Continuous Glucose Monitoring and Use of Alternative Markers To Assess Glycemia in Chronic Kidney Disease

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OBJECTIVE

In chronic kidney disease, glycated albumin and fructosamine have been postulated to be better biomarkers of glycemic control than HbA_{1c}. We evaluated the accuracy, variability, and covariate bias of three biomarkers (HbA_{1c}, glycated albumin, and fructosamine) compared with continuous glucose monitoring (CGM)–derived measurement of glycemia across estimated glomerular filtration rate (eGFR) in type 2 diabetes.

RESEARCH DESIGN AND METHODS

A prospective cohort study was conducted of 104 participants with type 2 diabetes, 80 with eGFR <60 mL/min/1.73 m² (not treated with dialysis) and 24 frequency-matched control subjects with eGFR ≥60 mL/min/1.73 m². Participants wore a blinded CGM for two 6-day periods separated by 2 weeks, with blood and urine collected at the end of each CGM period. HbA_{1c}, glycated albumin, and fructosamine were measured by high-performance liquid chromatographic, enzymatic, and colorimetric nitroblue tetrazolium methods, respectively.

RESULTS

Within-person biomarker values were strongly correlated between the two CGM periods ($r = 0.92$ – 0.95), although no marker fully captured the within-person variability of mean CGM glucose. All markers were similarly correlated with mean CGM glucose ($r = 0.71$ – 0.77). Compared with mean CGM glucose, glycated albumin and fructosamine were significantly biased by age, BMI, serum iron concentration, transferrin saturation, and albuminuria; HbA_{1c} was underestimated in those with albuminuria.

CONCLUSIONS

Glycated albumin and fructosamine were not less variable than HbA_{1c} at a given mean CGM glucose level, with several additional sources of bias. These results support measuring HbA_{1c} to monitor trends in glycemia among patients with eGFR <60 mL/min/1.73 m². Direct measurements of glucose are necessary to capture short-term variability.

Glycated hemoglobin (HbA_{1c}) is commonly used in people with diabetes to monitor long-term (~3 months) glycemic control. However, HbA_{1c} may not appropriately measure glycemic burden in those who also have chronic kidney disease (CKD), with both bias (defined as a difference in mean values) and more variability around the

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mean possible. In CKD, red blood cell (RBC) turnover is increased, providing less opportunity for hemoglobin glycation for a given level of glycemia. Even small changes in RBC life span can affect HbA_{1c} (1), and consequently, HbA_{1c} has been shown to underestimate glycemia in those with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m² and in those on dialysis (2,3). Anemia, which occurs commonly in CKD, and its treatment with iron supplements or erythropoietin-stimulating agents (ESAs), can affect HbA_{1c} levels (4–7). In addition, some studies suggest that HbA_{1c} differs by race (8–10), which further complicates its clinical use among the racially diverse population of patients with diabetes and CKD.

Glycated serum proteins, such as glycated albumin and fructosamine, may avoid issues related to RBC turnover and provide viable alternatives to HbA_{1c} as a marker of glycemia. Glycated albumin is the percentage of serum albumin to which a glucose molecule has been non-enzymatically attached, while fructosamine refers to all ketoamine linkages resulting from serum protein glycation (11). In particular, these biomarkers are not based on hemoglobin and hence, do not depend on RBC turnover (6,12). In addition, with a shorter half-life than hemoglobin, glycated proteins reflect average glycemic burden of the previous 2–3 weeks and so may be better suited than HbA_{1c} to monitor shorter-term changes in glycemia (2,11). Higher glycated albumin and fructosamine levels have both been linked to increased risk of cardiovascular outcomes and mortality as well as to other important clinical outcomes (13–16), and previous studies have suggested that glycated albumin and fructosamine may be comparable or superior to HbA_{1c} as a measure of glycemic control in patients treated with dialysis (3,17–21). However, there are few data evaluating the performance of these alternative biomarkers compared with a gold-standard measure of glycemia in patients with eGFR <60 mL/min/1.73 m² not treated with dialysis (2,19).

