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# Usefulness of Different Biochemical Markers of the Insulin-Like Growth Factor (IGF) Family in Diagnosing Growth Hormone Excess and Deficiency in Adults*

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**ABSTRACT**

The diagnostic approach to acromegaly and GH deficiency frequently includes measurement of several components of the insulin-like growth factor (IGF) system. IGF-I levels are reported to be good predictors of active and cured acromegaly, but are commonly found within the normal age-adjusted range in adult GH-deficient (GHD) patients. Circulating concentrations of IGF-binding protein-3 (IGFBP-3), acid-labile subunit (ALS), and free IGF-I reflect the GH secretory status, but their diagnostic accuracy is still debated. In this study serum levels of total and free IGF-I, IGFBP-3, ALS, and IGFBP-3-IGF-I and IGFBP-3-ALS complexes were determined in patients previously diagnosed with active (n = 67) or inactive (n = 16) acromegaly and adult GHD (n = 34) and compared with results obtained in 58 healthy controls.

In healthy subjects, IGF-I, IGFBP-3, ALS, and both IGFBP-3 complexes declined with age; a correlation was found between IGF-I and IGFBP-3 ($r = 0.59$; $P < 0.001$), ALS ($r = 0.67$; $P < 0.001$), and free IGF-I ($r = 0.40$; $P < 0.05$). Active acromegalic patients showed a significant increase in all parameters tested. IGF-I concentrations were above +2 SD in 100% of patients, whereas slightly lower sensitivities were shown for IGFBP-3 (85%), ALS (88%), and free IGF-I (94%). In this group, IGF-I exhibited a slightly higher correlation with IGFBP-3 ($r = 0.83$; $P < 0.001$) than with ALS levels ($r = 0.78$; $P < 0.001$). In cured acromegalic patients, we observed the normalization of all parameters but free IGF-I levels.

Adult GHD patients showed a significant reduction of all hormones. Unlike active acromegalic patients, all parameters had only a modest sensitivity in GHD; suppression below –2 SD was observed in 41% of GHD patients for IGF-I, 47% for IGFBP-3, 32% for ALS, and 35% for free IGF-I measurements. Previous radiotherapy and GH peak response below 3 $\mu$g/L were associated with significantly lower IGF-I, IGFBP-3, and ALS levels. IGF-I levels were significantly correlated to ALS ($r = 0.68$; $P < 0.001$) and IGFBP-3 ($r = 0.64$; $P < 0.001$) as well as with free IGF-I ($r = 0.67$; $P < 0.001$) levels. By multiple regression analysis, the number of anterior pituitary hormones impaired was the most predictive indicator of IGF-I, IGFBP-3, and free IGF-I levels in GHD patients; conversely, the GH peak response better anticipated ALS concentrations.

The pattern of IGFBP-3 complexes paralleled previous hormonal findings. In active acromegalic patients, IGFBP-3-IGF-I levels were 5.4-fold higher than in controls and were above +2 SD in 95% of patients, whereas IGFBP-3-ALS levels were elevated in 15% of cases. On the other hand, both IGFBP-3 complexes were able to predict GHD in only a minority of cases.

Taken together, these data support the diagnostic role of IGF-I in acromegaly and suggest that free IGF-I and the IGFBP-3-IGF-I complex can assist diagnostic strategies in this condition. All markers are of limited predictive value in adult GHD, as hormonal values are commonly found within the normal limits. In these patients, low IGFBP-3 and IGF-I concentrations can add further clinical information on the residual GH activity. (*J Clin Endocrinol Metab* **86:** 3001–3008, 2001)

DIAGNOSIS OF GH-RELATED disorders usually requires stimulatory testing to allow for the variability in determinations of spontaneous GH secretion (1, 2). Measurement of insulin-like growth factor I (IGF-I) has been proposed as an appropriate first-line investigation for diagnostic and therapeutic monitoring of acromegaly (1, 3–5). In pediatric GHD, approximately 80% of patients are expected to have IGF-I levels below –2 SD, for the age-related mean values (2, 6), but the relationship existing between IGF-I and both spontaneous and stimulated GH secretion is arguable in adult patients.

IGF-I circulates predominantly associated with IGF binding protein-3 (IGFBP-3) and the acid-labile subunit (ALS) in a ternary complex, which constitutes both a reservoir and a carrier system for IGF-I (7). Although GH is known to regulate the hepatic synthesis of these peptides, IGFBP-3 and ALS concentrations do not reflect the 24-h spontaneous GH secretion as accurately as IGF-I does. It is well established that both IGFBP-3 and ALS are increased in acromegaly and reduced in GH deficiency, but results can be paradoxically normal in both diseases (8–13). An altered status of GH secretion may be also reflected in a decreased free fraction of IGF-I, reported to be normally only 1% of the total IGF-I present in the circulation. Free IGF-I concentrations are elevated in the majority of patients with untreated acromegaly

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(14–16). In adult GHD, multiple regression analysis showed a significant correlation among free IGF-I concentrations and peak GH levels, disease duration, and residual pituitary function (17, 18). Nevertheless, the sensitivity of this marker seems to be affected by overlaps with normal levels both in acromegaly and GHD (16–19).

Until recently, the possibility of detecting immunocomplexes involving combined components of the IGF system had not been investigated (20). The determination of combined multiple markers potentially improves the accuracy of IGF axis immunoassays, as IGF-I, IGFBP-3, and ALS are all individually GH dependent. Two novel assays, capable of detecting IGFBP-3 complexed with IGF-I or ALS, showed these complexes to be significantly correlated to other markers of the GH/IGF-I axis (20).

Our study aimed at determining the usefulness of multiple IGF markers in the diagnostic approach to GH-related disorders. To achieve this goal, we assessed the circulating levels of GH, total and free IGF-I, IGFBP-3, ALS, and IGFBP-3-IGF-I and IGFBP-3-ALS complexes in a population of acromegalic and adult GHD patients.

## Subjects and Methods

### *Healthy controls*

Blood samples were obtained from 58 subjects (34 women and 24 men; age, 18–74 yr) working in or referred to the Department of Endocrinology, University of Naples. All participants were reportedly free from any endocrine or nonendocrine disease.

