Long-Term Glycemic Control Measurements in Diabetic Patients Receiving Hemodialysis

Melanie S. Joy, PharmD, William T. Cefalu, MD, Susan L. Hogan, PhD, and Patrick H. Nachman, MD

● **Cardiovascular morbidity is increased in patients with diabetes mellitus and there is a great prevalence of diabetes and cardiovascular disease among patients with end-stage renal disease (ESRD). Control of glycemia can decrease cardiovascular and end-organ damage. Because the validity of glycemic control tests have not been rigorously studied in patients with ESRD, we evaluated the value of various measures in these patients. The overall clinical goal was to investigate whether hemoglobin A_1C (A_1C) accurately reflects actual glycemic control as compared with other measures in light of the importance of attaining appropriately controlled blood glucose (BG). The commonly used tests of total glycated hemoglobin (GHb) and A_1C may be unreliable in patients with ESRD because of the presence of anemia, shortened red blood cell (RBC) survival, and assay interferences from uremia. The primary aim of this study was to assess the relationship of capillary BG measurements to A_1C, GHb, total glycated plasma proteins (GPP), and fructosamine (Fr) in diabetic patients receiving hemodialysis. Twenty-three patients were instructed to obtain BG evaluations twice daily for 7 days by using the Elite glucometer (Bayer Corporation, Elkhart, IN). These determinations included 6 fasting, 6 preprandial, and 3 separate 2-hour postprandial levels. Blood was obtained on day 7 for measurement of A_1C, GHb, GPP, and Fr. A_1C was analyzed by an immunoassay, GPP and GHb were assayed by affinity high-performance liquid chromatography (HPLC), and Fr by automated nitroblue colorimetric assay. Scatter plots were generated by plotting the average BG versus A_1C, GHb, GPP, or Fr. Linear regression was performed for each plot showing the following relationships: $A_1C = 0.0174 (BG) + 4.76$ ($r = 0.58$; $P < 0.05$): $GHb = 0.0371 (BG) + 3.57$ ($r = 0.584$; $P < 0.05$): $GPP = 0.0083 (BG) + 26.13$ ($r = 0.065$; $P = 0.77$): $Fr = 0.6865 (BG) + 250$ ($r = 0.345$; $P = 0.11$). Despite anemia and shortened RBC lifespan in patients with ESRD, A_1C in the range of 6% to 7% estimates glycemic control similarly to patients without severe renal impairment. A_1C values above 7.5% may overestimate hyperglycemia in patients with ESRD. Thus, diabetic patients receiving hemodialysis may have long-term BG that are more properly controlled than previously determined, reducing their risks of the macro- and microvascular complications of diabetes mellitus.**

INDEX WORDS: Hemoglobin A_1C; glycemic control; hemodialysis (HD); diabetes.

DIABETES MELLITUS represents 37% of all prevalent cases of end-stage renal disease (ESRD) patients receiving dialysis.[1] Although a higher frequency of ESRD is seen in patients with type 1 diabetes (40% develop ESRD), the actual prevalence is greater in type 2 diabetes because of the greater prevalence of type 2 diabetes in the population.[2] Other long-term complications resulting from diabetes include retinopathy, neuropathy, cardiovascular disease, and their sequelae. Control of hyperglycemia is known to decrease short-term morbidity, small-vessel disease, and cardiovascular risks.[3-5] In addition, control of other concomitant factors (hyperlipidemia, tobacco abuse, blood pressure, and dietary protein intake), as well as therapy with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, have slowed the progression of complications in patients with and without diabetes mellitus.[6-13]

Clinically, measurement of glycemic control in diabetic hemodialysis patients has been determined in a similar manner as for patients without ESRD; by monitoring blood glucose (BG) and glycated hemoglobin (hemoglobin A_1C [A_1C]). The same accepted normal ranges for A_1C are applied to all diabetic patients, regardless of their renal function. The good correlation between A_1C and BG in non-ESRD type 1 diabetic patients has been documented in the Diabetes Control and Complications Trial (DCCT).[14,15] An

From the Division of Nephrology and Hypertension, University of North Carolina, School of Medicine, Chapel Hill, NC; and the Endocrine, Diabetes, and Metabolism Unit, University of Vermont, College of Medicine, Burlington, VT.

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Address reprint requests to Melanie S. Joy, PharmD, Clinical Assistant Professor, University of North Carolina, Schools of Medicine and Pharmacy, Division of Nephrology and Hypertension, CB #7155, 348 MacNider Building, Chapel Hill, NC 27599-7155. E-mail: Melanie_Joy@med.unc.edu