We prospectively enrolled 81 participants with diabetes and moderate to severe CKD (defined as eGFR <60 mL/min/1.73 m²) not treated with dialysis and 24 frequency-matched control subjects with diabetes and no CKD to undergo continuous glucose monitoring

(CGM) in two periods over an average of 12 days (22). In this article, we evaluate the accuracy, variability, and covariate bias of HbA_{1c}, glycated albumin, and fructosamine compared with the CGM-derived measurement of glycemia, considered here to be a gold standard, across levels of GFR among people with diabetes.

RESEARCH DESIGN AND METHODS

Study Design and Population

Continuous Glucose Monitoring to Assess Glycemia in CKD (CANDY) was a prospective observational cohort study designed to characterize hypoglycemia in CKD and evaluate the performance of markers of glycemia in people with type 2 diabetes and moderate to severe CKD (22). Briefly, the study enrolled 105 participants between August 2015 and July 2017 with a clinical diagnosis of type 2 diabetes treated with sulfonylurea or insulin; 81 of these participants had moderate to severe CKD (eGFR 6 to <60 mL/min/1.73 m²), and 24 control subjects (eGFR ≥60 mL/min/1.73 m²) were frequency matched by age, duration of diabetes, HbA_{1c}, and use of glucose-lowering medication. Those with a history of kidney transplant, on dialysis, pregnant, currently using clinical CGM, currently being treated for cancer or with erythropoietin, unable to speak English, or <18 years of age were excluded from the study.

Participants wore a blinded CGM device for two nonconsecutive 6-day periods separated by ~2 weeks. Blood and spot urine samples were collected at the end of each CGM period; thus, biomarker measurements were separated by ~3 weeks. For this analysis evaluating the relative merits of biomarkers of glycemia compared with a CGM-based assessment of glycemia, we excluded one participant who did not have a complete set of biomarker measurements at either visit because of insufficient serum volume, for a final analytic sample of 104 participants.

The CANDY study was approved by institutional review boards of the University of Washington and the Puget Sound Veterans Affairs Health Care System. Each participant granted written informed consent.

Glycemia Markers

Biomarkers of glycemia were collected at the end of each CGM period, ~3

weeks apart. HbA_{1c} was measured from whole blood collected at the end of each CGM period using an NGSP network method (Tosoh G8 HPLC, Secondary Reference Laboratory, SRL 9), with long-term coefficients of variance (CVs) of 2.41% and 1.58% at levels of 5.1% and 10.4% HbA_{1c}. Glycated albumin was measured in serum by an enzymatic assay (Lucica GA-L kit; Asahi Kasei Pharma Corporation; kits were obtained directly from Asahi Kasei for research use only), and CVs were 4.2% and 2.1% for levels of 10.58% and 22.29%, respectively. Fructosamine was measured in serum using the colorimetric nitroblue tetrazolium assay (Roche Diagnostics Corporation, Indianapolis, IN), with CVs of 4.1% and 4.0% for levels of 230 and 370 μmol/L, respectively. Both glycated albumin and fructosamine assays were performed on the Roche cobas c 311 instrument.

CGM

Participants wore an iPro2 Professional blinded CGM Enlite sensor (Medtronic, Northridge, CA) to monitor glycemia over two nonconsecutive 6-day periods separated by ~2 weeks. Blood glucose concentrations were estimated every 5 min from interstitial glucose levels, with a detection range of 40–400 mg/dL. Participants calibrated the CGM at least twice daily with a fingerstick capillary blood glucose using a FreeStyle Lite glucose monitor (Abbott Laboratories, Alameda, CA). Study physicians reviewed each CGM report, identifying periods of biologic implausibility (defined as evidence of CGM malfunction or marked [>30%] dyssynchrony between CGM and fingerstick glucose values), which were excluded from subsequent analysis (22). Study physicians also intervened if severe hypoglycemia was noted on either CGM analysis to ensure safety of patients. The mean CGM blood glucose for each participant was calculated over all valid CGM measurements across both CGM periods and is assumed here to be a gold-standard measurement of glycemia. The glucose management indicator (GMI) was calculated as $3.31 + 0.02392 \times \text{mean CGM glucose (mg/dL)}$ (23). In a sensitivity analysis, we restricted analyses to the mean CGM blood glucose calculated only from the 6-day CGM period that immediately preceded the biomarker blood draw.