### *Acromegalic patients*

Eighty-three patients (47 women and 36 men; age, 22–73 yr) were diagnosed with acromegaly on the basis of signs and symptoms of disease, elevated GH levels (>2.5 $\mu$g/L) unsuppressed below 1 $\mu$g/L during an oral glucose tolerance test, high IGF-I concentrations, and radiological detection of pituitary adenoma. Patients were classified according to disease status: 67 patients (35 women and 32 men) were recruited at the time of diagnosis and were in the active disease stage. The remaining 16 (6 women and 10 men) were considered cured after pituitary surgery (all patients) and radiotherapy (n = 3): all exhibited normal GH levels in basal (<2.5 $\mu$g/L) and glucose-stimulated (<1 $\mu$g/L) conditions and the absence of residual tumor tissue on pituitary imaging.

### *Adult GHD patients*

Thirty-four hypopituitary subjects (19 women and 15 men; age, 18–60 yr) had been previously subjected to transsphenoidal and/or transcranial surgery for pituitary tumor, craniopharyngioma, or meningioma, except for 2 patients affected with idiopathic GHD. Eight of 32 patients previously operated on had been also subjected to external radiotherapy. Diagnosis of GHD was based on the GH peak response to the provocative test with arginine plus GHRH below 9 $\mu$g/L, as previously described (21). Six patients suffered from diabetes insipidus. Twenty-three patients were suffering from other anterior pituitary deficiencies in addition to GH: ACTH in 2; FSH and LH in 8; ACTH, FSH, and LH in 9; and FSH, LH, ACTH, and TSH in 4. All patients with multiple deficits were undergoing adequate replacement therapy at the time of testing.

### *Study procedure*

All subjects were included in the study after informed consent to participate and approval by the local ethics committee had been obtained. Subjects were analyzed under fasting conditions, while seated or recumbent. After blood drawing, samples were allowed to clot at room temperature and then were centrifuged, aliquoted, and stored at −80 C. Samples from each subject were run in duplicate. For GH measurements, individual values were calculated as follows: in normal subjects and acromegalic patients, the mean of three consecutive blood samples each drawn at 15-min intervals; in GHD patients, the maximal peak GH response to provocative tests (21).

### *Hormonal assays*

All measurements were performed using assays provided by Diagnostics Systems Laboratories, Inc. (Webster, TX), except for the GH assay.

Serum GH levels were measured by immunoradiometric assay (IRMA; HGH-CTK, Sorin, Italy). The assay sensitivity was 0.15 $\mu$g/L; the intra- and interassay coefficients of variation (CVs) were 4.5% and 7.9%, respectively. Total and free IGF-I, IGFBP-3, and ALS levels were measured with noncompetitive sandwich-type assays. Total IGF-I IRMA was performed after extraction; the assay sensitivity was 0.8 $\mu$g/L; intra- and interassay CVs for the low, medium, and high points of the curve were 3.4%, 3%, and 1.5% and 8.2%, 1.5%, and 3.7%, respectively. IGF-I reference concentrations were 101–503, 100–494, 101–303, and 78–258 $\mu$g/L for ages of less than 30, 30–40, 40–50, and above 50 yr, respectively. The free IGF-I IRMA was performed without extraction; the assay sensitivity was 0.03 $\mu$g/L, and the intra- and interassay CVs for the low, medium, and high points of the curve were 10.3%, 5.1%, and 3.3% and 7.7%, 3.6%, and 10.7%, respectively. The IGFBP-3 IRMA had a sensitivity of 0.5 $\mu$g/L; the intra- and interassay CVs for the low, medium, and high points of the curve were 1.8%, 3.2%, and 3.9% and 1.9%, 0.5%, and 0.6%, respectively. IGFBP-3 reference values were 2000–7290, 1730–7260, 2080–4310, and 2020–3990 $\mu$g/L for ages of less than 30, 30–40, 40–50, and over 50 yr, respectively. The ALS enzyme-linked immunosorbent assay (ELISA) (11) had a sensitivity of 0.07 $\mu$g/mL; the intra- and interassay CVs for the low, medium, and high points of the curve were 6.1%, 7.5%, and 3.8% and 8.6%, 2.8%, and 8.9%, respectively. The serum concentrations of the IGFBP-3-IGF-I and IGFBP-3-ALS complexes were measured by two ELISAs, recently described (20). Results were expressed as arbitrary units (AU) per L, calculated from a pool of 10 normal human sera, which were assigned a value of 100 AU/L. The sensitivity of the IGFBP-3-IGF-I assay was 0.12 AU/L; the intra- and interassay CVs were 3.5–6.6% and 4.8–9.7%, respectively. The IGFBP-3-ALS assay sensitivity was 1.13 AU/L; the intra- and interassay CVs were 4.3–7.6% and 5.3–10.6%, respectively.

### *Data analysis*

For each group, results are presented as the mean ± SEM. For statistical analysis, data from acromegalic and GHD patients were compared with the age-distributed ranges for healthy subjects, calculated by the simple least linear regression analysis, to express individual hormonal values as SD scores. The regression line represents the mean, and the prediction interval is represented by ±2 SD (residual deviation of the regression errors). To approximate a normal (Gaussian) distribution, data were introduced in the statistical analysis as log transformed. Unpaired Student's *t* test or Mann-Whitney rank-sum test was used for comparisons. Correlation coefficients were determined from simple and multiple least linear regression analysis. The test sensitivity was estimated from the percentage of acromegalic patients with hormonal concentrations above +2 SD and from the percentage of GHD patients with hormonal concentrations lower than –2 SD for the normal values for age. Significance was set at $P < 0.05$. Statistical analyses were performed with SPSS 10.0 (SPSS, Inc., Chicago, IL).