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average BG concentration can be estimated based on the glycated hemoglobin and vice versa.[14,15] Because the DCCT did not include patients with ESRD and very few patients had progressive renal disease (defined by increased proteinuria), this assumption may not be valid in this patient population.[16] There are reports in the literature of falsely increased values of A_1C and total glycated hemoglobin (GHb) in patients with renal failure caused by carbamylated hemoglobin interfering with the assay.[17] Other factors known to increase A_1C measurements include: increased hemoglobin F, hypertriglyceridemia, hyperbilirubinemia, opiate and alcohol addiction, lead poisoning, uremia, and high-dose aspirin therapy.[17,18] Falsely decreased A_1C measurements have been documented owing to decreased red blood cell (RBC) mass, decreased RBC survival, iron-deficiency anemia, phlebotomy, changes in iron or erythropoietin administration or hemolytic anemia (increases in erythrocyte pool), and hemodilution caused by blood transfusions.[17-23] Several of these factors that can effect A_1C measurements are found in patients with ESRD. These confounding factors suggest that the correlations of A_1C and BG concentrations may not be as predictable and accurate in patients with ESRD and, thus, may be a misleading assessment of the true degree of glycemic control. For this reason, there has been an interest in other markers of glycemic control such as glycated plasma proteins, that are independent of RBC survival. Unfortunately, the use of glycated plasma proteins may also be limited by various confounders. Falsely decreased values have been shown in the presence of low protein concentrations.[17] Falsely increased values have been measured in the presence of lipemia, hemolysis, high bilirubin and uric acid concentrations, uremia, and in patients receiving high doses of aspirin therapy.[17] The potential confounders to the use of glycated hemoglobin and glycated plasma proteins are summarized in Table 1.

Table 1. Contributing Factors Complicating Evaluation of Long-Term Glycemic Control

	Falsely Increased Values	Falsely Decreased Values
Glycated hemoglobin	Carbamylated hemoglobin	Reduced RBC lifespan
	Uremic acidosis	RBC transfusions
	Labile glycated compounds	Vitamins C and E
	Hemoglobin F	Pregnancy
	Hypertriglyceridemia	Iron deficiency
	Opiate addiction	Phlebotomy
	Lead poisoning	Hemolytic anemia
	Alcoholism	
	High doses of aspirin	
Glycated plasma proteins	Lipemia	Low serum protein concentrations
	Hyperbilirubinemia	
	Hemolysis	
	Increased uric acid	
	Uremia	
	High doses of aspirin	

This study was designed to evaluate 4 methods of measuring BG control in chronic hemodialysis patients with diabetes mellitus. The specific aims of the study were to: (1) measure and evaluate the correlation between A_1C, GHb, fructosamine (Fr), and total glycated plasma proteins (GPP) as indicators of glucose control when compared with BG testing in diabetic patients on chronic hemodialysis; (2) characterize RBC survival by erythrokinetic modeling to determine if this factor accounts for changes in correlation between A_1C and BG in patients with ESRD; and (3) develop a regression model to relate the BG values to corresponding A_1C values in the ESRD patient population receiving hemodialysis and compare this with the regression relationship published for diabetic patients with normal renal function based on the results of the DCCT.

METHODS

Diabetic patients and their controls receiving hemodialysis at the University of North Carolina Hospitals dialysis units were identified. Patient inclusion criteria consisted of: (1) age greater than 18 years, (2) receiving hemodialysis 3 times weekly, and (3) diagnosis of type 1 or type 2 diabetes mellitus. The exclusion criteria were: (1) receipt of a blood transfusion within the previous 3-month time period, (2) inability or unwillingness to perform the required BG measurements, (3) diagnosis of an immune or hereditary hemolytic anemia, (4) treatment with drugs known to interfere with the assays for glycemic control measures (eg, high-dose vitamin C, vitamin E, or aspirin, and opiate or alcohol addiction), and (5) serum albumin concentrations less than 3.0 g/dL. Patients were dialyzed in accordance with the recommendations presented in the Dialysis Outcomes Quality Initiative (DOQI) practice guidelines for hemodialysis adequacy.[24] Twenty-four patients signed a consent form to participate (23 were evaluable). Nine patients participated on a second occasion to determine intraindividual variations

across time. In addition, 8 nondiabetic dialysis patients were selected to serve as controls. All diabetic patients were instructed to use the Glucometer Elite BG meter (Bayer Corporation, Elkhart, IN) to obtain twice-daily BG measurements for 7 consecutive days. The product literature reported precision results from diabetic patients with mean high and low BG values of 260 mg/dL and 58.3 mg/dL, respectively. The within and overall coefficient of variations for the high and low were 3.1% and 4.1% (within) and 5.1% and 5.7% (overall), respectively.

The instruction for the glucometer was repeated several times by the study personnel. The patients had to show their competence in performing these tests before actually participating in the study. The BG measurements consisted of a predefined variation of 6 fasting, 6 preprandial, and 3 postprandial assessments (2 hour), obtained over a week. The DCCT used a 24-hour, 7-point, BG profile method in the intensive therapy arm. The 7-point BG profile was performed quarterly, whereas the A_1C was tested monthly.[14] Because of the day-to-day variation in caloric and carbohydrate intake, and level of physical stress imposed by the 3 times per week hemodialysis regimen, we felt that a 7-point BG profile over a single day would be less representative of glycemic control than a set of measures obtained over a week. The inter- and intradialysis treatment periods over the course of a week should more adequately reflect the fluctuations in insulin requirements seen in dialysis patients with diabetes. Therefore, we modified the BG sampling scheme to a 14-point scheme over a 7-day period. Patients would be less likely to alter their normal diet and subsequent BG levels during this longer time period, thus making these measurements more reflective of each patient's usual glycemic control. This scheme did allow for assessment of blood sugars preprandial, at bedtime, and postprandial (breakfast, lunch, and dinner), as defined.