Covariate Data

Demographics and medical history were ascertained through self-report, while trained research coordinators inventoried medications with assistance from electronic health records. Serum creatinine, hemoglobin, albumin, iron, and transferrin were measured using a DxC automated chemistry analyzer (Beckman Coulter, Brea, CA). Serum creatinine was measured using a modified Jaffe reaction, with results traceable to isotope dilution mass spectrometry. eGFR was calculated using the creatinine-only Chronic Kidney Disease Epidemiology Collaboration equation (24). Urine albumin and urine creatinine were collected from spot urines at the end of the second CGM period and measured using a turbidimetric method on a DxC automated chemistry analyzer.

Statistical Analysis

We used scatterplots and Pearson correlation coefficients to investigate the repeatability of glycemia markers between CGM periods as well as the relationship between change in the biomarker and change in the CGM mean glucose. Inferential comparisons of Pearson correlation coefficients used Fisher r -to- z transformation (25). We used linear regression of each biomarker on CGM mean glucose with robust Huber-White SEs (26) to quantify the variability and accuracy of each biomarker. From this regression, we calculated the proportion (denoted p_{10} , etc.) of observed biomarkers that fell within 10% (and 20% and 30%) of the value predicted by the regression. We also regressed the squared residuals on eGFR to evaluate whether the variability of the biomarker might vary by eGFR. Finally, we examined the covariate determinants of bias, defined as the difference in the mean biomarker comparing those with different values of the covariate but the same mean CGM glucose. To do this, we performed linear regression of the log-transformed glycemic marker on the potential determinant of bias adjusted for the CGM mean glucose. All primary regression analyses excluded one participant with a highly unusual HbA_{1c}-to-mean CGM glucose relationship.

For the primary analysis, we preferentially used the biomarker measurements taken from the end of the second CGM period; when these were

unavailable ($n = 11$ participants), we instead used measurements collected at the end of the first CGM period. In a sensitivity analysis, we repeated analyses in the subset of participants ($n = 92$) with complete glycemia marker measurements from the end of the second CGM period. In another sensitivity analysis, we restricted analysis to use only the CGM period that immediately preceded the biomarker collection. Finally, we performed a sensitivity analysis of the covariate determinants of bias of albumin-corrected fructosamine, defined as fructosamine divided by the albumin result from the glycated albumin measurement ($\mu\text{mol fructosamine}/\mu\text{mol albumin}$).

All analyses were conducted using the R 3.6.0 software environment (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed $P < 0.05$ was taken as evidence of statistical significance in all analyses.

RESULTS

Participant Characteristics

Of the 104 participants included in this study, 80 (77%) had eGFR < 60 mL/min/1.73 m², 67 (64%) were male, and 80 (77%) were White (Table 1). The mean age was 68 (SD 10) years, and BMI was 33 (SD 6) kg/m². Participants had a mean duration of diabetes of 19 (SD 10) years and HbA_{1c} of 7.7% (SD 1.3%) or 61 (SD 14.8) mmol/mol. During the CGM periods, the mean of each participant's mean CGM blood glucose was 168 (SD 38) mg/dL.