## Results

### *Healthy subjects*

Mean and age-distributed hormonal values for all groups are presented in Table 1 and Fig. 1. Total IGF-I ($r = -0.54$; $P < 0.001$), ALS ($r = -0.37$; $P < 0.01$), IGFBP-3-IGF-I ($r = -0.52$; $P < 0.001$), and IGFBP-3-ALS ($r = -0.41$; $P < 0.01$) concentrations declined with age. IGF-I levels were higher in females than in males (214 ± 19 *vs.* 177 ± 11 $\mu$g/L; $P < 0.05$) and were positively correlated to both IGFBP-3 and ALS

**TABLE 1.** Anthropometric and hormonal data (mean ± SEM) in healthy subjects and in patients with active acromegaly, cured acromegaly, and GH deficiency

| Parameters | Healthy subjects (n = 58) | Active acromegaly (n = 67) | Cured acromegaly (n = 16) | GH deficiency (n = 34) |
|---|---|---|---|---|
| Age (yr) | 38 ± 1.5 | 45 ± 1.7 | 44 ± 3.7 | 42 ± 2.7 |
| Sex (F/M) | 34/24 | 35/32 | 6/10 | 19/15 |
| Body mass index | 26.4 ± 0.2 | 27.6 ± 0.2 | 27.1 ± 0.3 | 27.9 ± 0.2 |
| IGF-I (μg/L) | 196 ± 11.6 | 800 ± 41[a] | 218 ± 20.7 | 89.2 ± 12.2[b] |
| IGFBP-3 (μg/L) | 3574 ± 84 | 6161 ± 191[a] | 3331 ± 184 | 2222 ± 13[b] |
| ALS (mg/L) | 16.7 ± 0.6 | 38.2 ± 1.5[a] | 16.6 ± 1.4 | 10.4 ± 1[b] |
| Free IGF-I (μg/L) | 1.2 ± 0.1 | 8.7 ± 0.6[a] | 3.4 ± 0.6[c] | 0.85 ± 0.2[b] |
| IGFBP-3-IGF-I (AU/L) | 51.4 ± 3 | 276 ± 30.2[a] | 61 ± 5.8 | 28.7 ± 5[b] |
| IGFBP-3-ALS (AU/L) | 98 ± 4.8 | 112 ± 5.3 | 68 ± 6.4[c] | 46 ± 4.3[b] |

Significance was set at 5%.
[a] Active acromegalic patients significant *vs.* controls.
[b] GH-deficient patients significant *vs.* controls.
[c] Cured acromegalic patients significant *vs.* controls.

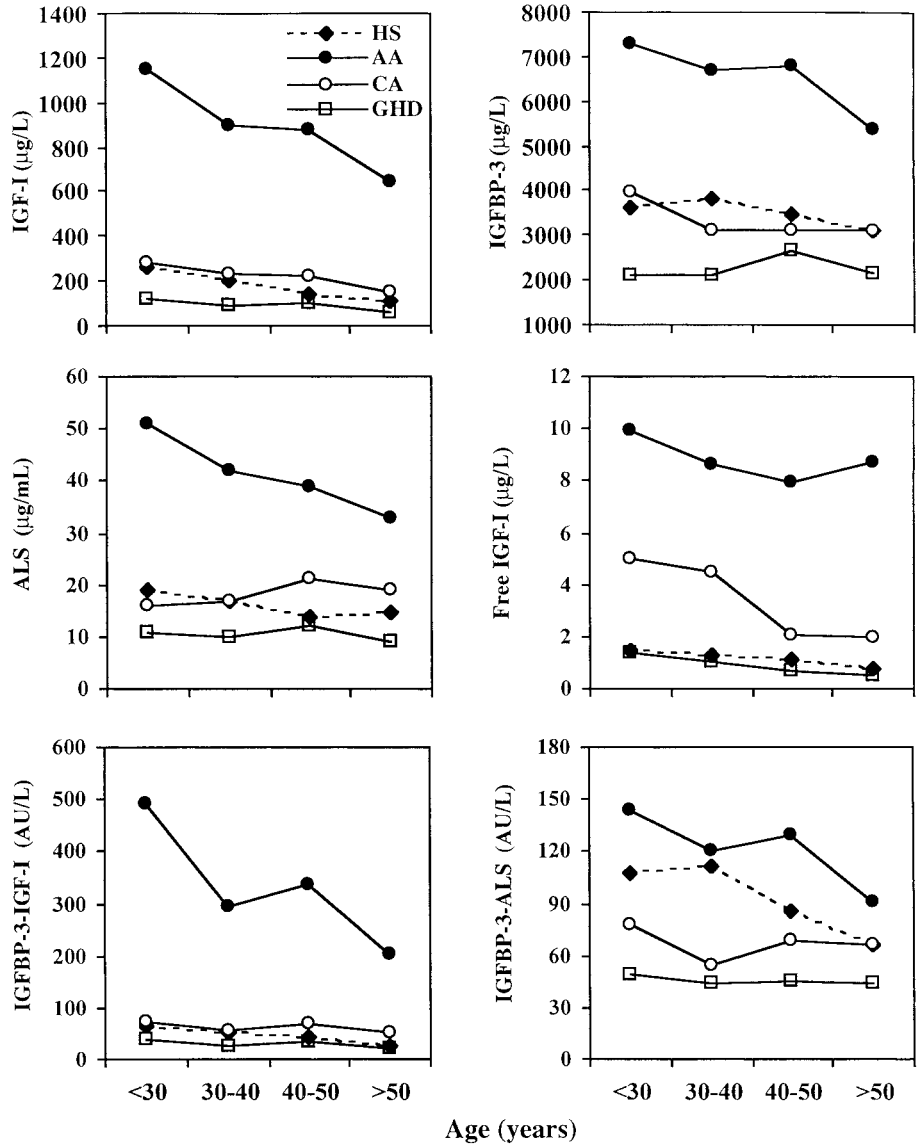


FIG. 1. Age-related mean hormonal concentrations assessed in healthy subjects (HS; ♦), active (AA; ●), and cured (CA; ○) acromegalic patients and GH-deficient patients (GHD; □).

levels (r = 0.59 and r = 0.67; $P < 0.001$ in both cases). A positive correlation also existed between free IGF-I and both total IGF-I (r = 0.40; $P < 0.01$) and the IGF-I/IGFBP-3 molar ratio (r = 0.38; $P < 0.01$). The free/total IGF-I ratio was below 1% in 48 subjects (83%). No gender-related differences were observed for IGFBP-3 complexes.