A data collection form was provided to each patient to record the test results. The glucometer stored the results of these tests in memory so that confirmation of the patient-reported data could be assessed for accuracy. When a discrepancy resulted between the written test results and those recorded in the glucometer's memory, the values stored in memory were used to calculate average BG. On the final day of testing (day 7 dialysis session), blood (15 mL) was collected for assessment of measures of glycemic control before infusion of heparin. The tubes were centrifuged at 2,000 $\times$ gravity, and blood, plasma, and serum were collected and assayed: A_1C (blood), GHb (blood), GPP (plasma), and Fr (serum). The control patients had their blood drawn for a random BG, A_1C, GHb, GPP, and Fr predialysis. The charts of all patients were reviewed for demographic, medical, medication, and additional laboratory information. This study was reviewed and approved by the institutional review board at the University of North Carolina and conducted according to the Declaration of Helsinki.

RBC Lifespan Method

The method of Uehlinger et al[25] was used to estimate RBC lifespans in our dialysis patients. A computerized model of this approach that incorporates administered dosages of erythropoietin α and measured hematocrit levels (EPOCALC) was supplied by Amgen, Inc. (Thousand Oaks, CA) for the purpose of this study. This model does not adjust for transfusions, intravenous iron administration, dialysis adequacy, hyperparathyroidism, and other factors known to affect erythropoietin sensitivity. We retrospectively entered erythropoietin doses and measured hematocrit levels into the EPOCALC program for a period of 3 to 6 months before our assessment periods for A_1C and GHb. Pharmacodynamic modeling provided information regarding RBC lifespan and individual sensitivity to erythropoietin for 18 of our patients. The RBC lifespan of patients not receiving erythropoietin α therapy could not be determined because the EPOCALC program is based on average erythropoietin sensitivity and RBC lifespan among ESRD patients receiving erythropoietin.

Assays

Hemoglobin A_1C concentrations were assessed by an immunoassay (Tina-Quant; Boehringer Mannheim, Indianapolis, IN), by using a BM/Hitachi 717 analyzer (Roche Diagnostics Corp, Indianapolis, IN). This method was without significant (-0.2% to 0.10%) A_1C changes caused by bilirubin ($\leq$50 mg/dL), lipemia ($\leq$800 mg/dL), ascorbic acid ($\leq$50 mg/dL), and rheumatoid factors ($\leq$750 IU/mL). The test was specific without cross-reactivity to carbamylated hemoglobin, glycated albumin, labile A_1C, or other hemoglobin A subfractions. The technical limits of the assay were 0.2 g/dL A_1C up to the value of the highest calibrator (2.5 g/dL), and hemoglobin concentrations of 6 to 24 g/dL. Calculation of A_1C was made by using the following formula from the product literature: $\%A_1C = (A_1C\ (g/dl)/Hgb\ (g/dl) * m) + b$, where Hgb = hemoglobin, m = slope, and b = intercept. Total coefficient of variation was less than 5.0% for A_1C. The bias in this method when compared with the DCCT trial high performance liquid chromatography (HPLC) method was -0.09 (for an A_1C of 6%) and -0.1 (for an A_1C of 8%). Previously reported data showed excellent correlation and similar line of identity between the DCCT HPLC and Tina-Quant methods for determination of A_1C values of 15% or less.[26,27]

GHb represents all subfractions of hemoglobin, including A_1C. The GPP measures all GPP. Both GHb and GPP were assayed by automated affinity HPLC on a Primus CLC-330 HPLC (Primus Corp., Kansas City, MO) as previously described.[28] Serum Fr, which measures the ability of ketamines to act as reducing agents, was assayed on a Cobas Mira Chemistry analyzer by using Roche reagents (Roche Diagnostic Systems, Nutely, NJ) as previously described.[29,30]

Statistical Analysis

The demographics and baseline measurements (body mass index [BMI], albumin) between diabetic and nondiabetic patients were compared by using Fisher's Exact tests for categoric measures and Wilcoxon rank tests for continuous measures. The relationship between BG and each of the 4 measures of glycemic control (A_1C, GHb, GPP, and Fr) were observed with graphic plots. Linear regression was used to model the relationship of BG with each of the 4 measures and Pearson's correlation coefficients were computed for each association. Multivariate regression analysis was used to control for factors such as race, sex, BMI, and RBC

lifespan. Only measures associated with BG (race) or measures found to be different between diabetic and nondiabetic patients (BMI) are reported in multivariate models. Other variables (sex, age, and albumin) did not add to the prediction of the models or confound the relationship between BG and any of the other measures. RBC lifespan was also controlled for in the subset of diabetic patients because there is a postulated relationship between survival of RBCs and subsequent results of glycemic control measures using hemoglobin subfractions. Model-adjusted correlation coefficients were reported for multivariate regression models for general comparison with the univariate correlation coefficients. Because some patients (n = 9) had a repeat testing period 6 months later, univariate regression analysis was used with the increased number of episodes (n = 32) to compare with the single-episode measurements. The regression lines evaluating BG and A_1C relationships from this study and the DCCT trial were graphed. The 95% confidence intervals for the slope and intercept from our study were computed. These values were not available for the DCCT trial.