Repeatability of Glycemia Markers

Ninety-two (88%) study participants had complete biomarker measurements at the end of the first and second CGM periods. Biomarker values at the end of the first period were strongly correlated with the values at the end of the second period (HbA_{1c}, $r = 0.95$; glycated albumin, $r = 0.93$; fructosamine, $r = 0.92$) (Table 2 and Supplementary Figs. 1 and 2). In contrast, there was markedly less concordance in the CGM mean blood glucose between the two periods ($r = 0.77$). The change in CGM mean glucose between the two periods was more strongly correlated with the change in glycated albumin ($r = 0.67$) than with the change in fructosamine ($r = 0.48$) or HbA_{1c} ($r = 0.26$). Within-person correlation of all biomarkers and the

correlation of change in each biomarker with the change in CGM mean glucose tended to be stronger among participants with eGFR < 60 mL/min/1.73 m² than among those with eGFR ≥ 60 mL/min/1.73 m².

Variability, Accuracy, and Bias of HbA_{1c}

HbA_{1c} showed strong correlation with CGM mean blood glucose among all participants ($r = 0.78$) and in those with eGFR < 60 mL/min/1.73 m² ($r = 0.78$) (23) (Table 3 and Fig. 1). Observed values of HbA_{1c} fell within 10% of the value predicted by the mean CGM glucose 77% of the time, and HbA_{1c} was significantly more variable as a marker of CGM mean glucose for participants with lower eGFR ($P = 0.02$) (Fig. 2). We saw little bias in HbA_{1c} for the mean CGM glucose by measured covariates, with the exception of albuminuria (urine albumin-to-creatinine ratio [uACR] $> 1,000$ mg/g) (Table 4 and Supplementary Fig. 3). There was no significant bias observed in HbA_{1c} by level of hemoglobin, serum iron, or transferrin saturation.

Variability, Accuracy, and Bias of Alternative Markers of Glycemia

Overall, there was a strong correlation among all markers of glycemia, particularly between glycated albumin and fructosamine; no marker of glycemia correlated with glucose variability as measured by CV% of glucose readings (Supplementary Table 2). We observed similar Pearson correlations of the three biomarkers of glycemia with CGM mean blood glucose among all participants (HbA_{1c}, $r = 0.78$; glycated albumin, $r = 0.77$; fructosamine, $r = 0.71$) and in those with eGFR < 60 mL/min/1.73 m² (HbA_{1c}, $r = 0.78$; glycated albumin, $r = 0.78$; fructosamine, $r = 0.71$) (Table 3). Observed values fell within 10% of the predicted value more often for HbA_{1c} ($p_{10} = 77\%$) than for either glycated albumin ($p_{10} = 56\%$) or fructosamine ($p_{10} = 61\%$) both in participants overall and in those with and without eGFR < 60 mL/min/1.73 m². Like HbA_{1c}, both glycated albumin and fructosamine were significantly more variable as a marker of CGM mean glucose for participants with lower eGFR (glycated albumin, $P = 0.03$; fructosamine, $P = 0.01$) (Fig. 2).

For participants with the same CGM mean blood glucose, statistically significant lower glycated albumin and