*Acromegalic patients*

*Active acromegaly.* As expected, all IGF parameters tested measured higher than those in controls (Tables 1 and 2, and Figs. 1 and 2). As in healthy subjects, age was associated with a decline in serum IGF-I ($r = -0.43$; $P < 0.001$), IGFBP-3 ($r = -0.44$; $P < 0.001$), ALS ($r = -0.40$; $P < 0.01$), and IGFBP-3-ALS levels ($r = -0.37$; $P < 0.01$; Fig. 1). IGF-I and IGFBP-3 concentrations were above +2 SD, respectively, in all and in 57 of 67 patients (sensitivities, 100% and 85%, respectively). A positive correlation existed between these 2 markers ($r = 0.83$; $P < 0.001$). The IGF-I/IGFBP-3 ratio was higher than that in control subjects ($P < 0.001$; Fig. 3).

Mean ALS levels were 2.2-fold higher than those in controls. ALS concentrations were above +2 SD in 59 patients (sensitivity, 88%) and strongly correlated with both IGF-I and IGFBP-3 ($r = 0.78$ and $r = 0.79$; $P < 0.001$ in both cases). Free IGF-I concentrations were above +2 SD in 63 subjects (sensitivity, 94%) and were only modestly correlated to the molar IGF-I/IGFBP-3 ratio ($r = 0.32$; $P < 0.05$). The free/total IGF-I ratio was more than 1% in 51% of cases. Discrepant results were obtained in the analysis of IGFBP-3 complexes. IGFBP-3-IGF-I concentrations were 5.4-fold increased compared with control values and were above +2 SD in 64 cases (sensitivity, 95%). Conversely, IGFBP-3-ALS values exceeded +2 SD in only 10 patients (sensitivity, 15%).

*Inactive acromegaly.* A progressive reduction of total ($r = -0.56$; $P < 0.001$) and free IGF-I levels ($r = -0.51$; $P < 0.001$) occurred with age (Fig. 1). In this subgroup we observed the normalization of total IGF-I, IGFBP-3, ALS, and IGFBP-3-IGF-I (sensitivity, 100% for all markers). In contrast, free IGF-I levels remained above +2 SD in 11 of 16 cases (sensitivity, 31%), and the free/total IGF-I ratio was, consequently,

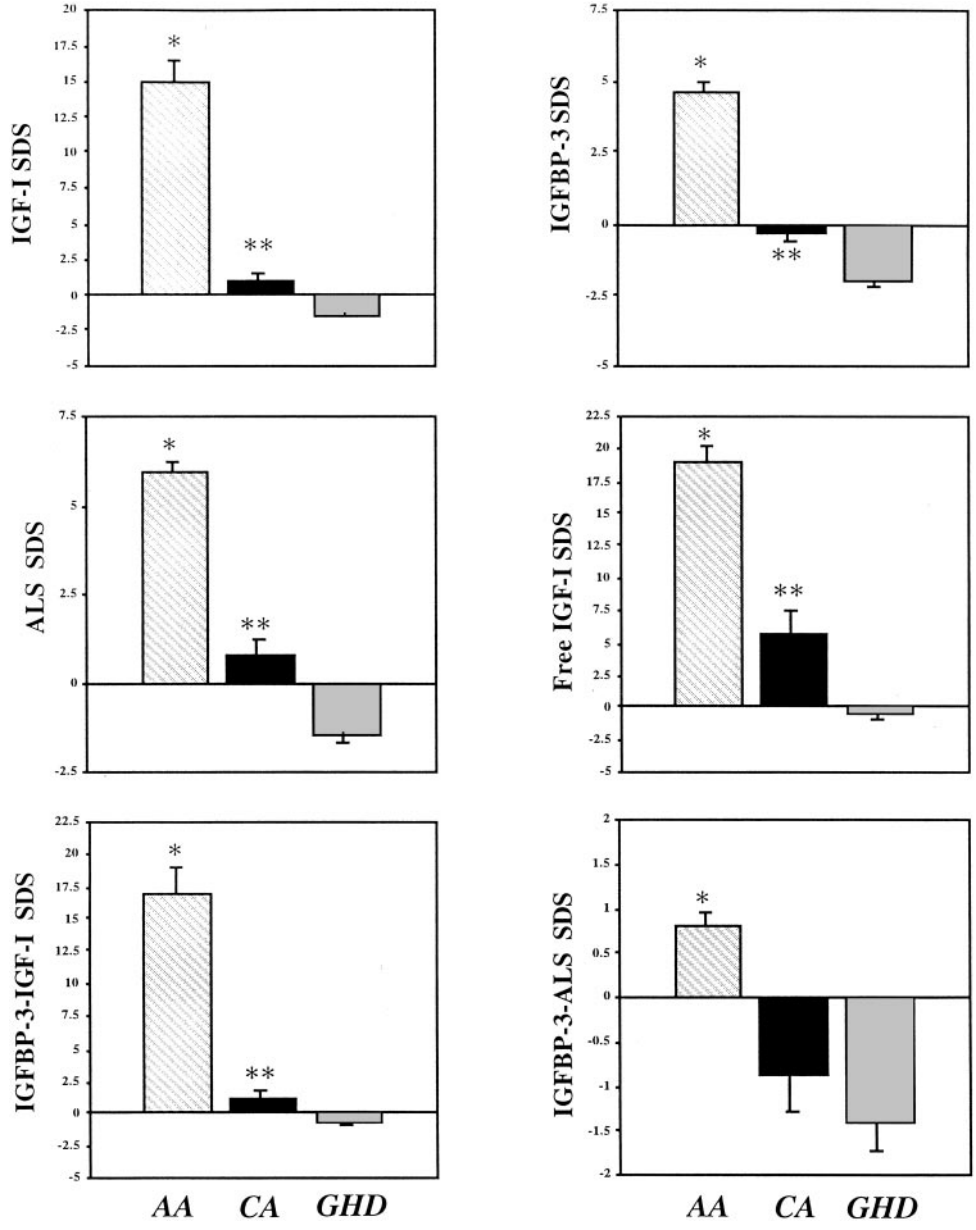


FIG. 2. Mean hormones concentrations expressed as SD scores (SDS) of age-matched mean control values. *, $P < 0.01$, active (AA) *vs.* both cured acromegaly (CA) and GHD; **, $P < 0.01$, cured acromegaly *vs.* GHD.

