overestimation of Phe and Tyr concentrations, which may cause mild overtreatment, i.e., a reduction in dietary Phe allowances in excess of metabolic tolerance. This is not critical with Phe concentrations at or above the upper limit of normal. Still, neurological outcome has also been demonstrated to be adversely affected by Phe deficiency (14). In view of an overestimation by up to 60 μmol/L in the low concentration range and a target range of 40–250 μmol/L in fresh samples, it would appear prudent to aim at Phe concentrations >100 μmol/L for samples measured after delayed preparation from whole blood.

In conclusion, the clinical practice of delayed measurement of Phe and Tyr in whole-blood samples mailed to the specialized laboratory leads to slightly increased readings. This is clinically safe, as long as Phe concentrations are kept above 100 μmol/L to prevent Phe deficiency.

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References

Mean Serum Concentration of Vitamin D-binding Protein (Gc Globulin) Is Related to the Gc Phenotype in Women, Anna Lis Lauridsen,1,2* Peter Vestergaard,3 and Ebba Nexo4 (1 Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; 2 Department of Clinical Biochemistry, Randers Central-sygehus, DK-8700 Randers, Denmark; 3 Department of Endocrinology and Metabolism, AAS, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; * author for correspondence: fax 45-89-49-30-60, e-mail all@dahl.net.dk)

Immunonephelometry has been reported (1) to be a suitable method for quantification of vitamin D-binding protein (also known as Gc globulin or Gc). We wished to develop such a method and examine the association between the mean serum concentration of Gc in women of known Gc phenotype and the phenotype of Gc.

Gc is a 52- to 58-kDa multifunctional plasma protein, synthesized mainly by hepatocytes. Polymorphisms in the Gc gene (codominant alleles) give rise to three major electrophoretic variants of Gc (Gc2, Gc1s, and Gc1f), which differ by amino acid substitutions as well as glycosylation (2, 3). The physiological significance related to the various phenotypes is yet to be discovered.

Gc is the major carrier protein of vitamin D and its metabolites in the circulation and is important for preservation of the vitamin (4, 5). Gc also transports components such as fatty acids and endotoxin (6, 7), and it is an important player in the actin scavenging system (8, 9). Gc binds actin released from cells upon injury, and the Gc-actin complexes are rapidly cleared from the circulation, thereby preventing the harmful effects of actin filaments in blood vessels. The resulting decrease in Gc concentration makes Gc usable as a prognostic indicator of survival of patients with significant tissue injury after trauma (10) and among patients with hepatic failure (11).

In addition to being a transporter and an actin scavenger protein, Gc may be of importance for bone formation and in the immune system. After in vitro removal of its galactose and sialic acid residues, Gc is converted to a very potent macrophage-activating factor, Gc-MAF (12). Administration of Gc-MAF to osteopetrotic rodents reversed their bone and immunological defects, probably by activating osteoclasts as well as macrophages (13). Finally, together with complement factors C5a and C5a des Arg, Gc may act as a co-chemotactic factor in facilitating chemotaxis of neutrophils and monocytes in inflammation (14, 15).

Despite the diverse and important roles of Gc, only a few methods for measurement of the protein in serum have been evaluated, and little attention has been paid to possible variation in the serum concentration of Gc as a function of phenotype.

We established an immunonephelometric method for quantification of serum Gc, essentially as described previously by Haughton and Mason (1), using a Behring Nephelometer II (Dade Behring) and reagents supplied by Behring (N Reaction Buffer, N Diluent, and N Supplement Reagent Precipitation). Rabbit Anti-Human Gc-Globulin
(Dako A/S) was filtered before use (CAMEO 13 N nylonsproejtefilter; Frisenette ApS). We used human lyophilized solid Gc-Globulin Mixed Type (Calbiochem-Novabiochem Corporation) to prepare the calibrator (200 mg/L Gc). Phosphate-buffered saline (pH 7.4; 10 mmol/L \( \text{PO}_4^{2-} \), 145 mmol/L NaCl) was used as the diluent.

The Behring Nephelometer II measures the light scattered by antigen-antibody complexes over a fixed period from 7.5 s to 6 min after mixture. We constructed five-point calibration curves, using calibrator dilutions of 1:5–1:80. Assay conditions were as follows: sample volume, 100 \( \mu \text{L} \); sample dilution, 1:20; antibody volume, 30 \( \mu \text{L} \); buffer volume, 80 + 80 \( \mu \text{L} \); volume of N Supplement Reagent Precipitation, 30 \( \mu \text{L} \).

The interassay CV for measurement of Gc was 2.6% and 4.6% for 275 and 137 mg/L Gc (n = 22), respectively, as judged from control samples analyzed over a period of 6 months. Sera from two blood donors were used as high (undiluted serum) and low (diluted 1.6:1 with phosphate-buffered saline) controls.