Table 1—Baseline characteristics of analytic population, overall and by eGFR

	Overall (N = 104)	eGFR <60 mL/min/1.73 m ² (n = 80)	eGFR ≥60 mL/min/1.73 m ² (n = 24)
Age (years), mean (SD)	67.5 (9.9)	68.4 (9.6)	64.3 (10.3)
Male, n (%)	67 (64)	52 (65)	15 (62)
Race, n (%)			
White	80 (77)	60 (75)	20 (83)
Black	13 (12)	11 (14)	2 (8)
Other	11 (11)	9 (11)	2 (8)
Hispanic ethnicity, n (%)	11 (11)	8 (10)	3 (12)
History of MI, n (%)	14 (13)	13 (16)	1 (4)
History of CHF, n (%)	19 (18)	18 (22)	1 (4)
History of stroke, n (%)	12 (12)	11 (14)	1 (4)
Duration of diabetes (years), mean (SD)	19.1 (10.0)	20.0 (10.4)	15.9 (8.4)
BMI (kg/m ²), mean (SD)	33.4 (5.7)	33.6 (5.6)	32.4 (6.2)
Systolic blood pressure (mmHg), mean (SD)	133 (21)	132 (21)	136 (17)
Diastolic blood pressure (mmHg), mean (SD)	73 (13)	72 (13)	78 (12)
eGFR (CKD-EPI) (mL/min/1.73 m ²), mean (SD)	48 (23)	38 (14)	83 (11)
<30 mL/min/1.73 m ² , n (%)	22 (21)	22 (28)	0 (0)
uACR (mg/g), median (IQR)	88 (16–604)	142 (31–675)	15 (8–37)
>1,000 mg/g, n (%)	16 (15)	14 (18)	2 (8)
Hemoglobin (g/dL), mean (SD)	12.4 (1.8)	12.2 (1.6)	13.1 (2.0)
Anemia, n (%)	38 (37)	30 (38)	8 (33)
Serum albumin (g/dL), mean (SD)	3.7 (0.4)	3.7 (0.4)	3.7 (0.4)
<3 g/dL, n (%)	6 (6)	4 (5)	2 (8)
Serum iron (μg/dL), mean (SD)	66 (23)	65 (23)	68 (23)
Transferrin saturation (%), mean (SD)	27 (11)	27 (11)	27 (11)
Mean CGM glucose (mg/dL), mean (SD)	168 (38)	170 (40)	158 (30)
GMI (%), mean (SD)	7.3 (0.9)	7.4 (1.0)	7.1 (0.7)
HbA _{1c} (%), mean (SD)	7.7 (1.3)	7.8 (1.4)	7.7 (1.3)
HbA _{1c} (mmol/mol), mean (SD)	61 (14.8)	61 (15.1)	60 (13.8)
Glycated albumin (%), mean (SD)	18.7 (4.8)	19.1 (5.0)	17.6 (3.6)
Fructosamine (μmol/L), mean (SD)	304 (59)	310 (61)	285 (49)

GMI is defined as $3.31 + 0.02392 \times \text{mean CGM glucose (mg/dL)}$ (23). Anemia is defined as hemoglobin <11.5 g/dL for females and <13 g/dL for males. CHF, congestive heart failure; IQR, interquartile range; MI, myocardial infarction.

fructosamine were observed in those of younger age; those with a higher BMI, lower serum iron, and lower transferrin saturation; and those with albuminuria (uACR >1,000 mg/g) (Table 4 and Supplementary Figs. 4 and 5). Estimates of bias in glycated albumin and fructosamine tended to be in the same direction and of similar magnitude (Supplementary Fig. 6). Fructosamine, among participants with the same CGM mean blood glucose, was also higher in those with higher serum albumin. Notably, there appeared to be little bias across the range of eGFR (Fig. 2).

These patterns of accuracy and bias persisted when restricting to the most recent CGM (Supplementary Tables 8 and 9) and to only those participants with biomarkers measured at the end of

the two CGM periods (Supplementary Tables 10 and 11). Results were also largely similar when excluding three influential participants (Supplementary Table 14). A sensitivity analysis of albumin-corrected fructosamine showed overestimation with higher age and lower eGFR and underestimation with higher BMI, hemoglobin, and serum albumin (Supplementary Table 7). We observed no statistically significant bias by sex or race/ethnicity for any marker of glycemia.

CONCLUSIONS

Among adults with type 2 diabetes and a range of eGFR who were not treated with dialysis or ESAs, neither glycated albumin nor fructosamine was a better surrogate of mean CGM glucose than HbA_{1c}. The

difference between measured HbA_{1c} and predicted HbA_{1c} on the basis of mean CGM glucose tended to be less variable than the corresponding differences between observed and predicted values for glycated albumin and fructosamine, and both glycated albumin and fructosamine had more sources of bias than HbA_{1c}. Results were consistent among participants with normal and reduced eGFR, although relatively few participants had eGFR <30 mL/min/1.73 m². Notably, changes in neither HbA_{1c}, glycated albumin, nor fructosamine captured the week-to-week or shorter-term variability of the CGM-derived measurement. Altogether, these findings support the use of HbA_{1c} over glycated albumin or fructosamine for monitoring of long-term glycemia in patients with type 2 diabetes and