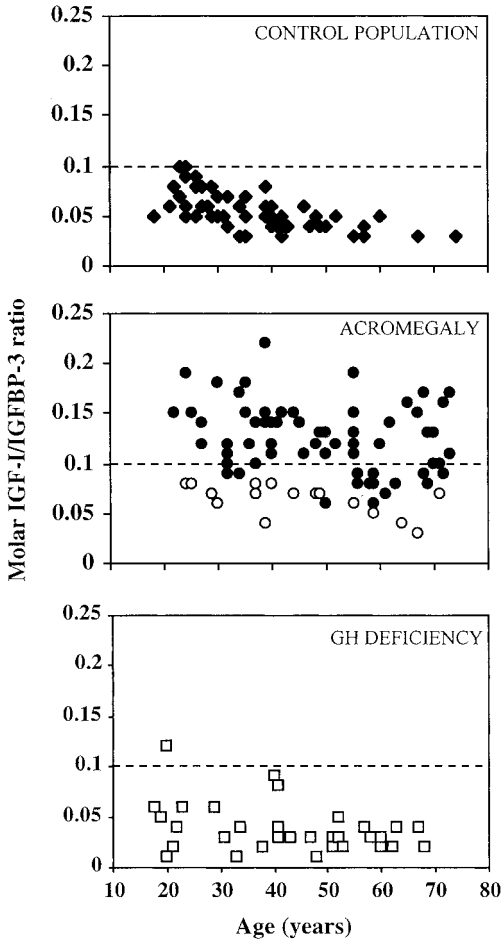


FIG. 3. Age distribution of individual IGF-I/IGFBP-3 ratio assessed in healthy controls (*upper panel*), active (●) and cured acromegalic (○) patients (*middle panel*), and GH-deficient patients (*lower panel*). The *dotted line* represents the cut-off value of 0.1 recorded in healthy subjects.

more than 1% in 6 cases. IGFBP-3-ALS levels were normalized in 13 cases and were below −2 SD in the 3 remaining patients (sensitivity, 81%; Table 1 and Figs. 1 and 2). We observed a significant correlation of free IGF-I with total IGF-I (r = 0.60; $P < 0.001$), but not with the IGF-I/IGFBP-3 ratio.

*Adult GHD*

All IGF parameters tested were significantly lower than those in normal subjects (Tables 1 and 2, and Figs. 1 and 2). Total (r = −0.34; $P < 0.05$) and free IGF-I levels (r = −0.35; $P < 0.05$) declined with increasing age (Fig. 1). IGF-I and IGFBP-3 concentrations were below –2 SD in 14 and 16 patients (sensitivities, 41% and 47%, respectively), 13 of whom had reached a GH peak response in the combined stimulatory test below 3 μg/L (Table 3). As expected, a positive correlation existed between IGF-I and IGFBP-3 concentrations (r = 0.64; $P < 0.001$), and both markers were negatively correlated to the number of anterior pituitary hormones impaired (r = −0.57 and r = −0.51, respectively; $P < 0.01$ in both cases). ALS concentrations were below –2 SD in 11 cases (sensitivity, 32%) and were well correlated to both IGFBP-3 and IGF-I levels (r = 0.85 and r = 0.68; $P < 0.001$ in both cases). Overall, IGF-I SD score, IGFBP-3 SD score, and ALS SD score were significantly reduced in patients with GH peak responses below 3 μg/L (Table 3) as well as in patients previously irradiated ($P < 0.05$).

**TABLE 2.** Comparison of IGF-I, IGFBP-3, ALS, free IGF-I, IGFBP-3-IGF-I, and IGFBP-3-ALS sensitivities in acromegalic and adult GHD patients, estimated from the percentage of acromegalic and GHD patients with hormonal concentrations respectively above +2 SD and below −2 SD the mean normal value for age

| Patient group | IGF-I | IGFBP-3 | ALS | Free IGF-I | IGFBP-3-IGF-I | IGFBP-3-ALS |
|---|---|---|---|---|---|---|
| Acromegaly | | | | | | |
| Active (> +2 SD) | 100 | 85 | 88 | 94 | 95 | 15 |
| Cured (< +2 SD) | 100 | 100 | 100 | 31 | 100 | 81 |
| Adult GH deficiency (< −2 SD) | 41 | 47 | 32 | 35 | 32 | 26 |

Values are percentages.

**TABLE 3.** Hormonal levels (mean ± SEM and z-scores) in 34 adult patients with GH deficiency, grouped according to the GH response to the provocative test with arginine plus GHRH (21)

| Parameters | GH <3 μg/L (n = 23) | GH >3 μg/L (n = 11) | *P* |
|---|---|---|---|
| Age (yr) | 43 ± 2.7 | 40 ± 6 | NS |
| IGF-I (μg/L) | 75.2 ± 14.2 | 118.2 ± 21.4 | 0.049 |
| z-Score | −1.7 | −1 | |
| IGFBP-3 (μg/L) | 2028 ± 146 | 2626 ± 242 | 0.048 |
| z-Score | −2.4 | −1.3 | |
| ALS (mg/L) | 8.4 ± 0.9 | 14.5 ± 2.8 | 0.017 |
| z-Score | −1.9 | −0.5 | |
| Free IGF-I (μg/L) | 0.7 ± 0.17 | 1.2 ± 0.4 | NS |
| z-Score | −0.8 | −0.04 | |
| IGFBP-3-IGF-I (AU/L) | 25.7 ± 5.9 | 34.9 ± 8.9 | NS |
| z-Score | −0.9 | −0.5 | |
| IGFBP-3-ALS (AU/L) | 41.3 ± 5 | 55.3 ± 7.8 | NS |
| z-Score | −1.5 | −1.1 | |

AU, Arbitrary units.

Free IGF-I levels were below –2 SD in 12 patients (sensitivity, 35%) and were inversely correlated to the number of anterior pituitary hormones impaired (r = −0.59; $P < 0.001$). Additionally, free IGF-I levels were correlated both with total IGF-I (r = 0.67; $P < 0.001$) and the molar IGF-I/IGFBP-3 ratio (r = 0.60; $P < 0.001$). The free/total IGF-I ratio was more than 1% in 11 cases. Mean IGFBP-3-IGF-I and IGFBP-3-ALS levels were reduced to 45% and 53% of normal values, respectively, but were below –2 SD in only 9 and 11 cases, respectively (sensitivity, 26% and 32%, respectively). None of the hormones tested showed any correlation with body mass index.

