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Biochemical investigations in diagnosis and follow up of acromegaly

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Abstract Measurements of human growth hormone (GH) and insulin-like growth-factor I (IGF-I) are cornerstones in the diagnosis of acromegaly. Both hormones are also used as biochemical markers in the evaluation of disease activity during treatment. Management of acromegaly is particularly challenging in cases where discordant information is obtained from measurement of GH concentrations following oral glucose load and from measurement of IGF-I. While in some patients biological factors can explain the discrepancy, in many cases issues with the analytical methods seem to be responsible. Assays used by endocrine laboratories to determine concentrations of GH and IGF-I underwent significant changes during the last decades. While generally leading to more sensitive and reproducible methods, these changes also had considerable impact on absolute concentrations measured. This must be reflected by updated decision limits, cut-offs and reference intervals. Since different commercially available assays do not agree very well, method specific interpretation of GH and IGF-I concentrations is required. This complexity in the interpretation of hormone concentrations is not always appropriately reflected in laboratory reports, but also not in clinical guidelines reporting decision limits not related to a specific analytical method. The present review provides an overview about methodological and biological variables affecting the biochemical assessment of acromegaly in diagnosis and follow up.

Keywords Immunoassay · Growth hormone · Insulin-like growth-factor I · Reference intervals · Biological variability

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Introduction

Acromegaly is a rare disease, which, in more than 95% of the patients, is caused by a growth hormone (GH) secreting pituitary adenoma. Diagnosis is frequently delayed due to the gradual onset of the disease with mostly unspecific symptoms [1]. As a result, the latency from first symptoms to diagnosis usually takes 7–10 years [2–5]. In spite of benignity of nearly all tumors, acromegaly is associated with increased morbidity and mortality. In addition to the characteristic changes in physiognomy, patients suffer from a variety of comorbidities affecting quality of life and reducing lifespan [3, 6–11]. Since most of these comorbidities are not, or only partially, reversible with treatment, early diagnosis and initiation of therapy is crucial to avoid long-term complications. Furthermore, surgery of pituitary adenomas is easier and the remission rate is higher when the tumor is still small and separated from surrounding tissues [12–14]. Therefore, sensitive and specific biochemical makers of disease activity are important. According to

current internationally recognized guidelines, the glucose tolerance test (OGTT) with 75 g glucose is the gold standard for diagnosing acromegaly, and the majority of experts in the field report to use the lowest GH concentration (GH nadir) after glucose load together with IGF-I as diagnostic criteria [15–17]. However, besides the general recognition of the value of GH and IGF-I measurements, the details of the biochemical evaluation of disease activity in initial diagnosis and during the course of therapy remain controversial and challenging. This is particularly due to the biological and analytical variability associated with the biochemical markers.

Physiologically, GH is secreted by the pituitary gland (or in case of acromegaly by a somatotropic adenoma), while IGF-I is primarily secreted by the liver following GH binding to hepatic GH receptors (GHR) [18]. Biochemically, quantification of GH provides a correlate of pituitary GH secretion, while measurement of IGF-I provides a biochemical equivalent for the peripheral response of the organism to circulating GH because most of the biological effects of GH are mediated by IGF-I [19]. In other words, both biochemical markers of the somatotropic axis provide us with different information. Furthermore, although the general concept is that IGF-I concentrations reflect the integrated GH secretory capacity of the pituitary, both components of the somatotropic axis can be modified independently by specific biological factors. Therefore, from a biological point of view, discrepancies between the two are to be expected under specific clinical conditions. However, since both parameters often are being used together to biochemically define disease activity, such discrepancies can make the diagnosis and monitoring of acromegaly challenging. Beyond biology, the availability of different GH and IGF-I assays with different standardization and specificity can pose further difficulties on the biochemical investigation. This is of particular interest when comparing results from different studies with measurements from different labs, but also if analytical methods are changed by the laboratory during the course of follow up in a patient.

For this review, we searched the PubMed database for the following keywords: diagnosis of acromegaly, biochemical diagnosis acromegaly, OGTT, acromegaly, discrepancies growth hormone IGF-I, discordant growth hormone IGF-I, divergence growth hormone IGF-I, growth hormone assay, IGF-I assay. Further publications were identified through the references of the initially selected literature.

