Further work is needed to determine whether changes in fsTnI and/or ssTnI are specific for a given disease (and if so, its severity) and particular muscle types. In addition, the performance of WB-DSA, like any other diagnostic assay using antibodies, is limited by antibody selection and the possibility that modifications of the target protein alter binding affinities and, hence, assay results. It will therefore be necessary to screen different patient cohorts with a variety of antibodies to overcome such limitations. Nevertheless, preferential or selective release of the two isoforms (and their modified products) into blood raises the possibility of improving the differential diagnosis of skeletal muscle injuries or disease, prognosis, and the evaluation of therapeutic effectiveness.

This work was supported by grants to S. I. and J. V. E. from the Canadian Institutes of Health Research (MOP 36339 and MT 14375), the Heart and Stroke Foundation of Canada (T-3759), and the Ontario Thoracic Society. We thank Spectral Diagnostics Inc. (Toronto, Canada) for generously providing one of the antibodies for this study.

References

Preanalytical Influences on the Measurement of Ghrelin, Michael Gröschl,* Roland Wagner, Jörg Dötsch, Wolfgang Rascher, and Manfred Rauch (Kinderklinik Erlangen, Loschengrассe 15, 91054 Erlangen, Germany; * author for correspondence: fax 0049-09131-8533745, e-mail michael.groeschl@kinder.imed.uni-erlangen.de)

Ghrelin is an acylated peptide with growth-hormone-releasing function (1, 2). It was first isolated from rat stomach during the search for an endogenous ligand to an “orphan” G-protein-coupled receptor (3). The peptide consists of 28 amino acids, with a n-octanoylation of the serine-3 residue, which is indispensable for biological activity. Human ghrelin differs from rat ghrelin by only two amino acids at positions 11 and 12. The peptide stimulates the release of growth hormone when administered intravenously to rats and given to rat primary pituitary cells (2).

In previous studies, serum was preferred for the determination of ghrelin. Experience with other sample materials obtained after administration of various anticoagulants/substances has not yet been described. It is therefore unknown which method of obtaining samples for ghrelin determination enables the most accurate and precise measurements. Furthermore, data on the stability of the hormone are still lacking, but are necessary for optimizing analytical conditions.

The objective of the present study was to compare the reliability of ghrelin measurements in serum and four different plasma samples and to evaluate data on stability under different storage conditions.

Blood samples were taken from apparently healthy volunteers (10 men and 4 women; age range, 18–40 years) who were not on medication and had normal blood pressure. The body mass index varied from 20 to 29 kg/m². Blood was taken between 1000 and 1100 by venipuncture (Multify® with 20-mL cannulas; Sarstedt) and immediately divided into tubes for plasma preparation with dipotassium EDTA (Kabe), citrate, fluoride, and lithium heparinate (Sarstedt) as anticoagulating substances. The content of liquid anticoagulating additive in citrate-plasma tubes was 118 ± 15 μL (n = 15; mean ± SD). Additionally, serum was prepared from each sample (Sarstedt). After clotting, samples were centri-fuged (10 min at 1500g).

Serum from five male volunteers was divided into two series of five aliquots each. One of the duplicate series was stored at 25 °C, the other was stored at 4 °C. Each day an additional sample from each series was frozen (−25 °C) until measurement.

To study the effect of repeated freezing and thawing, sera from healthy volunteers (n = 10) were divided into...
five identical aliquots. All aliquots were frozen immediately; four of these were rethawed the next day and then refrozen, with three then being rethawed, and so forth.

Additionally, blood from one male volunteer was supplemented with 150 or 500 ng/L recombinant human ghrelin. The blood was divided between diverse matrices as described above, and each sample was measured 10 times. Recovery of the added amounts was determined after subtraction of the basal ghrelin value of the sample.

Ghrelin was measured with a commercial RIA (Phoenix). Fifty percent binding occurred at 190 ng/L. The sensitivity of undiluted samples was 15 ng/L. Inter- and intraassay CVs, as given by the manufacturer, were 7.5% and 4.0%, respectively.

