Evaluation of the Comparability of Commercial Ghrelin Assays

To the Editor:

Several assays are available to measure human ghrelin (h-GHS), but the comparability of results among assays is unknown. As an addition to previous investigations concerning the stability of h-GHS (1), it was our objective to compare the two most commonly used commercial h-GHS RIAs, focusing on side-by-side determinations, recoveries, and assay imprecision.

The assays were purchased from Phoenix Pharmaceuticals (cat. no. RK-031-30) and Linco Research (cat. no. GHRT-89HK). Samples were stored at −80°C and assayed as recommended by the manufacturers.

For side-by-side comparisons, we used anonymized surplus sera (n = 151) from clinical studies of lean and obese individuals [mean body mass index (BMI), 26 kg/m²; range, 17–39 kg/m²]. Separate side-by-side comparisons were performed with 68 samples from two individuals (34/profile; BMI, 19.5 kg/m²) taken at 20-min intervals to evaluate the suitability of the assays for determining intraindividual changes. All participants gave informed consent.

For recovery and imprecision studies, serum was collected from four males (27–33 years of age; no drug use; normal blood pressure; BMI, 22–24 kg/m²). Assay calibrators were added to achieve added concentrations of 100, 200, 400, or 800 ng/L. Each was measured 20 times. Recovery was calculated by subtracting the basal value of the sample. The CV was determined for each sample as well as for a Lyphochek® control serum (cat. no. 40141; Bio-Rad), which was also measured 20 times. The interassay CV was calculated from repeated measurements of the Lyphochek control (n = 12) and pooled serum controls (n = 12) by the Phoenix method over a longer period. No data for the interassay CV of the Linco assay are available because it had not been used previously in our laboratories.

EVAPAK 3.0 evaluation software (Boehringer-Mannheim) was used for linear regression analysis (Passing–Bablok) (2).

Each assay uses 100 μL of sample. Linco provides ready-to-use calibrators; Phoenix provides a lyophilized preparation that must first be dissolved in assay buffer and then diluted repeatedly. Linco, in contrast to Phoenix, provides controls (two) per assay.

Both assays have a 100% cross-reactivity with h-des-octanoyl-GHS. Moreover, the Linco assay can be used for rodent and canine sera, whereas the specificity of the Phoenix antibody surprisingly changed between January 2003 (lot 500117) and August 2002 (lot 419523) to 1% cross-reactivity with non-human GHS.

Linco gives the concentrations in ng/L, whereas Phoenix gives concentrations in pg/tube, presented in a general protocol for peptide RIAs. The Phoenix calibration curve includes concentrations of 10–1280 ng/L. The calibrators of the Linco assay range from 100 to 10 000 ng/L. In each assay, a better curve fit was obtained when the two lowest calibrators were excluded.

The side-by-side comparison yielded linear regressions of r = 0.976 (n = 151) and r = 0.890 (intraindividual profiles). The measured values obtained with the Phoenix RIA ranged from 10 to 1240 ng/L (median, 340 ng/L), whereas the measured values obtained with the Linco assay were 10-fold higher (131–11 666 ng/L; median, 3282 ng/L). The regression is shown in Fig. 1. The 10-fold difference was observed neither when the calibrator of the Linco assay was replaced by the calibrator of the Phoenix assay nor vice versa.

Recovery of h-GHS was 54–104% [mean (SD), 75 (22)%] with the Linco assay and 67–123% with the Phoenix assay, with the higher recoveries at higher concentrations of added h-GHS.

Intraassay CV for the Phoenix method were 8–12% at the lower concentrations and 7–8% at higher concentrations. The CV of the Linco assay were 7–11% in the lower range and 5–9% in the upper concentration range.

We consider intraassay CV <10%
acceptable for studies of intraindividual variations in healthy individuals and patients with eating disorders (3, 4). Moreover, the precision of both assays was adequate based on the findings of the recovery experiments. Interassay CV were evaluated only for the Phoenix assay (12% at a concentrations of 500 ng/L; n = 12), which has been used in both laboratories for a longer period.

Despite a good correlation, the measured values obtained with the two assays differed by a factor of 10 (range, 3–38; median, 11). Exchanging the calibrators between the assays dramatically reduced the difference between results obtained with both assays. We therefore interpreted the discrepancy in h-GHS concentrations in the assays, it would be desirable to ascertain the true concentrations in the calibrators by a reference method, e.g., mass spectrometry, as has been done for other peptides (6, 7). Although it appears likely that the difference in values obtained with the two RIAs of exactly 10-fold is based on the omission of a mathematical adjustment, our findings are certainly not based on mistakes in recalculating the pg/tube data of the Phoenix assay into ng/L as the accepted measuring unit.

Overall, both assays appear analytically acceptable, although an adjustment to one reference standard is required. As long as such an adjustment is not performed, users must consider which specific assay has been used for published data. Moreover, switching between the two manufacturers within one study is not recommended.

Recently, Linco introduced an additional system for analyzing the so-called “active” h-GHS (cat. no. GHRA-88HK). This antibody was raised against a h-GHS epitope carrying an octanoyl group on the serine-3 position, which determines the biological function of the hormone by enabling binding to the receptor. To enable this analysis, however, special precautions must be taken, such as acidification of the sample to stabilize the labile side chain. Because a majority of studies performed at present refer to samples that have been collected and stored without fulfilling these requisites, we focused on comparison of those assays designed for the analysis of total GHS in human serum. It is likely, however, that measurement of the physiologically active GHS portion may, in the long term, be more relevant.

References

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Beckman Coulter Access Creatine Kinase MB Assay

To the Editor:

A recent article by Apple et al. (1) presents plasma 99th percentile reference limits and analytical characteristics for the leading cardiac troponin and creatinine kinase MB (CKMB) mass assays. It is important for the readers to understand that the CKMB assay used on the Access Immunoassay System for this study will no longer be available as of December 2003. In March 2003, Beckman Coulter released a new, reformulated assay with technical characteristics different from those reported by Apple et al.

Reference

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