levels of serum Gc-globulin; predictive value in fulminant hepatic failure.  


Effect of Hemoglobin Variants (Hb J, Hb G, and Hb E) on HbA1c Values as Measured by Cation-Exchange HPLC (Diamat), Li-Yu Tsai, 1* Shih-Meng Tsai, 2 Me-Nung Lin, 1 and Shu-Fen Liu 1 (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 A$_{nc}$ (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 A$_0$ 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 $\beta$-thalassemia heterozygotes (7). The occurrence of 24 abnormal Hbs (13 $\alpha$-chain variants and 11 $\beta$-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 220 000 blood samples in Canada, 23 cases of Hb J were identified (13). Given that the majority of hemoglobino pathic 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 AJ (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 AJ and 7.1–18.1 mmol/L and 9.8 ± 4.1 mmol/L, respectively, in the DM patients with Hb AG. 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; BioRad 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
The effects of Hb variants on HbA1c values determined by the HPLC system have been evaluated previously (3, 5, 6, 19, 20). An earlier report by Oshima et al. (5) of a study conducted in Japan described an abnormal chromatogram for HbA1c [as analyzed on an automated glycohemoglobin analyzer, HLC-723 Ghb V (Tosoh)] from a male DM patient with Hb JLome. In a study performed in Singapore, Wong et al. (19) observed an asymmetrical HbA1c peak with a right shoulder in nine HbA1c blood specimens analyzed by cation-exchange HPLC (Variant HbA1c program; Bio-Rad) from diabetic patients with Hb AE. In a study performed in Austria, Schnedl et al. (20) reported an additional peak at Hb A0, as well as falsely low HbA1c values, measured by cation-exchange HPLC (Diamat HbA1c) from a diabetic patient with Hb O Padova. Our results not only agree with those of Schnedl et al. (20), but also deal with Hb J, G, and E variants in a single study.

We conclude that Hb variants can contribute to mismanagement of patients with DM because of falsely low HbA1c values measured by HPLC. Careful interpretation of glycohemoglobin results is critical in populations with a relatively high prevalence of Hb variants, such as Hb AJ, AG, and AE.

**References**

Errors Caused by the Use of d,l-Octanoylcarnitine for Blood-Spot Calibrators, Donald H. Chace,¹ James C. DiPerna,² Barbara W. Adam,² and W. Harry Hannon² (¹ Neo Gen Screening, PO Box 219, Bridgeville, PA 15017; ² Centers for Disease Control and Prevention, 4770 Buford Hwy, NE, Atlanta, GA; * author for correspondence: fax 412-220-0784, e-mail dhchace@neogenscreening.com)

The use of tandem mass spectrometry (MS-MS) in the analysis of filter paper blood spots from newborns for acylcarnitines and amino acids has expanded significantly in recent years (1, 2). With estimates of 1 million specimens analyzed by MS-MS per year throughout the world, the demand is acute for assay standardization and harmonization. Programs exist at the CDC for amino acid standardization and quality assurance pertaining to newborn screening (3, 4). This program is being extended to include acylcarnitines, and the data in this report stem from that extension.

Five metabolites are key in the diagnosis of several disorders of fat and organic acid metabolism. Preliminary results demonstrated excellent linearity for each of the five acylcarnitines added to blood. However, an extremely unusual result was observed for octanoylcarnitine: the recovery of octanoylcarnitine was significantly lower than that of other acylcarnitines. This observation is supported by Turner and Dalton (5), who reported a 40% loss of octanoylcarnitine after addition to whole blood and plasma. We investigated the cause of this loss of octanoylcarnitine so that this serious error could be prevented or accounted for. The results of this study demonstrate the importance of assay standardization and the validation required in clinical screening that goes well beyond this particular quality-assurance/quality-control program for acylcarnitines.

We obtained isotope-labeled internal standards (L-2H5-propionylcarnitine, L-2H5-butrylcarnitine, L-2H5-octanoylcarnitine, L-2H5-myristoylcarnitine, and L-2H5-palmitoylcarnitine) from Cambridge Isotope Laboratories. Unlabeled standards (d,l-octanoylcarnitine, d,l-myristoylcarnitine, and d,l-palmitoylcarnitine) were obtained from Sigma, and L-propionylcarnitine, L-butryl carnitine, and L-octanoylcarnitine were obtained from Life Sciences Resources. The unlabeled standards were used to prepare a series of blood specimens at the CDC using procedures described previously (3, 4) with the following modifications: L-propionylcarnitine, L-butrylcarnitine, d,l-octanoylcarnitine, d,l-myristoylcarnitine, and L-palmitoylcarnitine were added to whole blood containing EDTA, whole blood containing heparin, and lysed cells at final concentrations of 0–14 μmol/L. Blood (25 μL) was applied to S&S Type 903 filter paper (Schleicher & Schuell), dried, and sent to Neo Gen Screening for analysis by MS-MS. A smaller subset of blood specimens containing heparin were prepared at Neo Gen Screening by the addition of an equimolar solution (31 μmol/L) of d,l-octanoylcarnitine or l-octanoylcarnitine to the internal standard, L-2H5-octanoylcarnitine.

For specimens obtained from the CDC, a 4.8 mm diameter spot was punched from each dried blood specimen, extracted with methanol containing deuterated L-acylcarnitine internal standards, and derivatized using a procedure described previously (6) with the following modification: dry, derivatized sample extracts were reconstituted immediately before analysis in acetonitrile-water (50:50 by volume) containing 0.2 mL/L formic acid and analyzed using electrospray MS-MS as described below. Specimens that contained both the internal standard and its unlabeled analog were extracted using methanol without the internal standard.

An Applied Biosystems/MDS Sciex Model API 3000 tandem mass spectrometer equipped with an electrospray ionization source was used for all analyses. A 10-μL aliquot of each specimen was injected using a Gilson 215 sample handler fitted with a Rheodyne Model 7010 injec- tor and a Perkin-Elmer LC Pump operating at a flow rate of 18 μL/min with acetonitrile-water (50:50 by volume) containing 0.2 mL/L formic acid as the mobile phase. Precursors of 85 Da scans and 103 Da scans were used, representing analyses for acyl and free carnitines (6). Concentration calculations were obtained by the following method: raw data (ion intensity data) were processed using an Apple script followed by its exportation to an Excel spreadsheet for further data reduction and calculations.

Excellent linearity (R² >0.98) was obtained for acylcarnitine calibration curves from blood containing either EDTA or heparin or in which the red cells were lysed. The slopes and intercepts for these addition assays are provided in Table 1. The results for the addition containing d,l-octanoylcarnitine had a slope of 0.59, which suggests a significantly reduced recovery. Similar results were reported by Turner and Dalton (5), who reported a significant loss of octanoylcarnitine of 40%. The stereoisomeric forms used in their study for octanoylcarnitine, however, were not noted. In an experiment in which l-octanoylcarnitine was used in an addition analysis, no significantly reduced recovery of l-octanoylcarnitine was observed (slope = 0.93). Repeat preparation of the l-octanoylcarnitine calibration curve using EDTA-treated blood did not show reduced recovery of l-octanoylcarnitine (data not shown).

Experiments were designed to further clarify and confirm the loss of d,l-octanoylcarnitine in blood. An equimolar mixture of d,l-octanoylcarnitine with L-2H5-octanoylcarnitine or of l-octanoylcarnitine with L-2H5-octanoylcarnitine was first analyzed as pure compounds. The MS-MS analyses of these mixtures demonstrated molar equivalents of d,l- or l-octanoylcarnitine (Fig. 1, A and B). An aliquot of this equimolar mixture was then...