Divergence between GH and IGF-I concentrations

There is an increasing number of reports where, in patients with active acromegaly, results from GH and IGF-I measurement do not agree. Patients can present with either elevated GH but normal IGF-I, or elevated IGF-I but apparently normal GH. The incidence of discrepant findings and the potential reasons to explain the discrepancy can be different at diagnosis and during treatment of the disease.

Recently, it has been reported that in 157 treatment naïve patients with clinically active acromegaly with elevated IGF-I levels investigated between 1996 and 2016, 31% had normal 24-h mean plasma GH levels [20]. Interestingly, the year of diagnosis had an influence on the incidence of discordant findings in this study: In the more recently diagnosed cases, the percentage of patients presenting with discordant laboratory findings was higher (49%) than during the rest of the observational period, potentially indicating the need to adjust reference intervals for the assays used [20]. In 2011 the same group had reported that in 40 untreated patients with acromegaly 33% had a GH nadir below 1 ng/mL, and 18% (n = 7) had a GH nadir below 0.4 ng/mL [21]. In another group of 25 newly diagnosed and untreated patients with acromegaly, five subjects with GH-secreting pituitary macroadenomas had basal GH levels below 1 ng/mL, and the same five patients (out of 15 patients who underwent an oGTT) also had a GH nadir below 1 ng/mL [22].

Discrepant findings were also found in patients with acromegaly after initiation of therapy. In 2008, data from the Belgian acromegaly registry (AcroBel) showed that discordant GH and IGF-I values can be found in approximately 35% of non-cured patients with acromegaly [23]. Another group reported persistent elevation of IGF-I despite GH nadir concentrations below 1 ng/mL in 13 out of 75 patients with acromegaly after treatment (surgery, radiotherapy and/or medical treatment) [24]. Tumor-size does not seem to have an influence on the incidence of discordant laboratory findings, but interestingly, discordant findings seem to occur more frequently after radiotherapy [20–22, 24, 25]. Furthermore, it has been suggested that one of the variants of the GHR (d3-GHR lacking exon 3) could have an impact on the frequency of discordant findings during treatment: In a study with 84 surgically treated patients, 20% exhibited discordant IGF-I and GH values, and 71% of those patients were carriers of the GHR variant [26]. Treatment with somatostatin analogs increased the proportion of discordant values in the total cohort to 31%, and 69% of them were d3-GHR carriers [26]. On the other hand, one study suggested that the percentage of discordant laboratory findings is significantly lower in patients treated with dopamine agonists [27].

Further examples for studies reporting discordant findings from IGF-I and GH measurements in untreated and treated patients with acromegaly are presented in Table 1. It is important for clinicans to be aware of the relatively high proportion of discordant biochemical findings and to reflect this in their diagnostic and therapeutic decisions.

Table 1 Studies reporting discordant findings from IGF-I and GH measurements

Subjects (n)	Disease status	Elevated IGF-I (%)	IGF-I assay	GH assessment modality	Designated GH cut off	Normal GH (%)	GH assay	Year	References
29	Post treatment	100	RIA, Nichols	GH nadir (100 g)	<1 ng/mL	50	2-site IRMA, DSL	2001	[28]
16	Untreated	100	2-site IRMA, DSL	GH nadir (100 g)	<1 ng/mL	50	ICMA, Nichols	2002	[29]
25	Untreated	100	RIA, DSL	GH nadir (100 g)	<1 ng/mL	20	IRMA, DSL	2003	[23]
15		100		Basal GH	<1 ng/mL	33			
35	Post treatment	100	2-site IRMA DSL	GH nadir (75 g)	<1 ng/mL	40	IRMA, BIOCODE	2004	[30]
51	Untreated	96	2-site IRMA DSL	Basal GH	<2.5 ng/mL	10	ICMA, Immulite	2008	[31]
					<1 ng/mL	4			
58	Post surgery	95			<2.5 ng/mL	9			
					<1 ng/mL	9			
42	Octreotide LAR	98			<2.5 ng/mL	31			
229	Untreated	89	ICMA, Nichols	Basal GH (mean of 3 values)	<2 ng/mL	24	ICMA, Nichols	2008	[20]
84	Post treatment	100	ICMA, Immulite	Mean GH (5 measurements every 30 min)	<2 ng/mL	20	ICMA, Immulite	2009	[26]
75	Post treatment	17 (n = 13)	2-site IRMA DSL	GH nadir (75 g)	<1 ng/mL	100	IFMA, inhouse	2010	[24]
40	Untreated	100	2-site IRMA DSL	GH nadir (100 g)	<1 ng/mL	33	ICMA, Nichols	2011	[22]
					<0.4 ng/mL	18			
38	Untreated	100	2-site IRMA DSL	24 h mean plasma GH	<4.3 ng/mL	63	ICMA, Nichols	2011	[32]
		100		GH nadir (100 g)	<0.4 ng/mL	5			
33 (multiple tests)	Post treatment (medication)	36.6	Different assays	Basal GH	<3.9 ng/mL	100	different assays	2011	[33]
		10.8		GH nadir (75 g)	<1 ng/mL	100			
27	Untreated	85	Different assays	GH nadir (75 g)	Depending on assay	4	Different assays	2012	[34]
111	Post treatment (no medication)	92				22			
89	Post treatment (medication)	86				13			
22	Healthy subjects	100	ICMA, Immulite	GH nadir (75 g)	<0.4 ng/mL	18	ICMA, Immulite	2014	[35]
88 women	Untreated	0	ICMA, Immulite	GH nadir (75 g)	<0.4 ng/mL	70	ICMA, Immulite	2015	[36]
72 men	Untreated					100			
70 women	Untreated			Basal GH		20			
72 men	Untreated					54			
157	Untreated	100	Different assays	24 h mean plasma GH	<4.7 ng/mL	31	Different assays	2016	[147]