Table 2—Within-person repeatability of glycemia markers and mean CGM glucose over ~3 weeks, overall and by eGFR

	Overall		eGFR <60 mL/min/1.73 m ²		eGFR ≥60 mL/min/1.73 m ²	
	Within-person correlation	Correlation of change in biomarker with change in CGM mean glucose	Within-person correlation	Correlation of change in biomarker with change in CGM mean glucose	Within-person correlation	Correlation of change in biomarker with change in CGM mean glucose
HbA _{1c}	0.95	0.26	0.96	0.31	0.88	0.05
Glycated albumin	0.93	0.67	0.94	0.73	0.94	0.38
Fructosamine	0.92	0.48	0.93	0.55	0.91	0.25
CGM mean glucose	0.77	—	0.76	—	0.78	—

Analyses exclude one participant with an implausible HbA_{1c}-to-mean CGM glucose relationship.

eGFR <60 mL/min/1.73 m² not treated with dialysis.

In this study, HbA_{1c}, glycated albumin, and fructosamine all had high within-person correlation (0.92–0.95) when measured ~3 weeks apart. The stability of these markers indicates low within-person variability over this time frame, which in turn provides reassurance for epidemiologic studies that use these measures. Over ~3 weeks, CGM mean glucose was observed to vary within an individual more than the biomarkers did, and change in none of the biomarkers fully reflected the change in CGM mean glucose. Changes in CGM mean glucose could reflect variability as a result of lifestyle, medication use, or in some cases, physician intervention. While HbA_{1c} would not be expected to vary greatly over this time period because of the longer life span of RBCs (~120 days), glycated albumin and fructosamine reflect a shorter-term (2–3 week) average glycemic burden and might be expected to have lower within-person

correlation. However, changes in glycated albumin reflected changes in mean glucose more closely than changes in fructosamine or HbA_{1c} did. While glycated albumin and fructosamine are conceptually similar, fructosamine reflects glycation of other proteins beyond albumin, such as globulins, which have both a longer half-life than albumin and a wide metabolic heterogeneity (27). We speculate that the contributions of other glycated proteins may explain why changes in fructosamine corresponded less closely than glycated albumin to changes in mean glucose.

None of the biomarkers were strongly correlated with CV% or time below range, clinically important measures of glucose variability and hypoglycemia. While the focus of this report was the evaluation of biomarkers as a tool to monitor long-term trends in glycemia, it should be noted that no biomarker captured clinically important minute-to-minute variability. In addition to quantifying mean glucose levels, CGM can be used to detect

and mitigate moment-to-moment alterations in blood glucose, allowing for improved glycemic management, particularly when results are available real time. As a result, direct measurement of blood glucose (e.g., with CGM) is necessary if capturing short-term variability and episodes of hypoglycemia are important.

Previous studies have raised questions about whether HbA_{1c} is an appropriate surrogate of glycemia among patients with reduced GFR. For example, a 2012 study (2) found a similar correlation of HbA_{1c} and mean glucose as our study in 25 subjects with diabetes and no kidney disease ($r = 0.66$) but a much lower correlation ($r = 0.38$) in 25 age- and sex-matched subjects with diabetes and eGFR <30 mL/min/1.73 m². In that study, HbA_{1c} significantly underestimated glycemia in patients with diabetes and eGFR <30 mL/min/1.73 m²; a 2010 study of 30 patients with diabetes and eGFR <60 mL/min/1.73 m² reached similar conclusions (19). In contrast, our

Table 3—Measures of correlation, variability, and accuracy of glycemia markers for mean CGM glucose, overall and by eGFR strata