To examine whether our method correctly quantified the serum Gc concentration, based on Gc phenotype, we analyzed four serum samples of each homozygous Gc-type, Gc2-2, Gc1s-1s, and Gc1f-1f, in six dilutions, from undiluted to 1:6. Data were subjected to linear regression analysis. The Gc phenotypes behaved alike (\( P = 0.291 \)). All samples showed a linear relationship between expected and observed concentrations of Gc (\( P < 0.001 \)), and the slopes and intercepts showed only small deviations from 1 and 0.

We determined the Gc phenotype of 586 healthy women not taking any hormonal medications [participants of the DOPS study (16) approved by the local ethics committee (no. 1990/1821)]. Because the Gc isoforms produced by different genotypes have different isoelectric points, they can be identified by isoelectric focusing. For that purpose we used Immobiline Dryplate Gels (pH 4.5–5.4) from Pharmacia Biotech. The running conditions were as follows: 10 °C, 3500 V, 5 mA, 15 W, 18–24 h. Gels were stained with Amido Black. Our results for gene frequencies (Gc2, 0.2640; Gc1s, 0.5844; Gc1f, 0.1516) did not differ from those reported in 1981 for a Danish population (n = 1674) (17). Using a z-test, we found \( P \) values of 0.63–0.86.

We measured the serum Gc concentrations in the 586 women. On the basis of visual inspection of histograms combined with calculation of means ± 4 SD, we decided to exclude two outliers. As seen from Fig. 1A, the mean Gc concentration varied from 226 mg/L for persons with the Gc2-2 phenotype to 274 mg/L for those with the Gc1s-1s phenotype. No statistically significant difference was observed for individuals bearing one or the other of the Gc1s or Gc1f types, whereas a strong significant difference was observed for Gc2-2, Gc2-1, and Gc1-1 (\( P < 0.001 \), Kruskal–Wallis test and ANOVA, least-significant difference; Fig. 1B). Using the nonparametric method based on rank numbers recommended by IFCC (18), we found the following 95% central reference intervals: Gc2-2, 183–268 mg/L (n = 40); Gc1-2, 201–310 mg/L (n = 228); Gc1-1, 218–346 mg/L (n = 316; Table 1). The central 95% reference interval for the 584 women altogether was 203–334 mg/L; the confidence interval of the lower limit was 199–210 mg/L, and the confidence interval of the upper limit was 323–341 mg/L.

Other assays for quantifying the serum concentration of Gc include radioimmunoassay (19), radial immunodiffusion (20), rocket immunoelectrophoresis (21), and ELISA (22). These methods are imprecise, technically demanding, time-consuming, or involve the use of isotopic tracers. The immunonephelometric assay for Gc (1) evaluated in this report therefore seems to be the most useful assay for routine use because it is fully automated and has a low imprecision.

The three genetic types of Gc differ in amino acid sequence (2) and in glycosylation. Gc2 is glycosylated...
with a terminal galactose, whereas Gc1f and Gc1s contain both galactose and sialic acid (3). These differences may cause variations in antigenicity and in binding affinity for the Gc antibodies. An important question concerning immunological assays for Gc, therefore, is whether the immunoreactivity of the Gc phenotypes is identical in that specific assay. Ideally one would like to show that equal amounts of each Gc phenotype produce the same signal in the assay. This is, however, impossible because pure Gc is available only as a mixture of the various phenotypes. In the assay. This is, however, impossible because pure Gc is available only as a mixture of the various phenotypes. In our study we chose to examine the linearity of the homozygous Gc types separately. All phenotypes behaved alike when the samples were serially diluted. This indicates that possible differences in immunoreactivity are unlikely to have any practical importance in our assay.

The observed relationship between Gc phenotype and Gc serum concentration is supported by a few previously published smaller studies (Table 1). Constans et al. (23) and Daiger et al. (24) analyzed 100 and 89 persons, respectively, but they did not distinguish between Gc1s and Gc1f. Braun et al. (25) determined the Gc concentrations of the three homozygous phenotypes (the number of participants was not specified), but their result for Gc1f-1f was much higher than ours. A possible explanation is that their assay recognized Gc1f-1f better than the other phenotypes.

The differences in Gc concentration among the Gc types are theoretically attributable to variations in either the production rate or the metabolic rate. From the glycosylation pattern of the Gc proteins one would expect Gc2-2 to be metabolized faster than the sialylated form, Gc1-1. Interestingly, the lowest serum concentrations were observed for Gc2-2. An increased metabolic rate for Gc2-2 is further supported by a study by Kawakami et al. (26), who injected radioactive Gc into four healthy young men. The metabolic rate of Gc was higher in the person with the Gc2-2 phenotype receiving Gc2 than in the person with Gc1-1 receiving Gc1 (32.6% vs 25.0% per day).