Initially, it is important to not delay diagnosis and initiation of treatment. In the monitoring of disease activity during treatment, discordant findings remain an issue, and it is of particular importance to understand the potential impact of specific therapeutic interventions on laboratory results.

Notably, discrepancies between GH and IGF-I have also been described in the absence of acromegaly: In a group of individuals with clinical suspicion of acromegaly, but normal IGF-I, 30% of women (n = 70) exhibited GH nadir concentrations >0.4 ng/mL, whereas in all men GH nadirs were below 0.4 ng/mL. In the same group, random baseline GH was above 0.4 ng/ml in 80% of the women and 46% of the men, respectively. Acromegaly was ruled out in all cases by extended biochemical testing, MRI and long term follow up [36].

Factors explaining discrepancies between GH and IGF-I

To explain discrepancies in the findings from measuring GH and IGF-I, methodological and biological factors have to be taken into account: The technical characteristics of the GH- and IGF-I assay used [37, 38], the use of different reference intervals of variable quality for interpretation of IGF-I concentrations, the different testing modalities, particularly for GH (fasted and non-fasted random GH, 8-, 12- and 24-h GH-profiles and the post glucose GH nadir) and, finally, biological confounders like comorbidities all can affect the agreement between GH and IGF-I concentrations. Furthermore, the time point of testing in relation to onset of the disease or inititation of treatment can have an influence. In this context, not only the impact of specific therapeutic interventions is important, but also the fact that the criteria to biochemically define active disease at diagnosis might differ from the criteria used to define cure after treatment.

Issues with GH assays

Before the 1990s a basal GH below 5 ng/mL was used to define cure after treatment of acromegaly [39, 40]. With the development of newer assays lower cut-off values were suggested. In the mid to late 1990s a basal GH below 2.5 ng/mL and a GH nadir below 2 ng/mL following oral glucose load were used as indication of successful treatment. Notably, already at that time some authors (using some assays) had proposed even lower cut-offs for GH during OGTT (<1 ng/mL) to define cure [41, 42]. In 2000, a consensus statement on diagnosis and treatment of acromegaly ("Cortina criteria") was published. In this statement, random GH concentrations below 0.4 ng/mL or GH nadir during OGTT below 1 ng/mL, both together with normal age- and gender-adjusted IGF-I concentration, were defined as exclusion criteria for acromegaly [43]. 10 years later, a revised consensus statement was released defining "control of disease activity" following therapeutic intervention using random GH concentrations below 1 ng/mL and GH nadir below 0.4 ng/mL (in combination with normal IGF-I). Interestingly, the most recent Endocrine Society Clinical Practice Guideline suggests the lack of suppression of GH to <1 ng/mL (together with elevated IGF-I) as a criterium for diagnosis, while a random GH <1 ng/mL (together with normal IGF-I) is suggested as a therapeutic goal [15]. In contrast, different other groups have suggested lower cut-offs [44–47], some of them emphasizing the need for sex adjusted cut-offs. For example, cut-offs of 0.27 and 0.34 ng/mL for GH following OGTT have been reported for men and women, respectively [45].