By multiple regression analysis, the number of anterior pituitary hormones impaired constituted the best predictive indicator of low IGF-I, IGFBP-3, free IGF-I, IGFBP-3-IGF-I, and IGFBP-3-ALS values; in contrast, ALS concentrations appeared to depend mostly on the GH peak response to the provocative test (Table 4).

## Discussion

The present study was designed to elucidate the diagnostic potential of the IGF system in adult GH disorders and to test the diagnostic accuracy of two combined GH-dependent

**TABLE 4.** Multiple linear regression analysis of the clinical variables influencing hormonal markers in 34 adults diagnosed with GH deficiency

| Variables | IGF-I | IGFBP-3 | ALS | Free IGF-I | IGFBP-3-IGF-I | IGFBP-3-ALS |
|---|---|---|---|---|---|---|
| Degree of hypopituitarism | 0.01 (−2.79) | 0.04 (−2.14) | 0.23 (−1.20) | 0.002 (−3.45) | 0.032 (−2.27) | 0.09 (−1.71) |
| Disease duration | 0.46 (−0.74) | 0.81 (−0.24) | 0.55 (−0.60) | 0.49 (−0.68) | 0.40 (−0.85) | 0.82 (0.22) |
| GH peak response | 0.78 (0.27) | 0.30 (1.05) | 0.03 (2.19) | 0.97 (−0.37) | 0.38 (−0.87) | 0.66 (0.44) |
| Age | 0.1 (−1.73) | 0.87 (0.19) | 0.35 (−0.95) | 0.11 (−1.63) | 0.26 (−1.14) | 0.64 (−0.46) |

The degree of hypopituitarism identified the number of anterior pituitary hormone deficits. *P* values are given, with *t* scores in *parentheses*.

markers recently developed. Our results show that all parameters tested were significantly increased in active acromegaly and reduced in GHD. There was a significant correlation among IGF-I, IGFBP-3, ALS ,and IGFBP-3-IGF-I concentrations in all groups evaluated, whereas total and free IGF-I were correlated only in the group of adult GHD patients. We observed that IGF-I measurements were highly reliable in the diagnosis of acromegaly. Conversely, the diagnostic significance of both IGF-I and IGFBP-3 measurements in adult GH deficiency was lower than that of the GH stimulatory testing. Finally, the concentrations of IGBP-3-IGF-I complex, free IGF-I, and ALS were more sensitive to GH excess than to GHD.

*Acromegaly*

The most accurate method for diagnosing acromegaly is a GH response greater than 1 $\mu$g/L to a glucose load (1, 3–5, 22). The employment of ultrasensitive assays allows discrimination of a lower GH threshold and optimizes diagnostic accuracy (23–25). However, postglucose suppression of GH levels seems not to definitely exclude active acromegaly (26). The diagnostic role played by IGF-I in acromegaly is well established (3–5, 25, 26) and was confirmed in the present study in both active and cured patients. Conversely, IGFBP-3 results were diagnostically unsuccessful in 10 active cases. Given the evidence that the measurement of IGFBP-3 concentrations, formerly proposed as a discriminatory indicator of acromegaly, can overlap normal levels in a considerable proportion of patients (9, 16, 26, 27), our results are in line with these observations.

One of our most striking observations was ALS and free IGF-I sensitivity in active acromegaly. ALS has been shown to reach concentrations 2.5-fold higher than those in controls (28) and to better predict the postoperative recurrence of acromegaly than does IGF-I (13). Some patients, however, can present with inappropriately normal ALS concentrations (11). In this study ALS sensitivity was generally lower than that of IGF-I in active acromegaly. This discrepancy could not be attributed to individual differences in age, disease duration, or GH levels. Conflicting results also exist regarding the usefulness of free IGF-I in acromegaly. Basal free IGF-I levels are usually elevated and generally correlate with both baseline and glucose-suppressed GH levels (14–16). Overlap with normal free IGF-I values has been reported in up to 73% of unsuccessfully treated acromegalic patients (16). In the current study a significant elevation of free IGF-I levels was found in as many as 94% of active patients, and as previously reported by van der Lely and co-workers (15), no correlation existed between free and total IGF-I levels. Despite these observations in active acromegaly, the utility of free IGF-I assays may be blunted by the persistence of high concentrations in the group of inactive acromegalic patients.

*Adult GHD*

Data analysis in GHD patients showed a greater variability of results. To date, no definitive conclusion exists on the reliability of the IGF system in diagnosing adult GHD. IGF-I is significantly decreased in childhood-onset GHD patients, and IGF-I reduction is generally greater in the case of organic pituitary disease, multiple hormonal insufficiencies, and younger onset age of GHD (29–34). However, the occurrence of normal IGF-I values can occur in 34–70% of adult-onset GHD patients (30, 34–37), and this result is confirmed in the present study. The diagnostic accuracy of IGFBP-3 measurements was slightly greater than that of IGF-I. In other studies, IGFBP-3 was shown to be more sensitive than IGF-I measurements in idiopathic GHD (38), but a comparison between the sensitivities of GH peak response and IGFBP-3 levels in short children did not confirm such a finding (39). Furthermore, up to 72% of adult-onset GHD patients can exhibit false negative IGFBP-3 results (18, 35, 37).

The impact of low GH levels on ALS and free IGF-I concentrations was even less clear. The age of onset and the severity of GHD have been proposed as determinant factors in reducing ALS levels (40–42). Despite the finding of low ALS levels in up to 72% of childhood-onset GHD patients (12), ALS concentrations in adult GHD have been found to overlap both normal and acromegalic values (11). We observed reduced ALS levels in only one third of cases; stratification of patients for GH peak response below 3 $\mu$g/L increased this percentage to 43% of cases. Similar figures were obtained for free IGF-I measurements. There is circumstantial evidence indicating that free IGF-I results vary depending on the assay techniques. Therefore, the reproducibility of data might depend on the methodology employed. By means of direct IRMA measurements, free IGF-I levels have been reported to be significantly reduced in over 70% of childhood-onset adult GHD patients (17) and to be below the lower normal limits in 52% of adult-onset GHD patients (18). Other researchers have reported results comparable to ours using ultrafiltration techniques (43).