RESULTS

The demographics of the diabetic and control hemodialysis patients are presented in Table 2. The age, race, sex, and albumin concentrations were similar between groups. The diabetic patients were nearly equally divided between sex and races. The BMI was significantly higher in patients with diabetes ($P = 0.04$). All study patients received hemodialysis for 3.5 to 4.5 hours, 3 times weekly. The blood and dialysate flow rates ranged from 250 to 450 mL/min and 450 to 600 mL/min, respectively. The delivered dialysis doses (KT/V) were similar for nondiabetic and diabetic patients ($P = 0.097$).

The values for glycemic control measures are presented in Table 3. Based on the recommended target value of A_1C (<7.0%), our diabetic patients on hemodialysis had, on average, reasonably good glycemic control (mean = 7.5%, range of A_1C, 5.8%–11.2%). The GHb averaged 9.6% (range 6.7%–16.5%). The nondiabetic dialysis patients exhibited A_1C and GHb of 5.4 ± 0.2 and 5.5 ± 0.4, respectively, which were within the normal ranges for nondiabetic patients who are not dialysis dependent. The GPP averaged 27.5% (range, 21.1%–27.7%) in diabetic dialysis patients. The Fr assay averaged 360 mcmoles/L (range, 292–586 mcmoles/L). Although a normal reference range has not been published for GPP or Fr in nondiabetic dialysis patients for comparison, our values were 20.6% ± 3.8% and 286.4 ± 59.6 mcmoles/L, respectively. Table 4 provides a comparison between the measures of glycemic control in nondiabetic patients who were hemodialysis dependent (from our study) versus literature values for patients without renal disease.[28,31,32] The ranges of these values for patients receiving hemodialysis overlapped with the ranges for patients without renal disease for all measures except Fr and GPP. For both Fr and GPP, the upper limit of the range was greater in hemodialysis patients than those not receiving hemodialysis. For all of these measures, the lower limits of the ranges were higher in patients receiving hemodialysis than in those without renal failure.

Average BG was plotted against A_1C, GHb, GPP, and Fr to determine the correlations and

Table 2. Patient and Control Demographics

	Diabetes (n = 23)	Nondiabetic Patients (n = 8)	*P* Value
Sex (men/women)	13/10	7/1	0.203
Race (W/AA)	10/13	5/3	0.433
Age (yr)	56 ± 11	57 ± 17	0.727
BMI	30.1 ± 5.9	24.8 ± 4.1	0.041
Albumin (g/dL)	3.8 ± 0.3	3.8 ± 0.4	0.928
Kt/V	1.41 ± 0.2	1.56 ± 0.2	0.097

Abbreviations: W, White; AA, African American.

Table 3. Glycemic Control Laboratory Results

	Diabetes (n = 23)	Nondiabetic Patients (n = 8)	*P* Value
BG (mg/dL)	160.2 ± 43.2	92.4 ± 23.7	0.0012
A_1C (%)	7.5 ± 1.3	5.4 ± 0.2	0.0004
GHb (%)	9.5 ± 2.7	5.5 ± 0.4	0.0004
GPP (%)	27.5 ± 5.5	20.6 ± 3.8	0.0071
Fr (mcmoles/L)	360 ± 85.9	286.4 ± 59.6	0.049

Table 4. Reported Reference Ranges for Glycemia in Patients Without Diabetes for Assay Methods Used in Current Study

	Nonhemodialysis	Hemodialysis*
Hemoglobin A_1C	4.0%–5.5%[31]	5.2%–5.6%
Total GHb	4.0%–8.0%[28]	4.7%–6.1%
Fr	165–303 mcmoles/L[32]	205–370 mcmoles/L
Total GPP	13.4%–25.0%[28]	17.7%–26.7%

*The ranges for patients on hemodialysis are derived from the current study.

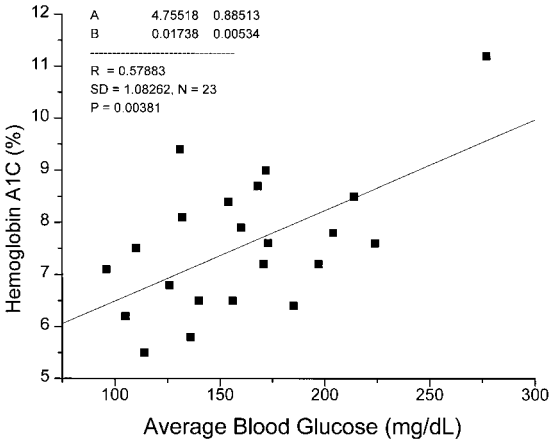

Fig 1. Linear regression analysis of average BG and hemoglobin A_1C showed similar correlation to the total glycosylated hemoglobin relationship (Fig 2): $A_1C = 0.0174$ (BG) + 4.76 (r = 0.579; $P < 0.05$).

equation of the line representing the relationships. A linear relationship between BG and each of the 4 measures of glycemic control (ie, A_1C, GHb, Fr, and GPP) were determined by graphic analysis.