The "evolution" of cut-off values to a large extent reflects the "evolution" of the analytical methods: Newer GH assays tend to be more sensitive, are based on monoclonal antibodies with higher specificity compared to older polyclonal antisera, and finally, most modern GH assays are calibrated against the latest international recombinant reference preparation IRP 88/624 or 98/574 (as opposed to the pituitary derived IRP 80/505 previously used). All these factors generally lead to lower absolute GH concentrations reported by the laboratories.

Until the early 90s, many of the traditional competitive GH assays exhibited quantification limits between 0.5 and 1 µg/L. The development of novel, non-isotopic two-site antibody assay allowed to reliably measuring GH at very low concentrations. Some of the assays demonstrated remarkable sensitivity down to 0.002 µg/L, leading to the discovery of the very low GH nadirs following OGTT in healthy subjects [48]. Apart from differences in the sensitivity, there were also changes in the specificity of the assays: From the 90s onwards many of the commercially available GH assays were based on high affinity monoclonal antibodies, while older assay had employed polyclonal antisera. Human growth hormone is an example of a protein that occurs in different molecular isoforms. Healthy pituitaries as well as pituitary adenomas mainly secrete the 22 kD GH isoform. However, a 20 kD GH isoform and other minor variants exist in considerable amounts. Furthermore, the isoforms form dimers and heteromers, leading to a broad spectrum of molecules that together constitute what is known as "growth hormone" [49]. The higher the specificity of the antibodies, the more likely they will recognize and bind only a certain subset of the molecular isoforms. This explains why different antibodies translate very different percentages of total GH into an assay signal, and therefore, why different GH assays can report very different concentrations of GH for the same sample.

The differential recognition of molecular isoforms by different GH assays also aggravated another problem in the standardization of GH assays: Traditionally, GH assays were calibrated against a poorly defined but internationally recognized reference preparation of pituitary origin (IRP 80/505). Apart from minor contaminations with other pituitary derived proteins this IRP contained a mixture of the various GH isoforms, although there had been an attempt to enrich the main 22 kD isoform. With the availability of a new international reference preparation 88/624 based on pure recombinant 22 kD human GH, some of the GH assays on the market were recalibrated. Because of the higher potency of the pure 22 kD GH in many of the immunoassays, GH concentrations reported for patients samples dropped. Meanwhile, the first recombinant preparation 88/624 has been replaced by a new preparation with identical physicochemical properties named 98/574 which is recommended to be used in all GH assay. The universal adoption of this standard is one key component of the attempts to improve standardization across GH assays [50–52].

The impact of assay methods on the absolute GH concentrations reported by the laboratory and, as a consequence, on clinical decisions has been reported repeatedly during the last decades [53–55]. Unfortunately, there has been little progress in standardization (or at least harmonization) of GH assays over time. Therefore, although to date the cut-offs for GH following oral glucose load most widely used by endocrinologists might be 0.4 ng/mL as suggested by the Cortina criteria [44] or 1 ng/mL as suggested by the latest Endocrine Soceity Practice Guideline [15], an universal adoption of these cut-offs is problematic in view of the huge methodological differences between GH assays. Given that many laboratories today are using modern sensitive GH assays from a methodological point of view the lower cut-offs (e.g. 0.4 ng/mL as opposed to 1.0 ng/mL) might be considered more widely applicable. However, recent studies repeatedly have demonstrated that in the same cohort of patients with acromegaly the decision whether GH is elevated or not after OGTT largely depends on the GH assay used [56], and that such dependency on the analytical methods severely limits the applicability of diagnostic criteria from consensus guidelines [57]. It remains important to recognize that any cut-off values mentioned in guidelines or consensus statements must be seen in the context of the analytical methods used to define them. For the most commonly used commercial assay methods published data on ideal method specific cut-offs for GH in well-defined patient populations must become available (and must be implemented by laboratories and clinicians).

Apart from the methodological issues it must not be forgotten that there is increasing evidence from recent studies that cut-off values for GH nadirs might also need to be adjusted for biological factors including gender. Table 2 lists mean GH nadirs reported from studies investigating the GH response to OGTT in healthy subjects by different GH assays. It is striking that almost all of the studies in healthy subjects published during the last 5 years report extremely low GH nadir concentrations. Although none of the studies specifcally addressed gender differences in a larger cohort, several studies suggest significant differences between women and men, with lower concentrations consistently reported for males. Furthermore, one study reported higher GH nadir concentrations in women in midcycle (0.44 ng/mL), making an influence of estrogens likely.