*IGFBP-3 complexes in acromegaly and adult GHD*

One unique feature of the present study is the extensive evaluation of two novel immunoassays, recently generated by pairwise combination of anti-IGFBP-3 with anti-IGF-I (IGFBP-3-IGF-I complex) or anti-ALS (IGFBP-3-ALS complex) antibodies (20). The theoretical advantage of these assays resides in the ability of directly measuring the combined

levels of several GH-dependent molecules in a single assay as well as reducing the effect of IGFBP-3 fragments and other IGFBPs. The present data confirm and extend previous findings. IGFBP-3-IGF-I levels appeared to be a reliable marker of acromegaly and reached mean concentrations 5.4-fold higher than those in controls; they were above +2 SD in 95% of patients. A lower sensitivity was shown for IGFBP-3-ALS complex. On the other hand, neither marker accurately distinguished adult GHD. The IGFBP-3-IGF-I ELISA is capable of quantifying any ALS-IGFBP-3-IGF-I ternary complex and any binary IGFBP-3-IGF-I complex that could be present in the circulation; the IGFBP-3-ALS ELISA interacts with both IGF-I- and IGF-II-based ternary complexes and, potentially, with any IGFBP-3-ALS combination. We cannot exclude, therefore, that the intrinsic immunoreactivity of the IGFBP-3-ALS ELISA as well as the dynamics of the ternary and binary complex in this condition (10) might contribute to the high IGFBP-3-IGF-I accuracy in acromegaly.

In conclusion, our data support the strong diagnostic role of IGF-I as marker of GH excess. IGFBP-3, ALS, and free IGF-I values provide complementary information, which can assist decision-making procedures in acromegaly. In agreement with previous studies, our findings in adult GHD patients indicate that a single ALS and free IGF-I determination does not offer significant diagnostic advantage over IGF-I and IGFBP-3 measurements. However, in the absence of a generally accepted gold standard test, it remains difficult to assess whether any marker can predict GHD better than the GH peak response to provocative testing. Finally, the results on IGFBP-3 complexes suggest that these markers, particularly the complex of IGFBP-3-IGF-I, can offer relevant information in diagnostic strategies for GH disorders and potentially open new diagnostic perspectives in conditions associated with GH/IGF-I alterations.

## References


1. **Melmed S, Ho K, Klibanski A, Reichlin S, Thorner M.** 1995 Recent advances in pathogenesis, diagnosis, and management of acromegaly. J Clin Endocrinol Metab. 80:3395–3402.
2. **de Boer H, Blok GJ, Van der Veen EA.** 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev. 16:63–86.
3. **Clemmons DR, Van Wyk JJ, Ridgway EC, Kliman B, Kjellberg RN, Underwood LE.** 1979 Evaluation of acromegaly by radioimmunoassay of somatomedin-C. N Engl J Med. 301:1138–1142.
4. **Barkan AL, Beitins IZ, Kelch RP.** 1988 Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. J Clin Endocrinol Metab. 67:69–73.
5. **Colao A, Lombardi G.** 1998 Growth hormone and prolactin excess. Lancet. 352:1455–1461.
6. **Rosenfeld RG, Wilson DM, Lee PD, Hintz RL.** 1986 Insulin-like growth factors I and II in evaluation of growth retardation. J Pediatr. 109:428–433.
7. **Baxter RC.** 1994 Insulin-like growth factor binding proteins in the human circulation: a review. Horm Res. 42:140–144.
8. **Grinspoon S, Clemmons D, Swearingen B, Klibanski A.** 1995 Serum insulin-like growth factor-binding protein-3 levels in the diagnosis of acromegaly. J Clin Endocrinol Metab. 80:927–932.
9. **de Herder WW, van der Lely AJ, Janssen JA, Uitterlinden P, Hofland LJ, Lamberts SW.** 1995 IGFBP-3 is a poor parameter for assessment of clinical activity in acromegaly. Clin Endocrinol (Oxf). 43:501–505.
10. **Baxter RC, Martin JL.** 1989 Structure of the Mr 140,000 growth hormone-dependent insulin-like growth factor binding protein complex: determination by reconstitution and affinity-labeling. Proc Natl Acad Sci USA. 86:6898–6902.
11. **Khosravi MJ, Diamandi A, Mistry J, Krishna RG, Khare A.** 1997 Acid-labile subunit of human insulin-like growth factor-binding protein complex: measurement, molecular, and clinical evaluation. J Clin Endocrinol Metab. 82:3944–3951.
12. **Juul A, Moller S, Mosfeldt-Laursen E, et al.** 1998 The acid-labile subunit of human ternary insulin-like growth factor binding protein complex in serum: hepatosplanchnic release, diurnal variation, circulating concentrations in healthy subjects, and diagnostic use in patients with growth hormone deficiency. J Clin Endocrinol Metab. 83:4408–4415.
13. **Arosio M, Garrone S, Bruzzi P, Faglia G, Minuto F, Barreca A.** 2001 Diagnostic value of the acid-labile subunit in acromegaly: evaluation in comparison with insulin-like growth factor (IGF)-I, and IGF-binding protein-1, -2, and -3. J Clin Endocrinol Metab. 86:1091–1098.
14. **Frystyk J, Skjaerbaek C, Dinesen B, Orskov H.** 1994 Free insulin-like growth factors (IGF-I and IGF-II) in human serum. FEBS Lett. 348:185–191.
15. **van der Lely AJ, de Herder WW, Janssen JA, Lamberts SW.** 1997 Acromegaly: the significance of serum total and free IGF-I and IGF-binding protein-3 in diagnosis. J Endocrinol. 155(Suppl 1):S9–S13.
16. **Stoffel-Wagner B, Springer W, Bidlingmaier F, Klingmuller D.** 1997 A comparison of different methods for diagnosing acromegaly. Clin Endocrinol (Oxf). 46:531–537.
17. **Juul A, Holm K, Kastrup KW, et al.** 1997 Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. J Clin Endocrinol Metab. 82:2497–2502.
18. **Musolino NR, Da Cunha Neto MB, Marino Junior R, Giannella-Neto D, Bronstein MD.** 1999 Evaluation of free insulin-like growth factor-I measurement on the diagnosis and follow-up treatment of growth hormone-deficient adult patients. Clin Endocrinol (Oxf). 50:441–449.
19. **Kawai N, Kanzaki S, Takano-Watou S, et al.** 1999 Serum free insulin-like growth factor I (IGF-I), total IGF-I, and IGF-binding protein-3 concentrations in normal children and children with growth hormone deficiency. J Clin Endocrinol Metab. 84:82–89.
20. **Khosravi J, Diamandi A, Mistry J, Krischna RG.** 1999 The high molecular weight insulin-like growth factor-binding protein complex: epitope mapping, immunoassay, and preliminary clinical evaluation. J Clin Endocrinol Metab. 84:2826–2833.
21. **Colao A, Cerbone G, Pivonello R, et al.** 1999 The growth hormone (GH) response to the arginine plus GH-releasing hormone test is correlated to the severity of lipid profile abnormalities in adult patients with GH deficiency. J Clin Endocrinol Metab. 84:1277–1282.
22. **Giustina A, Barkan A, Casanueva FF, et al.** 2000 Criteria for cure of acromegaly: a consensus statement. J Clin Endocrinol Metab. 85:526–529.
23. **Hattori N, Shimatsu A, Kato Y, et al.** 1990 Growth hormone responses to oral glucose loading measured by highly sensitive enzyme immunoassay in normal subjects and patients with glucose intolerance and acromegaly. J Clin Endocrinol Metab. 70:771–776.
24. **Chapman IM, Hartman ML, Straume M, Johnson ML, Veldhuis JD, Thorner MO.** 1994 Enhanced sensitivity growth hormone (GH) chemiluminescence assay reveals lower postglucose nadir GH concentrations in men than women. J Clin Endocrinol Metab. 78:1312–1319.
25. **Freda PU, Post KD, Powell JS, Wardlaw SL.** 1998 Evaluation of disease status with sensitive measures of growth hormone secretion in 60 postoperative patients with acromegaly. J Clin Endocrinol Metab. 83:3808–3816.
26. **Chang-DeMoranville BM, Jackson IM.** 1992 Diagnosis and endocrine testing in acromegaly. Endocrinol Metab Clin North Am. 21:649–668.
27. **Juul A, Main K, Blum WF, Lindholm J, Ranke MB, Skakkebaek NE.** 1994 The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. Clin Endocrinol (Oxf). 41:85–93.
28. **Baxter RC.** 1997 The binding protein's binding protein: clinical applications of acid-labile subunit (ALS) measurement. J Clin Endocrinol Metab. 82:3941–3943.
29. **Growth Hormone Research Society (GRS).** 1998 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. 83:379–381.
30. **Shalet SM, Toogood A, Rahim A, Brennan BM.** 1998 The diagnosis of growth hormone deficiency in children and adults. Endocr Rev. 19:203–223.
31. **Toogood AA, Beardwell CG, Shalet SM.** 1994 The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. Clin Endocrinol (Oxf). 41:511–516.
32. **Attanasio AF, Lamberts SW, Matranga AM, et al.** 1997 Adult growth hormone (GH)-deficient patients demonstrate heterogeneity between childhood onset and adult onset before and during human GH treatment. Adult Growth Hormone Deficiency Study Group. J Clin Endocrinol Metab. 82:82–88.
33. **Svensson J, Johannsson G, Bengtsson BA.** 1997 Insulin-like growth factor-I in growth hormone-deficient adults: relationship to population-based normal values, body composition and insulin tolerance test. Clin Endocrinol (Oxf). 46:579–586.
34. **Hilding A, Hall K, Wivall-Helleryd IL, Saaf M, Melin AL, Thoren M.** 1999 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. 84:2013–2019.
35. **Hoffman DM, O'Sullivan AJ, Baxter RC, Ho KK.** 1994 Diagnosis of growth-hormone deficiency in adults. Lancet. 343:1064–1068.

