Measurement of the acid-labile subunit of the insulin-like growth factor binding protein complex in human serum: a comparison of four immunoassays

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Abstract

The acid-labile subunit (ALS) of the high molecular weight insulin-like growth factor binding protein complex is a liver-derived glycoprotein which is regulated by growth hormone and serves as a serum marker of growth hormone action. We have compared the measurement of ALS by four immunoassay methods (two RIAs, two ELISAs) utilizing various polyclonal and monoclonal antibodies raised against natural or recombinant human ALS, or synthetic ALS peptides. Despite the variety of methodologies and reagents, results obtained by the four methods were highly correlated for 125 sera from various patient groups, and when compared for individual groups of sera

from healthy children and adults, growth hormone-deficient children and adults, and subjects with acromegaly. Some weaker correlations among methods were seen when measuring ALS levels in groups of sera from pregnant subjects and subjects with chronic renal failure. An assay using antibodies raised against recombinant ALS yielded lower apparent values than the other methods in patient sera, the discrepancy probably being attributable to a difference in standardization. We conclude that a variety of assay formats and reagents can yield serum ALS values of potential clinical utility.

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Introduction

The optimal assessment of growth hormone (GH) secretory status has been a problem for endocrinologists for many years (Ranke & Haber 1992, Hoffman *et al.* 1994). Since GH itself has a pulsatile pattern of secretion and is influenced by stress, exercise and other factors, a random serum GH measurement is of limited diagnostic value. Multiple sampling at short time intervals, quantitation of the peak GH response to physiological or pharmacological stimuli, and the measurement of GH-dependent proteins such as insulin-like growth factor (IGF)-I, are among the many approaches that have been taken to overcome this problem (Albertsson-Wikland & Rosberg 1992). While multiple sampling yields a direct measure of the GH secretory profile, its applicability is limited, due to the many GH determinations needed for each profile. Less sampling is required to determine the GH response to stimulation by exercise, insulin-induced hypoglycemia, or agents such as arginine, clonidine, GH-releasing hormone

and short synthetic GH-releasing peptides (Ranke & Haber 1992). However, these agents do not always provoke a GH response in every patient, and the establishment of the threshold response required to define normal GH secretion has been controversial.

GH acts on the liver and other tissues to stimulate the production of IGF-I, an anabolic and mitogenic peptide which mediates the growth-promoting activity of GH (Humbel 1990). The normal synthesis of IGF-I is dependent on the activation of hepatic GH receptors. Serum IGF-I levels are markedly reduced in GH-deficient subjects, or in subjects with adequate GH secretion but absent, inactive or down-regulated GH receptors (Donaghy & Baxter 1996, Rosenfeld 1996). Like the structurally related but less GH-dependent peptide, IGF-II, IGF-I is carried in the circulation in complexes with IGF binding proteins (IGFBPs). This family of six homologous proteins is characterized by strong conservation of structure in both their amino- and carboxy-terminal domains, and high binding affinities ($K_a = 10^9$ – 10^{11} mol/l)

for IGF-I and IGF-II (Baxter 1997a). The majority of the IGFs in serum are associated with the most abundant binding protein, IGFBP-3, which can combine with another protein, known as the acid-labile subunit or ALS, to form heterotrimeric complexes containing IGF, IGFBP-3 and ALS (Baxter & Martin 1989). ALS is a leucine-rich glycoprotein of approximately 85 kDa, the protein core of which is predicted to be largely toroidal (Leong *et al.* 1992, Janosi *et al.* 1999b). It has recently been shown that ALS can also form similar ternary complexes with IGFBP-5 and IGFs (Twigg & Baxter 1998), although IGFBP-5 complexes would be expected to be relatively minor carriers of IGFs compared with IGFBP-3, due to the 5- to 10-fold lower serum IGFBP-5 concentration (Baxter & Martin 1986, Mohan *et al.* 1995).