Issues with IGF-I assays

Many of the analytical issues discussed for GH assays above also apply to IGF-I assays [52, 63], For example, the change from competitive assays based on polyclonal antisera to sandwich type immunoassays based on monoclonal antibodies has modified not only sensitivity, but also specificity of the IGF-I assays. In case of IGF-I assays, epitope specificity and assay setup can have dramatic impact on measured IGF-I concentrations because of the presence of several high affinity IGF-I binding proteins. These binding proteins interfere with different assays to a different degree, and not all assays have implemented the same, effective measures to prevent interference from binding proteins [37]. Furthermore, standardization of IGF-I assays has been an issue because a reference preparation used by many assays in the past (and still being used by some manufacturers) was impure and poorly defined [64]. Changing the reference preparation to a newer, recombinant standard [65] is recommended by consensus guidelines, but the change in absolute concentrations reported by re-calibrated assays needs to be taken into account. Such changes need to be reflected by new reference intervals for correct interpretation of IGF-I values, which have to be implemented by laboratories and communicated to clinicians. Unfortunately, there is indication that the latter steps are frequently omitted; this makes different interpretations of the same IGF-I values generated by the same analytical methods in different local laboratories an issue [57]. Given all these potential analytical and methodological pitfalls, it is not surprising that—in a clinical setting—classification of patients with acromegaly and agreement between interpretation of GH and IGF-I results can be different depending on the IGF-I assay used [66].

Reference intervals are of particular importance for correct interpretation of IGF-I concentrations measured by any assay method. Given the methodological differences between assays, it is obvious that such reference intervals have to be established for each analytical method

Table 2 GH nadir concentrations during OGTT in healthy controls and patients with impaired glucose tolerance* and diabetes mellitus**

Mean GH nadir (ng/mL)		n		GH assay	GH calibrator	Year	References
f	m	f	m				
0.15		37		EIA, inhouse	NHPP HS2243E	1990	[58]
0.13*		20*					
0.14**		22**					
0.25	0.029	6	9	ICMA, Nichols	WHO IS 80/505	1994	[48]
0.84		25		RIA, inhouse	NHPP AFP-4793B	1998	[47]
0.09				IRMA, DSL	WHO IS 88/624		
0.04				ELISA, DSL	WHO IS 88/624		
0.09	0.08	20	26	IRMA, DSL	WHO IS 88/624	2001	[28]
0.1	0.06	30	26	IFMA, inhouse	NHPP AFP-4793B	2002	[59]
0.14		39	43	IRMA, Sorin	not mentioned	2003	[60]
0.08		46		IRMA, DSL	WHO IS 88/624	2004	[46]
0.087	0.051	44	50	IRMA, BIOCODE	WHO IS 88/624	2004	[30]
<0.34	<0.27	25	25	ICMA, Nichols	WHO IS 98/574	2006	[45]
0.11	0.02	38	34	IFMA, AutoDELFIA	WHO IS 80/505	2006	[53]
0.19	0.05	35	25	ICMA, Siemens (Immulite 2000)	WHO IS 80/505		
0.3	0.11	120	80	IFMA, AutoDELFIA	WHO IS 80/505	2008	[61]
0.6	0.25	120	80	ICMA, Immulite	WHO IS 98/574		
0.2	0.1	120	80	IRMA, DSL	WHO IS 88/624		
0.13		147	66	Immulite 2000	WHO IS 98/574	2008	[56]
0.06				ICMA, Nichols	WHO IS 98/574		
0.015				ELISA, DSL	WHO IS 88/624		
0.097		3	5	IFMA	WHO IS 98/574	2010	[24]
0.1	0.05	31	14	IFMA, AutoDelfia		2011	[32]
0.12 $_{\text{follicular phase}}$	0.096	13	11	ICMA, Immulite 2000, Siemens		2011	[62]
0.44 $_{\text{midcycle}}$							
0.19 $_{\text{late luteal phase}}$							
0.07	0.04	18	7	ICMA, IDS-iSYS	WHO IS 98/574	2012	[33]
0.11	0.08			ICMA, Immulite 2000			
0.07	0.05	21	20	Ultrasensitive ICMA, Beckman Coulter	WHO IS 98/574	2013	[35]