The regression equations defined in Figs 1 and 2 are as follows:

$$A_1C = 0.0174\ (BG) + 4.76\ (r = 0.579;\ P < 0.05)$$

$$GHb = 0.0371\ (BG) + 3.57\ (r = 0.584;\ P < 0.05)$$

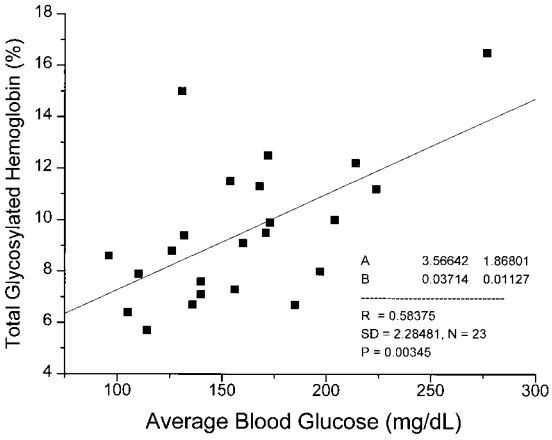

Fig 2. Linear regression analysis of average BG and total glycosylated hemoglobin showed similar correlation to the hemoglobin A_1C relationship (Fig 1): GHb = 0.0371 (BG) + 3.57 (r = 0.584; $P < 0.05$).

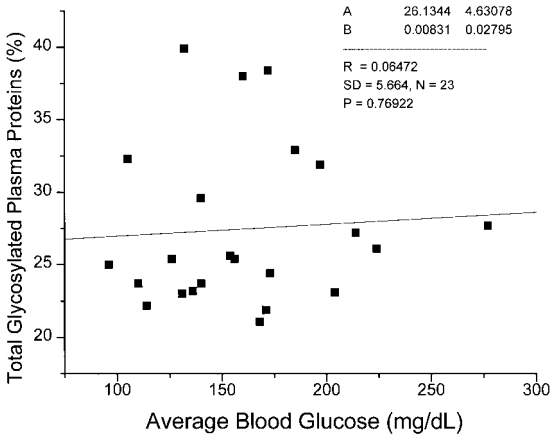

Fig 3. Linear regression analysis of average BG and total glycosylated plasma proteins showed the weakest correlation of all the measures: GPP = 0.0083 (BG) + 26.13 (r = 0.065; $P = 0.77$).

When compared with Fr and GPP, the graphs that plotted A_1C or GHb and average BG showed the best correlations. The relationship between BG and GPP was the least strongly correlated. This relationship is defined by the following regression equation and Fig 3:

$$GPP = 0.0083\ (BG) + 26.13\ (r = 0.065;\ P = 0.77)$$

Fructosamine was poorly correlated with BG. The following regression equation and Fig 4 defines this relationship:

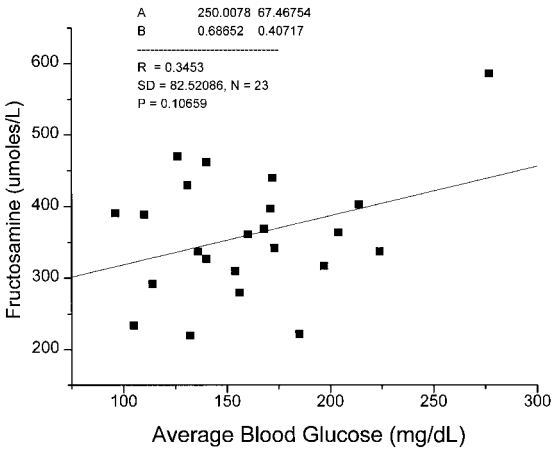

Fig 4. Linear regression analysis of average blood glucose and fructosamine showed a poor correlation when compared with total glycosylated hemoglobin and hemoglobin A_1C: Fr = 0.687 (BG) + 250 (r = 0.345; $P = 0.11$).

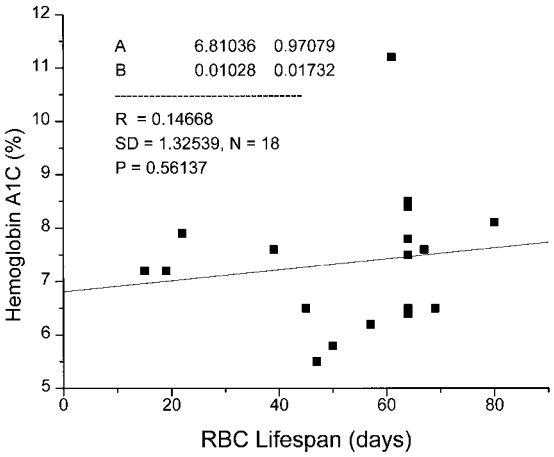

Fig 5. A poor correlation between RBC lifespan and hemoglobin A_1C was shown: A_1C = 0.010 (RBC lifespan) + 6.810 (r = 0.147, *P* = 0.56).

$$Fr = 0.687\ (BG) + 250\ (r = 0.345;\ P = 0.11)$$

Because of the poor correlation between Fr and BG, we sought to determine the role, if any, albumin concentration may have because the literature suggests a relationship between plasma albumin and Fr.[32] The relationship between albumin and Fr was poorly and nonsignificantly correlated. We calculated the Fr concentrations adjusted for the degree of hypoalbuminemia as suggested by Howey et al.[33] A graph of BG versus adjusted Fr showed a similar regression equation and correlation coefficient as for the unadjusted Fr graph (data not shown). Based on the similarity between groups, this adjustment did not appear to be necessary for our hemodialysis patients with albumin concentrations of 3.0 mg/dL or greater. All of the earlier-mentioned regression models were redrawn with the addition of the repeated studies with 9 patients (n = 32). The equations representing these models were similar to the single-measurement models, except for the correlation between BG and Fr, which became significant with the additional measurements [Fr = 1.092 (BG) + 201 (r = 0.481; *P* = .005)].