Like IGF-I, both IGFBP-3 (Baxter & Martin 1986) and IGFBP-5 (Ono *et al.* 1996) are GH-dependent proteins, and IGFBP-3 measurement in serum has been used to evaluate GH secretory status in children and adults (Blum *et al.* 1993, Juul *et al.* 1997). Synthesis of the third member of the complex, ALS, appears to occur exclusively in the liver, and is similarly dependent on GH (Dai *et al.* 1994), with levels in the serum of hypophysectomized rats being less than 5% of those in normal controls (Fielder *et al.* 1996). While serum ALS levels are also markedly GH-dependent in humans (Baxter 1990), there has been limited detailed evaluation of the value of ALS as an index of GH secretory status or action, mainly because the assay has remained in very restricted use since its introduction in 1990 (Baxter 1997b). A variety of independent assay methods have recently become available, and this study was therefore designed to evaluate and compare their performance with serum samples from subjects in a variety of different physiological and pathological states.

Materials and Methods

Standard ALS preparation

ALS was isolated from human serum as previously described (Baxter *et al.* 1989), except that the final high-performance ion-exchange chromatography was performed on a 4.6 × 250 mm Bakerbond PEI column (Phenomenex, Torrance, CA, USA). Recombinant human ALS was produced in Chinese hamster ovary (CHO) cells at Genentech (San Francisco, CA, USA). The protein concentrations of native and recombinant human ALS were determined by quantitative amino acid analysis. The calculated molecular mass of ALS, based on its predicted amino acid sequence, is 63.1 kDa, but both the native and recombinant proteins appear as a glycoprotein doublet of 84–86 kDa by SDS-PAGE. Based on these observations, we assume here that 1 nmol ALS is equal to 63.1 µg nonglycosylated protein or 85 µg glycosylated protein. For the purpose of comparison, all

Figure 1 Representative standard curves for the four immunoassay methods. For Methods 1 and 2, B/B₀ represents the ratio of radioactivity bound in the presence of standard (B) to that bound in the absence of standard (B₀), where both B and B₀ have been corrected for nonspecific binding (NSB). Method 1: B₀=42% of total radioactivity, NSB=3.6%; Method 2: B₀=45% of total radioactivity, NSB=1.6%. For Methods 3 and 4, blank absorbance (less than 0.1 Absorbance unit) has been subtracted. 100 fmol=8.5 ng glycosylated ALS.

results are expressed in molar units (100 nmol/l=8.5 mg/l glycosylated ALS).

Assay methods

Method 1 This is a conventional in-house radioimmunoassay first described in 1990 (Baxter 1990). This assay uses a rabbit polyclonal antibody (designated AL3) raised against human serum ALS. In brief, serum samples (25 µl of a 1:100 dilution) or standards were incubated for 16 h at 22 °C with ¹²⁵I-labeled ALS (approx. 10 000 c.p.m., 1 ng) and ALS antiserum at a final concentration of 1:100 000, in a total volume of 0.5 ml. Separation was by precipitation with goat anti-rabbit immunoglobulin G (IgG) and polyethylene glycol. Method 1, calibrated in molar units, covers an analytical range of 1–250 fmol/tube (0.085–21.3 ng/tube), equivalent to 4–1000 nmol/l (0.34–85 mg/l) in serum samples assayed at 25 µl of a 1:100 dilution.

Method 2 This is a commercial radioimmunoassay (Bioclone Australia Pty. Limited, Sydney, NSW, Australia) using a rabbit polyclonal antibody raised against human

Table 1 Estimates of within- and between-assay precision of the 4 assay methods

	Within-assay precision			Between assay precision		
	Low	Medium	High	Low	Medium	High
<i>Method 1</i>						
Mean $\pm$ S.D. (nmol/l)	35.0 $\pm$ 2.3	139.6 $\pm$ 5.4	536.4 $\pm$ 10.0	35.0 $\pm$ 4.7	139.6 $\pm$ 9.3	536.4 $\pm$ 2.3
CV (%)	6.6	3.9	1.9	13.4	6.7	7.9
n	10	10	10	10	10	10
<i>Method 2</i>						
Mean $\pm$ S.D. (nmol/l)	48.5 $\pm$ 2.6	207 $\pm$ 5.8	488 $\pm$ 20.0	50.6 $\pm$ 2.1	208 $\pm$ 8.2	344 $\pm$ 16.7
CV (%)	5.4	2.8	4.1	4.2	3.9	4.9
n	22	22	22	39	39	39
<i>Method 3</i>						
Mean $\pm$ S.D. (nmol/l)	19.4 $\pm$ 1.2	90.8 $\pm$ 6.8	343.2 $\pm$ 13.2	26.1 $\pm$ 2.2	92.9 $\pm$ 2.6	354.5 $\pm$ 31.4
CV (%)	6.1	7.5	3.8	8.6	2.8	8.9
n	8	8	8	9	9	9
<i>Method 4</i>						
Mean $\pm$ S.D. (nmol/l)	43.4 $\pm$ 1.4	61.5 $\pm$ 6.7	—	43.4 $\pm$ 5.7	61.5 $\pm$ 3.1	—
CV (%)	3.3	10.9	—	13.1	5.1	—
n	8	8	—	8	8	—