WHO IS World Health Organization International Standards from the National Institute of Biological Standards and Controls (NIBSC, Hertfordshire, UK), *NHPP* Preparations from the National Hormone and Pituitary Program (NHPP, Torrance, USA), *EIA* Enzyme immunoassay, *ICMA* Immunchemiluminometirc assay, *RIA* Radio immunoassay, *IRMA* Immunradiometric assay, *IFMA* Immunfluorometric assay, *ELISA* Enzyme linked immunosorbent assay

separately. They need to be based on large cohorts selected from an appropriate, carefully characterized background population. The recent consensus statement called for transparency in a sense that origin and characteristics of the reference population, number of individuals in each age cohort as well as all mathematical and statistical procedures involved in the generation of reference intervals need to be published in peer-reviewed journals. The availability of such publications does not remove the differences related to analytical methods, but allows a direct comparison of quality and appropriateness of the reference intervals used. As demonstrated in a recent multicenter study to establish method specific reference intervals for a new automated IGF-I assay [67], in very large cohorts the impact of geographic origin, medications and comorbidities on the robustness of the reference intervals becomes negligible. In smaller studies, however, such factors can significantly impact on the definition of "normal" IGF-I. Furthermore, a compilation of IGF-I reference intervals from studies published during the last decades (Supplemental Table 1 in [67]) revealed not only significant differences regarding size and composition of the cohorts investigated, but also regarding statistical methods used to calculate the reference intervals. This is remarkable because the "normal range"—even if calculated from the same reference population—can be significantly different when different statistical methods are employed. Interestingly, a very recent study [68], which used samples from the same cohort of approximately 1000 adults to establish reference intervals for four different IGF-I assays, revealed that—even when using the same

statistical approach—the reference intervals are significantly different between the assays. This was not only true for absolute concentrations (which could be explained by differences in assay calibration), but also for the shape of the centiles and the width of the reference intervals in different age groups: some assays gave significantly broader reference intervals with particularly higher "upper limits of normal" than other assays. Whether this is related to differences in the method employed to remove interference from binding proteins or to differences in specificity (leading to differences in the recognition of IGF fragments) is unknown. However, it clearly demonstrates that reference intervals and standard deviation scores (SDS) cannot be mathematically converted between assays.

Different testing modalities for GH

Baseline fasting or random GH, mean GH in day profiles and nadir GH during OGTT all have been suggested for the diagnosis of acromegaly as well as for evaluation of treatment success. Multi-point sampling for day profiles requires a lot of time, personnel and resources, and is not practical for outpatient care. In patients with elevated IGF-I, it has been suggested that basal GH above 5 ng/mL in men and 10 ng/mL in women provide sufficient specificity to diagnose acromegaly without further multi-point measurements [69]. Although simple and fast, such an approach bears the risk of misclassifying patients. High GH peaks can occur physiologically, with stress, after physical exercise or in the fasting state. Falsely elevated IGF-I values are not uncommon. Therefore, although suggested as diagnostically relevant in the past, the diagnosis of acromegaly should not be solely based on measurement of random GH. The pulsatile nature of GH secretion makes random GH values less specific, making multi-point measurement such as an OGTT or mean GH from GH profiles necessary for robust diagnosis [60, 70–72]. The relevance of mean GH concentrations assessed over various time periods is also controversial. Although generally correlated to IGF-I and GH nadir [29, 73–76], mean GH concentrations can remain within the normal range particularly in mild cases of acromegaly [29]. Furthermore, not only secretion of very high concentrations of GH, but also tonic secretion of comparably low GH concentrations can result in elevated IGF-I [77–79]. Therefore, an apparently normal mean GH in a profile does not rule out acromegaly. To better reflect the impact of pulsatile versus continuous GH secretion, one group suggested to complement mean GH concentrations by analysis of minimum GH from a GH day profile. The combination of both parameters showed good correlation with IGF-I [34].

The majority of experts in the field prefer to diagnose or rule out clinically suspected acromegaly on the basis of age- and gender adjusted IGF-I in combination with the GH nadir during OGTT [15–17]. The OGTT is an easy, cost-effective diagnostic procedure, which rarely leads to complications and is applicable to nearly all patients. It can be diagnostically relevant even in patients with impaired glucose metabolism if metabolic state is controlled: In diabetic patients without acromegaly several studies have shown suppression of GH following oral glucose intake, and none of the patients encountered test-related complications [36, 58]. In turn, performing an OGTT in suspected acromegaly has the advantage that at the same time of diagnosing acromegaly one can also diagnose disturbances in glucose metabolism. Such disturbances are common in patients with acromegaly, and should be treated early. The usefulness of GH nadir concentrations during OGTT has also been demonstrated in the evaluation of success of surgical procedures. Normalization of GH nadirs can be observed as early as 1 week postoperatively while normalization of IGF-I can be delayed up to 12 months [80–83].