Multivariate models controlling for race, BMI, and RBC lifespan were evaluated for each equation (A_1C, GHb, GPP, and Fr) with BG. Because no appreciable differences in parameter estimates, correlation coefficients, and statistical significances were seen with these models, the unadjusted equations are reported for all associations.

Erythrokinetic modeling for the diabetic patients receiving erythropoietin therapy showed an average RBC lifespan of 53.1 ± 18.6 days. This value is below the average RBC lifespan (68 days) determined in hemodialysis patients by Uehlinger et al.[25] A plot of RBC lifespan versus A_1C showed a poor relationship and correlation (A_1C = .010 [RBC lifespan] + 6.810, r = .147, *P* = .56) (Fig 5). This model was not improved significantly with the addition of the repeated measurements that were performed in a small group of patients. Analysis of variance testing to control for BG in the model of RBC lifespan and A_1C did not improve the relationship.

When compared with the DCCT trial of diabetic patients without severely compromised renal function, there was a trend for A_1C values to be associated with lower BG in diabetic dialysis patients at A_1C values greater than 7% (Fig 6). On the other hand, at lower A_1C values (<6%), higher BG values were observed in hemodialysis patients than in DCCT patients. The maximum limit of the 95% confidence interval for the mean regression line estimated for the diabetic dialysis patients approaches the regression line of the DCCT trial (Fig 6). However, no measures of variation were available from the DCCT data (personal communica-

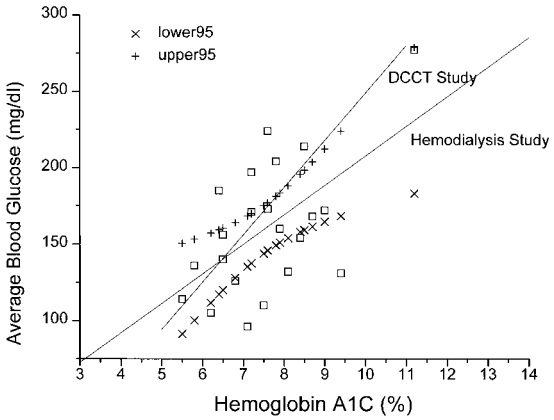

Fig 6. The upper limit of the 95% confidence interval for the diabetic dialysis patients overlaps with the regression line of the DCCT trial. For the DCCT, the published regression equation was: BG = 30.9 (A_1C) − 60.2. The regression equation for our data was BG = 19.28 (A_1C) + 14.85. The 95% confidence intervals for our data were computed for slope (7.7, 30.9) and intercept (−74.0, 103.7). Variation estimates were not available from the DCCT investigators.

tion), preventing statistical comparisons of the 2 lines. These results suggest that A_1C may underestimate the long-term level of glycemic control in diabetic patients requiring hemodialysis, especially at A_1C levels above 7.5%.

The DCCT data showed the following regression relationship between average BG and A_1C[15]:

$$BG = 30.9\ (A_1C) - 60.2$$

Based on these data, there was a trend for our hemodialysis patients to have lower BG concentrations than the DCCT patients with normal renal function for A_1Cs greater than approximately 7.5%. In hemodialysis patients, an A_1C increase of 1% related to a 20 mg/dL increase in BG. This is in contrast to the DCCT in which an increase in A_1C of 1% was related to a 30 mg/dL increase in BG.[15,16] Thus, each 1% increase in A_1C represents a 33% lower BG in dialysis patients than those without renal insufficiency.

Although we did not directly compare measurement of A_1C by the Tina-Quant and HPLC assays with our specimens, we were justified in comparing our results with the DCCT data because there is a direct correlation, similar line of identity, and small amount of bias between the 2 methods.[26,27]

DISCUSSION

The results of the DCCT showed the benefit of intensive insulin therapy and BG assessment; 34% reduction in retinopathy, 35% reduction in microalbuminuria, and 60% reduction in neuropathy in primary prevention.[16] A difference in mean GHb level of only 2% resulted in this marked reduction in complications.[16] Although these profound reductions in complications have been shown with routine monitoring, estimates in the literature report that only about 30% to 40% of diabetic patients routinely self-monitor their blood sugars.[34,35]