serum ALS (Senese *et al.* 1997). The protocol was broadly similar to that of Method 1, except that serum samples were added as 100 μ l of a 1:200 dilution, approx. 2.5 ng ALS tracer ($\sim$ 25 000 c.p.m.) were used, and separation of bound from free radioactivity depended on the use of magnetizable beads coated with an anti-rabbit second antibody. Method 2, expressed in molar units, covers an analytical range of 5.3–503 fmol/tube (0.45–42.8 ng/

tube), equivalent to 10.6–1006 nmol/l (0.90–85.5 mg/l) in serum samples assayed at 100 μ l of a 1:200 dilution.

Method 3 This is a commercial two-site enzyme-linked immunosorbent assay (ELISA) (DSL Inc., Webster, TX, USA) using goat antisera raised against synthetic amino- and carboxy-terminal peptides of human ALS (Khosravi *et al.* 1997). In brief, standards and samples were pretreated

Figure 2 Mean values $\pm$ S.E.M. for ALS measured by four methods, as indicated, in sera from groups of normal boys ($n=15$), girls ($n=16$), adults ($n=10$), GH-deficient (GHD) children ($n=10$), GHD adults, either untreated ($n=21$) or treated (tr, $n=11$) with GH, acromegalic adults ($n=22$), pregnant women ($n=10$), and adults with chronic renal failure ($n=10$).

Figure 3 Difference plots showing the relationship between ALS values, in nmol/l, for all 125 subjects in the study, measured using Method 1, and values measured by Methods 2 (top), 3 (middle) and 4 (bottom).

in SDS to optimize their immunoreactivity, and aliquots (20 μ l of a 1:100 dilution) were incubated in microtiter plates coated with capture antibody for 1 h at 22 °C. The standard solution was made in an assay buffer matrix containing partially purified ALS from human serum as previously described (Khosravi *et al.* 1997), calibrated against the same natural (glycosylated) ALS reference preparation as in Methods 1 and 2. Enzyme-labeled detection antibody was added, incubated at 22 °C for 30 min, wells were aspirated and washed, chromogen solution was added, and color development stopped after 10 min. Absorbances were read on a plate reader at 450 nm. The Method 3 standards, calibrated in mass units expressed as glycosylated ALS, range from 0.16–11 ng/

well (1.9–129 fmol/well), equivalent to 0.8–55 mg/l (9.4–650 nmol/l) in serum samples assayed at 20 μ l of a 1:100 dilution.

Method 4 This is a two-site ELISA developed at Genentech Inc. (South San Francisco, CA, USA) using monoclonal antibodies raised against recombinant human (rh) ALS. Standards (rhALS) and diluted samples (50 μ l of 1:50, 1:150, 1:450 and 1:1350 dilutions) were added to microtiter plate wells coated with capture antibody, and incubated for 2 h at 22 °C together with enzyme-labeled detection antibody. After washing, chromogen solution was added and color development was stopped after 15 min. Absorbances were read on a plate reader at

450 nm. Method 4 standards, when converted from their calibrated protein mass, ignoring glycosylation, to the equivalent mass of glycosylated ALS, cover the range of 0.11–6.75 ng/well (1.2–79 fmol/well), equivalent to 0.32–20.2 mg/l (3.7–238 nmol/l) in serum samples assayed at 50 µl of a 1:150 dilution.

Serum samples

The 125 serum samples used in this trial were selected to allow the best comparison of different assay methods on sera containing a wide range of ALS concentrations, and not to provide reference ranges for various clinical conditions. All samples were obtained with the permission of the relevant institutional ethics committees. They comprised samples from healthy non-pregnant adults ($n=10$), pregnant women ($n=10$), healthy girls aged 10–17 years ($n=16$), healthy boys aged 10–15 years ($n=15$), untreated GH-deficient adults ($n=21$), GH-deficient adults replaced with hGH (0.125 U/kg/week, s.c.) for 6 months ($n=11$), GH-deficient children ($n=10$), adults with acromegaly ($n=22$), and adults with chronic renal failure ($n=10$).