Biological factors modifying GH and IGF-I concentrations

Under physiological conditions, GH is secreted in a pulsatile fashion. Amplitude and frequency of GH pulses vary with time during the day, gender, age, menstrual cycle, nutrition, exercise and body composition [84, 85]. Consequently, adjustment of clinical decision limits for GH based on gender, age and body mass index has been discussed [56, 62]. In contrast to GH, IGF-I is secreted continuously, has a longer half-life and exhibits more stable concentrations in blood [86, 87]. These properties make IGF-I an excellent surrogate marker of GH action and the best biomarker for disease activity in acromegaly [88]. Nevertheless, IGF-I levels can be modified by a variety of physiological and pathological factors. Understanding the biological variables affecting each of the two components of the GH/IGF axis is crucial for correct interpretation of biochemical findings in a patient.

GH concentrations generally are higher in healthy premenopausal women and change with phases of the menstrual cycle [72, 89, 90]. GH is highest during mid-cycle in younger woman [62, 91]. It has also been shown that GH nadir concentrations during OGTT are higher in younger as compared to older women [30, 45, 48, 53, 56, 59, 61, 92]. In 2001, a comparison of GH nadirs following oral glucose load in 26 men and 20 women revealed higher basal GH in women, but no significant difference in the nadir [28]. Treatment with oral estrogens (oral contraceptives or hormonal replacement therapy) generally increases GH levels [28, 93–95].

Sex specific differences have been reported in some, but not all studies in patients with acromegaly. One

study in patients with acromegaly did not find any differences related to sex in basal GH and GH nadir [28], while another study in 151 patients (79 women and 72 men, age 19–77 years) clearly demonstrated higher GH nadir concentrations in women [96]. In this study, basal GH and GH nadir concentrations were also negatively correlated with age in both sexes. Although this had not always been observed in studies investigating healthy subjects beyond the age of 50 [53, 61, 96], the age-dependent decline in mean GH pulse amplitude and pulse duration has already been described more than 20 years ago [90]. Consistent with this, in 2006 one group suggested the use of age-adjusted cut-off values for mean GH (in a diurnal profile) and for the GH nadir to determine remission after surgical therapy of acromegaly [13].

In contrast to higher GH levels in women, lower IGF-I levels compared to men of the same age have been reported in some studies. However, while more important during childhood, the impact of sex on IGF-I concentrations is minor beyond puberty [52, 67]. The sex-related differences have been explained by a mild hepatic GH resistance caused by estrogen [72, 90, 97, 98]. Interestingly, in women on estrogen therapy the route of estrogen administration significantly influences IGF-I concentrations: While oral estrogens reduce IGF-I levels and increase IGF-I binding proteins, transdermal estrogens have no impact on IGF-I levels [94, 99]. In patients with acromegaly, lower IGF-I levels in women compared to men have also been described [96, 100, 101]. Before more specific treatment options became available, the IGF-I suppressive effect of oral estrogens had been used to ameliorate signs and symptoms of acromegaly [102, 103]. Parenteral administration of testosteron, in turn, can increase IGF-I [104]. The multiple influences of sex steroids on the GH/IGF axis, and the changes in sex steroids with age further support the use of gender- and age-specific reference values [105].

Several studies have demonstrated that GH nadir concentrations during OGTT in healthy subjects can be significantly <1 ng/mL, but the degree of suppression depends on sex and body mass index (BMI) [75–79]. Recently, the impact of age, sex and BMI on 24-h pulsatile GH secretion has been demonstrated in a group of 130 healthy adults (85 women, 45 men, 20–77 years, BMI 18.3–49.8 kg/m^2) [72]. Age was negatively correlated with basal and pulsatile 24-h GH secretion, while BMI was negatively correlated only with basal GH. Another study in 200 healthy adults did not find a correlation of BMI and GH nadir [61], but all subjects had comparably low BMI (BMI 18.5–27 kg/m^2). In healthy obese subjects, but also in obese patients with acromegaly, lower concentrations for basal and nadir GH have been reported before [56, 106]. A number of other studies, however, did not confirm the correlation between BMI and GH [28, 30, 45, 48, 92].