Values in different matrices were compared by Passing-Bablok regression (4). The CV for any sample matrix was the mean intraassay CV from all of the different samples. ANOVA with the Bonferroni multiple comparison test was used to examine alterations in hormone values under various storage conditions and to assess the influences of repeated freezing and thawing. Alterations exceeding ±2 intraassay CVs were defined as being stability dependent. \( P < 0.05 \) was considered significant.

The mean intraassay CV of ghrelin measurements from serum was 3.8%. Because the determination of ghrelin from serum is commonly used, all further descriptions of different matrices are related to the ghrelin measurements in this matrix.

As in serum, we found a low intraassay CV of 3.8% for ghrelin measurements in dipotassium-EDTA plasma. The linear regression equation between both matrices was as follows: dipotassium-EDTA plasma = 1.01 × serum + 12.3 ng/L. The differences from the respective serum values were -33% to +14% with a correlation of \( r^2 = 0.97 \). There was no significant difference between ghrelin concentrations from matched serum and EDTA-plasma samples.

The intraassay CV of ghrelin measurements from lithium-heparinate plasma was 4.8%. The differences from the corresponding serum values were -29% to +39% with a correlation of \( r^2 = 0.95 \), and the regression equation was as follows: lithium-heparinate plasma = 1.07 × serum - 17.8 ng/L. This matrix generally yielded significantly lower results (mean, 7%; \( P < 0.01 \)) compared with the matched serum samples.

In fluoride-plasma tubes, the intraassay CV of ghrelin measurements was 4.5%. Here, the differences from the matched serum values were -30% to +15% with a correlation of \( r^2 = 0.90 \). The regression equation was as follows: fluoride plasma = 0.84 × serum + 14.4 ng/L. Significant differences in comparison with serum values were not found.

Significantly lower concentrations of ghrelin were measured in citrate plasma in comparison with serum (\( P < 0.001 \)). The results were generally 25% lower than in the serum samples with a range of -51% to +2%. The correlation to the matched serum samples was \( r^2 = 0.94 \). The CV was 2.8%. The regression equation was as follows: citrate plasma = 0.71 × serum + 13 ng/L.

After subtraction of the basal ghrelin content measured in each of the five sample matrices, we calculated recoveries (%) of the added amounts of recombinant ghrelin. Recovery of 150 ng/L recombinant ghrelin (mean ± SD) was 100% ± 13% in serum, 99% ± 12% in EDTA plasma, 94% ± 4% in lithium-heparinate plasma, 100% ± 11% in fluoride plasma, and 83% ± 10% in citrate plasma. The recoveries for 500 ng/L ghrelin added to various matrices were 101% ± 5% in serum, 98% ± 6% in EDTA plasma, 100% ± 7% in lithium-heparinate plasma, 97% ± 7% in fluoride plasma, and 88% ± 6% in citrate plasma.

Ghrelin was stable when stored at 4 °C for up to 3 days, whereas storage at 25 °C for >1 day produced significantly lower results (Fig. 1). Repeated freezing and thawing had no influence on the concentrations of the peptide (\( P = 0.39 \)).

Since its discovery in 1999, many studies on ghrelin have been published. Most studies describe the use of serum (5, 6) whereas only a few used plasma, and these studies had no further explanation regarding plasma use (7, 8). Because of the increased interest in measuring ghrelin, a standardized method for sample collection is required. Consequently, our aim was to compare the equivalence of ghrelin values measured from different specimens and to determine the optimal sample matrix for accuracy and reliability, as we recently described for human leptin (9). Our additional aim was to investigate the stability of ghrelin under various storage conditions because previous studies have described the influence of storage conditions on the analysis of various endocrine substances, such as steroids (10, 11) and peptide hormones, including human growth hormone (12), luteinizing hormone, follicle-stimulating hormone, and prolactin (13).