Data analysis

Method 1 was published in 1990 (Baxter 1990) and was the only existing ALS assay for some 8 years after its introduction. It is therefore regarded as the *de facto* standard method, and was used as the reference method in this study. Mean values for groups were compared by analysis of variance followed by Fisher's Protected Least Significant Difference (PLSD) test, performed using StatView 4.02 (Abacus Concepts, Berkeley, CA, USA). Slopes and intercepts of regression lines, with 95% confidence limits, were calculated using UltraFit 3.0 (Biosoft, Cambridge, Cambs, UK).

Results

Typical standard curves for the 4 methods are shown in Fig. 1. For the RIA methods (Methods 1 and 2), the response variable is B/B_0 , the radioactivity bound in the presence of each standard, expressed as a percentage of that bound in the absence of standard. For the ELISA methods (Methods 3 and 4), the response variable is absorbance at 450 nm. Methods 1 and 4 gave half-maximal responses (estimated as the half-maximal measured response) in the range 15–20 fmol/tube or well, whereas for Methods 2 and 3, the half-maximal responses were in the range 40–50 fmol/tube or well. Estimates of the within- and between-assay precision for the 4 assay methods are summarized in Table 1.

A set of 125 serum samples was run in all four assay methods. For the 125 samples, the mean value ($\pm$ s.d.) obtained by Method 1 was 230.6 ± 124.7 nmol/l; for

Table 2 Comparisons between values for all samples, determined by Methods 2, 3, or 4, and values determined by Method 1. Values are shown with 95% confidence limits

	Slope	Intercept	Correlation coefficient
Comparison with Method 1			
Method 2	0.916 ± 0.045	27.4 ± 11.8	0.964
Method 3	0.953 ± 0.056	10.7 ± 14.7	0.950
Method 4	0.615 ± 0.032	16.3 ± 8.4	0.959

Method 2, 238.6 ± 118.4 nmol/l; for Method 3, 230.3 ± 125.0 nmol/l; and for Method 4, 158.1 ± 79.9 nmol/l (significantly lower than the means for the other three methods, $P<0.0001$). Figure 2 shows the mean values $\pm$ s.e.m. for each assay method, for all subject groups. In each case, means for Methods 1–3 were in close agreement, whereas the mean for Method 4 was lower.

Figure 3 shows difference plots comparing results for all samples obtained by Methods 2, 3 and 4 with those obtained by Method 1, which is regarded as the reference method. Only the comparison between Methods 1 and 4 shows evidence of a systematic dose-dependent discrepancy, consistent with a difference in standardization of the two methods. The same data obtained by Methods 2, 3 and 4 were also compared with those obtained by Method 1 in simple linear regression analyses. The slopes and intercepts of the regression lines derived from these analyses are presented in Table 2, with 95% confidence limits. Despite the different methodologies (radioimmunoassay and ELISA), and the use of antibodies raised against natural protein, recombinant protein or synthetic part-sequence peptides, all methods gave remarkably consistent results, with values for the correlation coefficient (r) for the various comparisons of at least 0.950, and intercepts close to zero. Slopes were close to unity for the comparisons Method 2 vs Method 1 and Method 3 vs Method 1. In contrast, the slope for the comparison Method 4 vs Method 1 was 0.615. Together with the lower overall mean value for Method 4, this is consistent with the difference plots in suggesting a discrepancy in calibration between the standard used in Method 4 and those used in the other methods.

When the rhALS standard used in Method 4 was calibrated against the same native ALS reference preparation used to calibrate the other Method standards, values obtained for unknown samples were elevated by a factor of 1.73, which would normalize the slope of the calibrated Method 4 vs Method 1 comparison plot to approximately 1.0 (data not shown).