The clinical significance of the differences in serum Gc concentration among Gc phenotypes is yet to be investigated. Regarding the actin scavenging system, it is hardly crucial. The half-life of Gc-actin complexes is only 30 min, suggesting that differences in the initial concentrations of Gc are of minor importance. Regarding interpretation of serum concentrations of substances carried by Gc, notably vitamin D, it may well be important to know the Gc concentration and, possibly, the Gc phenotype.

In conclusion, we evaluated an automated assay for quantification of Gc and showed that the serum concentration of Gc depends on the Gc phenotype.

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Effect of Hemoglobin Variants (Hb J, Hb G, and Hb E) on HbA1c Values as Measured by Cation-Exchange HPLC

On HbA1c Values as Measured by Cation-Exchange HPLC (Diamat), Li-Yu Tsai,1* Shih-Meng Tsai,2 Me-Nung Lin,3 and Shu-Fen Liu4 (1 Department of Clinical Biochemistry, School of Technology for Medical Science, and 2 Department of Public Health, School of Medicine, Kaohsiung Medical University, Kaohsiung 80702, Taiwan; * author for correspondence: fax 866-7-2370544, e-mail tslyu@cc.kmu.edu.tw)

Hemoglobin A1c (HbA1c) is used for the long-term management of patients with diabetes mellitus (DM) (1, 2). Hb variants other than HbA1c and e-N-lysine-glycated Hb A0 may cause analytical interference in determinations of HbA1c (3–6). In one study, the authors estimated the prevalence of thalassemia in Taiwan as 7%; moreover, ~1% of the people in northern Taiwan are α-thalassemia heterozygotes (7). The occurrence of 24 abnormal Hbs (13 α-chain variants and 11 β-chain variants), including Hb G-Taipei, in populations in the Silk Road area of Northwestern China has been presented in a review (8). The frequency of thalassemia has been estimated to be ~1 in 2350 in Japan (9) and even higher in North Africa (10). Hb E is the second most prevalent Hb variant worldwide and the third most prevalent variant in the US, after Hb S and C. Hb E is found primarily in Southeast Asia, especially among the Thai population (11). In the northeastern region of India, the gene frequency of Hb E is 10.9% (12). In a study of 222,000 blood samples in Canada, 23 cases of Hb J were identified (13). Given that the majority of hemoglobinopathic cases are from families of Asian, Southeast Asian, and Asian Indian ancestry (7–12, 14–16), the aim of this study was to investigate the influence of selected Hb structural variants on HbA1c values measured by cation-exchange HPLC.

We collected 17 EDTA-anticoagulated whole blood specimens from DM patients with Hb J (6 patients), Hb AG (10 patients), or Hb AE (1 patient had a fasting sugar of 10.4 mmol/L) to analyze HbA1c. The ranges and mean values for fasting sugar were 8.2–17.8 mmol/L and 12.6 ± 3.9 mmol/L, respectively, in the DM patients with Hb AG and 7.1–18.1 mmol/L and 9.8 ± 4.1 mmol/L, respectively, in the DM patients with Hb AE. In addition, one specimen from a nondiabetic patient with the Hb AG variant (fasting sugar, 4.4 mmol/L) and another from a nondiabetic patient with Hb AE (fasting sugar, 5.2 mmol/L) were analyzed. HbA1c and glycated Hb were measured by cation-exchange HPLC (Diamat HbA1c program; Bio-Rad Laboratories) and by boronate ion capture (IMx analyzer; Abbott Laboratories). Both methods had a CV <5%, and both reported results as percentage of HbA1c. When Tiran et al. (4) comparatively evaluated five glycated Hb assay methods, including the Abbott IMx glycated Hb ion capture assay, they found that the methods showed generally acceptable precision and good accordance with the Bio-Rad Diamat system. Bon et al. (17) determined the accuracy of the IMx assay by comparison with a reference HPLC assay for 603 specimens; the correlation coefficients were 0.88–0.96. In addition, several investigators have shown that glucose, bilirubin, triglycerides, labile fraction, and Hb variants do not interfere with the Abbott IMx assay (18). Moreover, the IMx assay is not sensitive to interference by cyanate derived from spontaneous dissociation of urea. In the present study, a boronate-affinity analytical method on a CLC 385 analyzer (Primus Corporation) served as the comparison method because of the high specificity and the negligible interference of Hb variants in that method (1). The Hb variants were identified by electrophoretic separation of Hb on cellulose acetate membranes. Specimens for which HPLC chromatograms suggested the presence of abnormal peaks underwent hemoglobinopathy studies.

The Abbott IMx boronate ion-capture method showed no important effects from any of the Hb variants tested, and its results for HbA1c agreed well with those of the