Our results show only slight differences in the ghrelin measurements in serum and different plasmas obtained from identical blood samples. Data on the intraassay CV for serum were in good accordance with data provided by the manufacturer. All five matrices showed low intraass-

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**Fig. 1.** Stability of human ghrelin after storage at 25 and 4 °C for up to 5 days (mean ± SD). Dotted lines indicate clinical acceptable range (±3 intraassay CVs).
say CVs. The high precision of the results may enable single measurements instead of multiple determination, thereby reducing costs.

The significantly lower values for ghrelin in citrate plasma may only partially (~12%) be explained by dilution with the anticoagulating liquid in the tubes (118 ± 15 μL). The discrepancy between citrate plasma and serum was ~25%, a discrepancy that is too high for a recommendation for citrate plasma. In contrast, results for lithium-heparin plasma were in only ~7% lower than serum results, which we consider to acceptable.

It should be kept in mind that the magnitude of the difference between values from matched sample matrices might be influenced by the assay system used, as has been shown for the determination of cardiac troponin T and I (14, 15). Our findings are based on the use of a direct RIA that is commercially available and is currently widely used for research.

As we have determined, storage of serum under cooled conditions allows stable results for up to 3 days. Storage at warm temperatures for >1 day should be avoided. This is very important when samples are transported by mail. Because no significant decrease in the ghrelin values was observed after repeated freezing and thawing, there should be no problems if sample tubes are used several times, e.g., for repeating an assay or using material after determination of other analytes.

In conclusion, ghrelin is relatively stable when stored under cooled conditions. This, as well as the fact that several sample matrices can be used as alternatives, is a good precondition for further studies on this interesting peptide hormone.

References


Biological Variation of Glycohemoglobin, Curt Rohlfing,1† Hsiao-Mei Wiedmeyer,1 Randie Little,1 V. Lee Grotz,2 Alethea Tennill,1 Jack England,1 Richard Madson,1 and David Goldstein1 (1 University of Missouri School of Medicine, Columbia, MO 65212; 2 McNeil Specialty Products Company, New Brunswick, NJ 08903; *address correspondence to this author at: Department of Child Health, University of Missouri–Columbia, 1 Hospital Dr., M772, Columbia, MO 65212; fax 573-884-4748, e-mail RohlfingC@health.missouri.edu)

Glycohemoglobin (GHB) is a measure of long-term mean glycemia that predicts risks for the development and/or progression of diabetic complications in patients with type 1 and type 2 diabetes (1, 2). Several reports have suggested, however, that although the within-subject variation in GHB unrelated to glycemia is minimal, there is substantial between-subject variation in GHB, e.g., “low glycators” and “high glycators” (3–5). These reports have suggested that because of this large between-subject variation, GHB may not be useful for diabetes screening or diagnosis and that when GHB is used for routine management of patients with diabetes, different patients may require very different GHB target values to achieve the same overall glycemic status. We therefore examined the biological variation of GHB and fasting plasma glucose (FPG) in nondiabetic individuals.

Individuals without diabetes (n = 48) participated in a study of an artificial sweetener that has no effect on GHB or plasma glucose concentrations [Submission to Food and Drug Administration. McNeil Specialty Products Company food additive petition 7A3987 (Sucralose), 1987–1997]. Because the study was designed to detect minimal changes in plasma glucose concentrations, all participants were men to avoid the effects of cyclic hormonal changes on insulin (and therefore, plasma glucose) concentrations. At the prestudy screening, all individuals were healthy on the basis of a medical history, physical examination, and electrocardiography results; results of hematology and blood chemistry studies, urine examination, and measures of blood glucose control (FPG, insulin, C-peptide, and hemoglobin A1c) were all within their respective reference intervals. Participants who failed a baseline oral glucose tolerance test [fasting >7.8 mmol/L (140 mg/dL), 1 h >11.1 mmol/L (200 mg/dL), and/or 2 h >7.8 mmol/L (140 mg/dL)] were excluded. Those who took medications that could affect glucose