GHb has been routinely used to assess glycemic control of patients with diabetes mellitus. It is formed slowly and nonenzymatically from hemoglobin and glucose. It is recognized as an excellent marker of glycemic control because its rate of formation is proportional to the BG concentration over the average RBC lifespan (mean 120 days). The validity of A_1C in monitoring the glycemic control of patients with ESRD has been incompletely evaluated. In question is not only whether A_1C correlates with glycemic control in patients with ESRD, but whether A_1C measures correspond to the same average serum glucose concentrations in patients with ESRD and patients with normal renal function. It is important to determine if currently used measures of GHb over- or underestimate glycemic control in patients with ESRD. Of particular concern would be if A_1C overestimates glycemic control in patients with ESRD (ie, measured A_1C is lower than expected for the same glucose concentrations in patients without renal insufficiency). This would in effect expose diabetic patients with ESRD to an increased risk for long-term small- and large-vessel disease associated with poor glycemic control, such as retinopathy and neuropathy. In addition, because 50% of patients commencing renal replacement therapy suffer from cardiovascular disease, the impact of inadequately controlled diabetes on cardiovascular and cerebrovascular risks may be significant in this population.[36]

In this study, we aimed to evaluate the correlation and accuracy of common clinically used measures of glycemic control (A_1C and GHb) in chronic hemodialysis patients with diabetes mellitus. Because of the potential for limitations on the accuracy of measures of glycemic control that are based on hemoglobin, we evaluated GPP and Fr as possible alternative measures, perhaps better suited to the ESRD population. The rationale for using glycated serum proteins for determination of glycemic control are: (1) control can be measured consistently over a shorter time frame because of the shorter half-life of serum proteins (albumin 14 days), and (2) serum proteins could be used in instances in which RBC lifespans (and accuracy of GHbs) are altered. Comparisons of glycated proteins (Fr and GPP) to GHb in patients with diabetes mellitus without concurrent renal diseases have been reported.[37,38] The validity of the Fr assay and its use in assessment of short-term glycemic control have also been described.[29,30]

Hemoglobin A_1C is a posttranslational modification of hemoglobin A. The hemoglobin A subfractions were initially measured by ion-exchange chromatography and gel electrophoresis, but are now also measured by immunoassay methods (eg, Tina-Quant).[27] GHb describes all

GHb species as quantified by affinity chromatography, which detects structural differences instead of charge selectivity (ion exchange chromatography and gel electrophoresis). Interferences with GHb assay methods are common and are listed in Table 1.[17,18] In addition, the results of the various assays measuring GHb are not compared with a single GHb standard, thus limiting the potential to directly compare results across all tests and laboratories. A recent review of the Tina-Quant A_1C immunoassay (standardized to the HPLC method used in the DCCT trial) showed no significant interference from bilirubin and triglycerides up to levels of 64.5 mg/dL and 2,000 mg/dL, respectively.[26,27] None of the patients evaluated in our study had bilirubin or triglyceride concentrations in the assay interference range. In addition, no interference from hemoglobin F was shown for this method.[27] Our regression analysis indicated nearly identical correlation coefficients when BG was plotted against either A_1C or GHb.

Like GHb, the Fr and GPP methods also have assay interferences that are delineated in Table 1.[17] Immunoassay methods for Fr have been developed to counter some of the interferences. There has been considerable debate regarding whether the Fr assay should be corrected for total protein or albumin concentration, especially if albumin concentration is below the normal range.[33,39-41] The GPP test measures albumin and all other plasma proteins; as such, it may be less influenced by variations in 1 protein. Both the Fr and GPP tests are indicative of glycemic control over the previous 1 to 3 weeks, instead of the previous 8 to 16 weeks (range of RBC lifespans in hemodialysis) because the half-lives of the plasma proteins are less than that of the RBC. Because the current guidelines (DOQI) recommend an adequate protein intake in hemodialysis patients to maintain a normal albumin concentration, hypoalbuminemia may become less of a significant issue in assessing glycemic control with methods based on glycated proteins or Fr.[42] Nevertheless, we sought to limit the effect of albumin concentration by eliminating patients with albumin concentrations of less than 3.0 g/dL. In addition, we corrected our patient's albumin concentrations to the normal range when graphically analyzing the correlations between BG and Fr by using the method of Howey et al.[33] The normalization of these concentrations had no effect on these correlations. As suggested in our results, additional patients to the data set may improve the correlation between Fr and BG. The lower reference ranges for all tests of glycemic control were higher in our ESRD patients than reported in patients without renal disease. In addition, for Fr and GPP, a higher upper reference limit was shown in patients with ESRD.

Testing that measures glycated proteins appears to have less assay interference from carbamylated proteins when the thiobarbituric acid method is used. To avoid assay interference with our measurements of GPP, we used affinity HPLC and a thiobarbituric acid method to measure GPP and Fr, respectively. Avoidance of ion-exchange and gel electrophoresis methods in measuring glycated proteins is suggested in patients receiving hemodialysis. Although we sought to eliminate interferences that might alter the interpretation of our results, the measurement of GPP did not appear to correlate well with BG in our ESRD patients.

Several articles have recently evaluated various measures of glycemic control in patients with chronic renal failure, though none addressed the issue of accuracy of these methods.[20-22] Increases in hemoglobin A_1 (13%–22%) have been shown in uremic patients by ion-exchange chromatography,[22] but assay methods such as HPLC, colorimetry, or immunoassay appear to produce less elevations in GHb measurements owing to the uremic state or the dialysis therapy.[20,21] We evaluated A_1C and GHb by immunoassay and affinity HPLC methods, respectively, to eliminate interference with our measurements. It is necessary to note that when clinically evaluating the A_1C, one needs to consider the assay methodology used and reported reference range. For instance, though the normal reference range for the A_1C immunoassay we used was 4.0% to 5.5%, a published boronate affinity chromatography assay report shows a much wider normal reference range of 4.5% to 7.0%.[21,31] Our data revealed that measurement of GHb and A_1C correlated well with mean BG concentrations. GPP correlated poorly with average BG measurements.