To examine possible differences between the four methods in greater detail, comparisons were performed on subsets of samples representing various categories of subjects. Figure 4 shows difference plot analyses for the 41

Figure 4 Difference plots showing the relationship between ALS values, in nmol/l, for 41 normal children and adults, measured using Method 1, and values measured by Methods 2 (top), 3 (middle) and 4 (bottom).

normal, nonpregnant subjects. As seen in Fig. 3, only the comparison between Methods 1 and 4 shows evidence of a systematic dose-dependent discrepancy, consistent with the standardization difference. For the 41 normal, nonpregnant subjects' samples, the mean value ($\pm$ s.d.) obtained by Method 1 was 226.3 ± 53.5 nmol/l; for Method 2, 236.1 ± 45.7 nmol/l; for Method 3, 213.9 ± 57.8 nmol/l; and for Method 4, 157.2 ± 41.6 nmol/l. As shown in Table 3, correlation coefficients

derived from linear regression analyses were high (in the range 0.84–0.89), although the slope of the Method 2 vs Method 1 comparison was only 0.76, due to a large Y-axis intercept. Similar comparisons for groups of subjects with GH disorders (all GH-deficient subjects, all acromegalic subjects) are shown in Table 3; again, correlation coefficients were high (in the range 0.82–0.98), and the slopes of regression lines for comparisons of Method 4 vs Method 1 were consistently lower than for comparisons between

Table 3 Comparisons between values for various subject groups, determined by Methods 2, 3, or 4, and values determined by Method 1. Values are shown with 95% confidence limits

Subject group	Comparison with Method 1	Slope	Intercept	Correlation coefficient
Normal ^a (n=41)	Method 2	0.760 ± 0.127	64.0 ± 29.5	0.889
	Method 3	0.908 ± 0.190	8.5 ± 44.1	0.840
	Method 4	0.694 ± 0.114	0.1 ± 26.4	0.893
GH-deficient ^b (n=42)	Method 2	0.987 ± 0.071	14.0 ± 11.3	0.976
	Method 3	1.031 ± 0.123	10.4 ± 19.6	0.937
	Method 4	0.718 ± 0.055	0.8 ± 8.8	0.972
Acromegalic (n=22)	Method 2	0.772 ± 0.250	93.4 ± 107.9	0.821
	Method 3	0.985 ± 0.211	2.4 ± 91.1	0.908
	Method 4	0.587 ± 0.182	17.5 ± 78.5	0.832
Pregnant (n=10)	Method 2	0.294 ± 0.522 ^{NS}	222.8 ± 161.6	0.417
	Method 3	0.559 ± 0.410	140.0 ± 126.9	0.744
	Method 4	0.535 ± 0.229	58.2 ± 70.8	0.885
Chronic renal failure (n=10)	Method 2	0.724 ± 0.183	47.4 ± 28.9	0.956
	Method 3	0.406 ± 1.067 ^{NS}	78.8 ± 168.8	0.297
	Method 4	0.560 ± 0.166	29.2 ± 26.3	0.940

^aPooled data for children and adults. ^bPooled data for all GH-deficient groups. ^{NS}Slope not significantly different from zero.

Methods 2 or 3 vs Method 1, consistent with a systematic effect of assay calibration for Method 4.

The final comparisons were for samples from pregnant subjects and patients with chronic renal failure, both conditions where proteolysis of IGFBP-3 is reported to occur (Hossenlopp *et al.* 1990, Powell *et al.* 1993). In these relatively small sample groups, greater discrepancies among some of the methods were seen than in the other patient groups (Table 3). For the pregnancy sera, the Method 2 vs Method 1 comparison showed a slope not significantly different from zero, and for the chronic renal failure sera, the Method 3 vs Method 1 comparison had a non-significant slope. However, it should be noted that for both the pregnancy and chronic renal failure sample groups, the mean values obtained for the 10 sera in each group were virtually identical for Methods 1–3, and were systematically lower only for Method 4 (Fig. 2).

Discussion

This study demonstrates strong overall correlations among the values obtained in the four ALS immunoassay methods tested, despite the different analytical approaches used, and the likely differences in the epitopes recognized by the antibodies. This uniformity of results suggests that, at least for healthy subjects and those with GH disorders, ALS probably circulates in a substantially intact form; i.e. there are no significant concentrations of ALS fragments, or alternative conformations of ALS, that might be detected differentially by the different antibodies. Methods 1, 2, and

4 detect denatured (i.e. acidified) ALS poorly, if at all, and are believed to detect complexed ALS as effectively as free ALS, whereas Method 3 relies on the denaturation (by SDS) of ALS to increase detection by 200-fold (Khosravi *et al.* 1997). The close parallel in the values for different patient groups detected by these different methodological approaches indicates that most or all of the ALS in the circulation is in the native conformation, since a significant concentration of denatured ALS would lead to systematically lower values by Method 1 compared with Method 3.