36. **Bates AS, Evans AJ, Jones P, Clayton RN.** 1995 Assessment of GH status in adults with GH deficiency using serum growth hormone, serum insulin-like growth factor-I and urinary growth hormone excretion. Clin Endocrinol (Oxf). 42:425–430.
37. **Thissen JP, Ketelslegers JM, Maiter D.** 1996 Use of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in the diagnosis of acromegaly and growth hormone deficiency in adults. Growth Regul. 6:222–229.
38. **Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB.** 1993 Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab. 76:1610–1616.
39. **Phillip M, Chalew SA, Kowarski AA, Stene MA.** 1993 Plasma IGFBP-3 and its relationship with quantitative growth hormone secretion in short children. Clin Endocrinol (Oxf). 39:427–432.
40. **de Boer H, Blok GJ, Popp-Snijders C, Stuurman L, Baxter RC, van der Veen E.** 1996 Monitoring of growth hormone replacement therapy in adults, based on measurement of serum markers. J Clin Endocrinol Metab. 81:1371–1377.
41. **Thoren M, Hilding A, Baxter RC, Degerblad M, Wivall-Helleryd IL, Hall K.** 1997 Serum insulin-like growth factor I (IGF-I), IGF-binding protein-1 and -3, and the acid-labile subunit as serum markers of body composition during growth hormone (GH) therapy in adults with GH deficiency. J Clin Endocrinol Metab. 82:223–228.
42. **Labarta JI, Gargosky SE, Simpson DM, et al.** 1997 Immunoblot studies of the acid-labile subunit (ALS) in biological fluids, normal human serum and in children with GH deficiency and GH receptor deficiency before and after long-term therapy with GH or IGF-I respectively. Clin Endocrinol (Oxf). 47:657–666.
43. **Skjaerbaek C, Vahl N, Frystyk J, et al.** 1997 Serum free insulin-like growth factor-I in growth hormone-deficient adults before and after growth hormone replacement. Eur J Endocrinol. 137:132–137.

## Erratum

In the article "A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the Multiple Outcomes of Raloxifene Evaluation Clinical Trial" by Paul Lips, Tu Duong, Anna Oleksik, Dennis Black, Steven Cummings, David Cox, and Thomas Nickelsen for the Multiple Outcomes of Raloxifene Evaluation Study Group (*The Journal of Clinical Endocrinology & Metabolism* **86:**1212–1221), the name of Louis G. Ste-Marie, M.D. (Centre-Hospitalier de l'Université de Montréal—Campus St. Luc, Montréal, Quebéc, Canada), was inadvertently omitted from the list of investigators who participated in the MORE trial. The authors regret the error.