To assess the accuracy of the A_1C test, we compared the relationship of A_1C measurements

in diabetic patients on dialysis (as determined in our study) with the results for that test in patients with preserved renal function (as determined in the DCCT trial). There are several limitations to this approach: different assay methodologies were used to assess A_1C, and we were unable to obtain 95% confidence interval data from the DCCT trial. In light of the first of these theoretical limitations, a comparison of the A_1C results from the Tina Quant (our study) and HPLC (DCCT) methods showed nearly identical measures of A_1C (with a bias of -0.09 to -0.1 only).[26] Comparison of the regression lines between A_1C in patients with preserved renal function (DCCT) versus ESRD (our study) revealed that A_1C tends to underestimate glycemic control in the latter group. Thus, for any value of A_1C above 7.5%, the serum glucose measurements tend to be lower in ESRD patients on hemodialysis than in patients with preserved renal function (as defined by the DCCT). The difference between the 2 A_1C-versus-BG curves tends to increase as A_1C increases. On the contrary, A_1C overestimates mean glucose concentrations at values near and below the target of 6.5% for patients with ESRD. Because large trials similar to the DCCT have not been reported using GHb, we were unable to infer the accuracy of this measurement in predicting BG in our dialysis patients.

Because the Tina-Quant assay was documented to be free from interference caused by carbamylated hemoglobin and other interference factors were not present, the increased reported A_1C in our dialysis patients was most likely caused by uremia. Indeed, the difference in glucose concentrations can be substantial at higher A_1C levels. Thus, for example, an A_1C of 7.5% would correspond to a BG of 159 mg/dL in a patient with ESRD and 172 mg/dL in a diabetic patient without renal failure. At A_1Cs of less than 6%, the corresponding A_1C per level of BG is lower for patients receiving hemodialysis, possibly reflecting an influence from other documented confounders. The differences in measured A_1C we found between the DCCT data and our hemodialysis patients is preliminary data. The magnitude of this difference may be less apparent with greater numbers of evaluable hemodialysis patients. Also, the DCCT incorporated data from quarterly evaluations for 6.5 years in 1,441 patients, which would tend to tighten the fit of the regression line.

For clinical purposes, the character of this relationship is such that A_1C remains a very useful test to guide the therapy of patients on hemodialysis. A hemodialysis patient with an A_1C near the recommended target of 7.0% would have better glycemic control than a patient without compromised renal function. On the other hand, a very elevated A_1C value (eg, $>8.5\%$) would still relay the message of poor glycemic control to the patient and treating physician. Also, for centers that report only GHb, it is reasonable to feel confident in the correlation with BG in patients with ESRD.

Since the length of the RBC lifespan is presumed to determine the accuracy of A_1C and GHb measurements because of exposure time to BG concentrations, assessment of the lifespan may be useful to evaluate these measures. The life of the RBC in dialysis patients may be altered as compared with the normal lifespan of 120 days in patients not receiving dialysis and without renal insufficiency. One model suggests a mean RBC lifespan of 68 days in hemodialysis patients with an interindividual variability of 34%.[25] RBCs with a shortened lifespan have a shorter exposure to the ambient glucose concentrations in the blood. This may result in a reduced measured A_1C per level of glycemia when compared with patients with normal RBC lifespans. Our patients had average RBC lifespans of 53 days versus 120 days in patients without renal disease.[25] However, based on our regression analysis, a good correlation between RBC lifespan and A_1C failed to exist. The poor fit of the erythrokinetic model in our patients (mean residual of plot of the regression line $= 1.22 \pm .55$) may reflect the fact that RBC lifespan has less of an impact on measured A_1C compared with BG concentrations and/or the presence of other concurrent factors. It may also be the result of our relatively small sample size and narrow range of RBC lifespans. This model was not improved significantly with the addition of the repeated measurements that were performed in a small group of patients or by controlling for level of BG. Based on our limited data, there is little use in predicting RBC lifespan to predict accuracy of A_1C or GHb measurements.

CONCLUSIONS

Glycated hemoglobin (A_1C or GHb) appears to correlate most accurately with measured BG in patients with ESRD receiving hemodialysis, whereas Fr and GPP, as measures of GPP, appear to correlate poorly with glycemic control. Our study implies that there is an underestimation of glycemic control by assessment of A_1C in hemodialysis patients, especially when A_1C is greater then 7.5%. If the benefits of tight glycemic control as shown by the DCCT trial apply to the population of patients on dialysis, a higher A_1C in hemodialysis patients is less worrisome than in the overall population because this represents a lower BG concentration. Hemodialysis patients with good glycemic control, as defined by A_1C of less than 6.0%, appear to have higher levels of BG than that published for the DCCT in patients without coexisting renal disease, suggesting a role of possible confounders in the immunoassay method. The reference ranges and specificity need to be considered when interpreting the results of measurements of glycemic control in ESRD patients.

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