25. Fowke JH, Matthews CE, Yu H, *et al*. Racial differences in the association between body mass index and serum IGF1, IGF2, and IGFBP3. *Endocr Relat Cancer.* 2010;17(1):51-60. doi:10.1677/ERC-09-0023.

26. Maskarinec G, Williams AE, Kaaks R. A cross-sectional investigation of breast density and insulin-like growth factor I. *Int J Cancer.* 2003;107(6):991-996. doi:10.1002/ijc.11505.

27. Erotokritou-Mulligan I, Bassett EE, Cowan DA, *et al*. Influence of ethnicity on IGF-I and procollagen III peptide (P-III-P) in elite athletes and its effect on the ability to detect GH abuse. *Clin. Endocrinol.* 2009;70(1):161-168.

28. 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(2):146-155. doi:10.1016/j.ghir.2008.08.005.

29. Heald AH, Cade JE, Cruickshank JK, Anderson S, White A, Gibson JM. The influence of dietary intake on the insulin-like growth factor (IGF) system across three ethnic groups: a population-based study. *Public Health Nutr.* 2003;6(2):175-180. doi:10.1079/PHN2002414.

30. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev.* 1994;15(1):80-101. doi:10.1210/edrv-15-1-80.

31. Underwood LE, Thissen JP, Lemozy S, Ketelslegers JM, Clemmons DR. Hormonal and nutritional regulation of IGF-I and its binding proteins. *Horm Res.* 1994;42(4-5):145-151. doi:10.1159/000184187.

32. Isley WL, Underwood LE, Clemmons DR. Dietary components that regulate serum somatomedin-C concentrations in humans. *J Clin Invest.* 1983;71(2):175-182. doi:10.1172/jci110757.

33. Isley WL, Underwood LE, Clemmons DR. Changes in plasma somatomedin-C in response to ingestion of diets with variable protein and energy content. *JPEN J Parenter Enteral Nutr.* 1984;8(4):407-411. doi:10.1177/0148607184008004407.

34. Kaklamani VG, Linos A, Kaklamani E, Markaki I, Koumantaki Y, Mantzoros CS. Dietary fat and carbohydrates are independently associated with circulating insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 concentrations in healthy adults. *J. Clin. Oncol.* 1999;17(10):3291-3298.

35. Larsson SC, Wolk K, Brismar K, Wolk A. Association of diet with serum insulin-like growth factor I in middle-aged and elderly men. *Am J Clin Nutr.* 2005;81(5):1163-1167. doi:10.1093/ajcn/81.5.1163.

36. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2002;11(9):852-861.

37. Hoppe C, Molgaard C, Juul A, Michaelsen KF. High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. *Eur J Clin Nutr.* 2004;58(9):1211-1216.

38. Lieberman HR, Fulgoni VL, III, Agarwal S, Pasiakos SM, Berryman CE. Protein intake is more stable than carbohydrate or fat intake across various US demographic groups and international populations. *Am J Clin Nutr.* 2020;112(1):180-186.

39. Wittig F, Hummel E, Wenzler G, Heuer T. Energy and macronutrient intake over the course of the day of German adults: A DEDIPAC-study. *Appetite* 2017;114:125-136. doi:10.1016/j.appet.2017.03.018.

40. Amrein K, Scherkl M, Hoffmann M, *et al*. Vitamin D deficiency 2.0: an update on the current status worldwide. *Eur J Clin Nutr.* 2020;74(11):1498-1513. doi:10.1038/s41430-020-0558-y.

41. Jones GRD, Haeckel R, Loh TP, *et al*; Intervals obotICoR. Limits D. Indirect methods for reference interval determination – review and recommendations. *Clinical Chemistry and Laboratory Medicine (CCLM).* 2019;57(1):20-29.

42. Bidlingmaier M, Valcour A, Schilbach K, Kuehnle T, Diederich S, Rogge T, Cavalier E, Katayev A. *Bidlingmaier_Differences in IGF-I between EU and US populations_supplemental materials.pdf*. figshare; 2021:Online resource. doi:10.6084/m9.figshare.17138375.v4.

43. Katayev A, Fleming JK, Luo D, Fisher AH, Sharp TM. Reference intervals data mining: no longer a probability paper method. *Am J Clin Pathol.* 2015;143(1):134-142. doi:10.1309/AJCPQPRNIB54WFKJ.

44. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med.* 1992;11(10):1305-1319. doi:10.1002/sim.4780111005.

45. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth hormone & IGF research: official journal of the Growth Hormone Research Society and the International IGF Research Society* 2003;13(4):113-170.

46. CDC. https://www.cdc.gov/obesity/data/prevalence-maps.html.

47. Pokrajac A, Wark G, Ellis AR, Wear J, Wieringa GE, Trainer PJ. Variation in GH and IGF-I assays limits the applicability of international consensus criteria to local practice. *Clinical endocrinology* 2007;67(1):65-70. doi:10.1111/j.1365-2265.2007.02836.x.

48. Algeciras-Schimnich A, Bruns DE, Boyd JC, Bryant SC, La Fortune KA, Grebe SK. Failure of current laboratory protocols to detect lot-to-lot reagent differences: findings and possible solutions. *Clin Chem.* 2013;59(8):1187-1194. doi:10.1373/clinchem.2013.205070.

49. Ameri P, Giusti A, Boschetti M, *et al*. Vitamin D increases circulating IGF1 in adults: potential implication for the treatment of GH deficiency. *Eur J Endocrinol.* 2013;169(6):767-772. doi:10.1530/EJE-13-0510.

50. Lewitt MS, Dent MS, Hall K. The insulin-like growth factor system in obesity, insulin resistance and type 2 diabetes mellitus. *Clin. Med.* 2014;3(4):1561-1574. doi:10.3390/jcm3041561.

51. Frystyk J, Vestbo E, Skjaerbaek C, Mogensen CE, Orskov H. Free insulin-like growth factors in human obesity. *Metab Clin Exp.* 1995;44(10 Suppl 4):37-44. doi:10.1016/0026-0495(95)90219-8.

52. Ricart W, Fernandez-Real JM. No decrease in free IGF-I with increasing insulin in obesity-related insulin resistance. *Obesity research* 2001;9(10):631-636. doi:10.1038/oby.2001.83.

53. Maccario M, Ramunni J, Oleandri SE, *et al*. Relationships between IGF-I and age, gender, body mass, fat distribution, metabolic and hormonal variables in obese patients. *Int. J. Obes.* 1999;23(6):612-618.

54. Janssen F, Bardoutsos A, Vidra N. Obesity prevalence in the long-term future in 18 European Countries and in the USA. *Obesity facts* 2020;13(5):514-527. doi:10.1159/000511023.

55. Parent A-S, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon J-P. The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev.* 2003;24(5):668-693. doi:10.1210/er.2002-0019.

56. Holmes DT, Buhr KA. Widespread incorrect implementation of the Hoffmann Method, the correct approach, and modern alternatives. *Am J Clin Pathol.* 2019;151(3):328-336. doi:10.1093/ajcp/aqy149.

57. Katayev A, Fleming JK, Holmes DT, Buhr KA. Widespread implementation of the Hoffmann Method: a second opinion. *Am J Clin Pathol.* 2019;152(1):116-117. doi:10.1093/ajcp/aqz015.

58. van Esdonk MJ, van Zutphen EJM, Roelfsema F, *et al*. How are growth hormone and insulin-like growth factor-1 reported as markers for drug effectiveness in clinical acromegaly research? A comprehensive methodologic review. *Pituitary* 2018;21(3):310-322. doi:10.1007/s11102-018-0884-4.