The impact of body composition on IGF-I is complex. However, in severe obesity IGF-I seems to be significantly reduced, an effect which is reversible after weight loss [107–109]. Similarly, prolonged fasting and malnutrition have also been shown to reduce IGF-I [99, 110–112]. This effect, however, was not observed in patients with acromegaly [113]. Overall, fasting and nutrition differentially affect GH and IGF-I: In states of acute and chronic malnutrition as well as in anorexia nervosa GH is increased and IGF-I decreased due to the peripheral GH resistance [114–117].

Several diseases have an impact on circulating GH and IGF-I. In chronic renal failure increased GH release and reduced GH clearance lead to higher GH concentrations [118, 119]. However, although GH is increased, IGF-I is unchanged or even decreased. It has been shown that uremia leads to GH resistance due to impaired JAK/STAT signaling at the GH receptor [120, 121]. Additionally, IGF-I binding proteins have been shown to be elevated in patients with chronic kidney disease [122, 123], potentially reducing bioavailability of IGF-I.

In patients with type 2 diabetes and insulin resistance, suppression of GH release by glucose is impaired, resulting in higher GH concentrations compared to patients with normal insulin sensitivity [124]. Elevated GH concentrations have also been reported in patients with type 1 diabetes, most likely related to a reduction in somatostatin release and therefore increased GH secretion [125–127]. Furthermore, it has been demonstrated that insulin treatment enhances spontaneous pulsatile GH secretion, explaining increased random GH levels without underlying acromegaly [36, 128]. On the other hand, chronic hyperglycemia has been shown to be associated with a suppression of GH release. Differential effects of acute and chronic hyperglycemia have to be taken into account when GH secretion is studied in diabetic patients [129]. The neuropeptide galanin has been reported to paradoxically decrease GH in active acromegaly independent of disorders of glucose metabolism [130, 131]. Therefore, if available, the galanin test could be helpful in diagnosing acromegaly in patients with diabetes mellitus. Recent reviews have also discussed the important interactions exist between insulin levels and hepatic GH receptor expression and hepatic GH sensitivity [132].

As already indicated by its name, IGF-I shares almost 50% homology to insulin in amino acid sequence. It is not surprising that the IGF-I level is influenced by glucose metabolism [133], Insulin induces hepatic IGF-I synthesis via modulation of the GH receptor [134], and insulin can decrease IGF-I binding proteins and thereby increase free IGF-I [135]. However—despite these mechanisms potentially leading to higher IGF-I—IGF-I concentrations usually are within the lower part of the age- and sex-adjusted

reference intervals. This is due to the coexisting hepatic GH resistance, which reduces IGF-I synthesis [124, 136].

Finally, in acute critical illness GH concentrations can be increased due to higher peaks, higher pulse frequency and peripheral GH resistance which, in turn, leads to a decrease in IGF-I levels [137–141]. In contrast, in chronic critical illness pulse amplitude and frequency can be reduced and GH levels can be normal [142, 143]. Dissociation of GH and IGF-I with high GH and low IGF-I due to GH resistance can occur in states of systemic inflammation, chronic liver disease and cirrhosis [144–146]. The GH resistance seen in cirrhosis has been attributed to a significant reduction of hepatic GH receptor mRNA [31].

Conclusion and expert opinion

In clinical practice, the occurrence of divergent findings for GH and IGF-I in diagnosis and monitoring of acromegaly provides problems. Understanding how "numbers" reported by laboratories depend on the analytical methods employed, but also how the differences in analytical methods can be handled by application of appropriate method specific decision limits and reference intervals is important. Modern GH assays generally report much lower concentrations than assays previously used, requiring continuous adaptation of traditional cut-offs. Reference intervals for IGF-I can be very different depending on the methods used, and clinicians should demand from laboratories to provide transparent, method specific reference intervals from appropriately sized studies. Furthermore, since a wide spectrum of potential biological factors differentially can modify GH and IGF-I concentrations, it remains crucial for the clinician to base any interpretation of laboratory data on the clinical information available for the patient. While there is good evidence showing that—beyond puberty—sex and a wide range of BMIs only marginally affect IGF-I, recent findings suggest to develop assay-specific cut-off values for GH during OGTT adjusted for sex and BMI. Finally, specific therapeutic interventions and comorbidities must be taken into account in the assessment of laboratory findings.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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