Despite the strong correlations among the four methods for most samples, it was evident that results obtained using Method 4 were systematically lower than those for the other three methods, suggesting a difference in calibration. Standards used in Methods 1–3 were all calibrated against the same reference preparation of natural ALS, whereas Method 4 used a different primary standard of recombinant human ALS derived from CHO cells. Concentrations of both primary standards were determined by quantitative amino acid analysis in the same laboratory. However, the recombinant protein showed ~1.73 times the immunoreactivity of the natural protein, and parallelism with native ALS, when the native and recombinant proteins were compared in Method 4 (data not shown).

As the full amino acid sequence of human ALS was deduced from the cDNA sequence, with only partial sequencing of the natural protein (Leong *et al.* 1992), the possibility of a difference in primary structure between serum-derived and recombinant ALS cannot be excluded. However, quantitative amino acid analyses of the natural and recombinant proteins have suggested that both

molecules have the amino acid composition predicted by the cDNA. A more likely difference between the two preparations is in the extensive glycosylation, which accounts for some 25% of the apparent molecular mass of 85 kDa of the fully glycosylated protein (Baxter *et al.* 1989). This carbohydrate appears to play an essential role in ternary complex formation (Janosi *et al.* 1999a). Further physico-chemical characterization will be required to define the apparent differences between rhALS and native ALS which our data suggest. Nevertheless, the present comparison has demonstrated that all four methods are capable of distinguishing ALS values in a wide variety of patient samples to a similar degree.

The two sample groups in which some discrepancies among methods was seen were those from pregnant subjects, and from subjects with renal failure. These groups were included in the analysis as examples of 'difficult' samples, since IGFBP physiology is abnormal in both conditions. In pregnancy, a serum protease is known to cause partial proteolysis of IGFBP-3 (Hossenlopp *et al.* 1990); however, it still forms a normal ternary complex with ALS and appears to transport IGFs normally (Suikkari & Baxter 1992). The functional abnormality in pregnancy IGFBP-3 is thus still controversial. In contrast, the proteolyzed form of IGFBP-3 seen in renal failure serum has an obvious functional abnormality since it circulates at a low molecular weight and fails to complex with ALS (Powell *et al.* 1993, Baxter *et al.* 1994).

Although there may be some disruption to ALS complexes in pregnancy, the apparent discrepancy in ALS values between Methods 1 and 2 when assaying pregnancy sera is, nevertheless, difficult to explain, since the ALS antisera used were raised against the same natural ALS preparation and are believed to have similar specificities (data not shown). The discrepancy is probably exaggerated by the small sample number and the clustered ALS concentrations in these samples; indeed, when one of the 10 data points was omitted, the slope of the regression line between these two sets of data became significant, with a correlation coefficient of 0.768. In contrast, the discrepancy between Methods 1 and 3 when measuring renal failure samples did not appear to be due to a single outlying point. Method 3 uses antisera raised against amino- and carboxy-terminal peptides of ALS, whereas Methods 1, 2, and 4 use antibodies raised against the full-length protein. This may result in discrepancies among the four methods in rare samples containing fragments or truncated forms of ALS, with Method 3 more likely to detect only full-length molecules. Whether amino- or carboxy-terminally truncated fragments of ALS actually circulate in renal failure or any other condition is unknown, but this is clearly an area for further investigation.

The clinical utility of serum ALS determination remains to be fully evaluated (Baxter 1997b). As a GH-dependent, hepatocyte-derived protein its measurement may provide

a unique index of hepatic GH response in conditions such as disorders of GH secretion, catabolic states, and hepatic cirrhosis (Baxter 1997b). Serum ALS levels show significant rises in response to GH administration in GH deficiency (De Boer *et al.* 1996) and to GH secretagogues in prolonged critical illness (Van den Berghe *et al.* 1998). As illustrated by the values obtained in the present comparative study, ALS levels differ markedly among samples from subjects with GH deficiency, normal GH status, and GH excess, so that its measurement may prove useful as a marker of GH secretory status. While recent studies support this idea (Juul *et al.* 1998), the ultimate acceptance of the measurement of ALS as an important tool in clinical practice will await a more extensive evaluation of its diagnostic value in many laboratories. The recent release of several commercial ALS assays should certainly accelerate progress towards this goal.

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