Metric	Overall			eGFR <60 mL/min/1.73 m ²			eGFR ≥60 mL/min/1.73 m ²		
	HbA _{1c}	Glycated albumin	Fructosamine	HbA _{1c}	Glycated albumin	Fructosamine	HbA _{1c}	Glycated albumin	Fructosamine
Pearson r	0.78	0.77	0.71	0.78	0.78	0.71	0.76	0.72	0.63
Spearman r	0.74	0.69	0.63	0.75	0.66	0.63	0.65	0.79	0.68
Absolute residuals, median (IQR)	−0.0 (−0.4 to 0.5)	−0.1 (−1.7 to 1.4)	−4.7 (−19.4 to 21.9)	0.0 (−0.4 to 0.5)	0.0 (−1.8 to 1.3)	−7.1 (−20.7 to 19.2)	−0.3 (−0.4 to 0.4)	−0.2 (−1.5 to 1.0)	8.7 (−32.6 to 23.2)
P ₁₀ (%)	77	56	61	75	55	64	78	52	43
P ₂₀ (%)	92	81	83	90	79	84	100	91	83
P ₃₀ (%)	99	94	97	99	92	95	100	100	100

Correlations are the correlation of the biomarker with CGM mean glucose. Residuals come from a linear regression of the biomarker on the mean CGM glucose; units for residuals are percent for HbA_{1c} and glycated albumin and micromoles per liter for fructosamine. P₁₀, P₂₀, and P₃₀ are the proportion of observed biomarkers that fall within 10%, 20%, and 30% of the predicted value of the biomarker from the linear regression, respectively. Analyses exclude one participant with an implausible HbA_{1c}-to-mean CGM glucose relationship. IQR, interquartile range.

Figure 1—HbA_{1c} and GMI by CKD stage. GMI is defined as $3.31 + 0.02392 \times \text{mean CGM glucose (mg/dL)}$. Dashed lines indicate the line of identity.

study showed that HbA_{1c} performed reasonably well among participants with mild to moderate CKD. We found that HbA_{1c} had good correlation ($r = 0.78$) with CGM mean glucose and low variability ($p_{10} = 77\%$); these values among participants with $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ were similar to those with normal eGFR. Notably, we observed in this study no bias by eGFR, even among those with $\text{eGFR} < 30 \text{ mL/min/1.73 m}^2$, although there were relatively few ($n = 22$) participants in this range, and participants in our study were not treated with ESAs. However, we found evidence that the difference between observed HbA_{1c} and the expected value on the basis of mean CGM glucose was significantly more variable for those with lower eGFR. In another study of moderate to severe CKD, Lo et al. (28) found a correlation of mean CGM glucose with HbA_{1c} ($r = 0.79$) similar to that in our study among patients with $\text{eGFR} 30\text{--}59 \text{ mL/min/1.73 m}^2$, which did not differ significantly from an indirect comparator group from the A1C-Derived Average Glucose (ADAG) study ($r = 0.74$). In that study, the correlation was substantially lower

($r = 0.34$) among participants with $\text{eGFR} < 30 \text{ mL/min/1.73 m}^2$, although it should be noted that nearly all of these used ESAs. Future studies should focus on people with $< 30 \text{ mL/min/1.73 m}^2$.

In contrast to prior research, we found few non-GFR sources of bias in HbA_{1c}. Previous studies have noted concerns about poor estimation of HbA_{1c} in patients with CKD because the production and life span of RBCs are reduced (29), while medications such as ESAs used to treat the condition can change the fraction of young red cells (30). We found no bias in HbA_{1c} by level of hemoglobin, serum iron, or transferrin saturation; one possible explanation for the differing results is that the level of CKD studied here was too mild to affect RBC turnover. These results are generalizable only to patients not treated with ESAs, for whom results may differ. Among patients with $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$, many of whom were treated with ESAs, Lo et al. (28) found significant bias in HbA_{1c} by anemia status and by ESA treatment. However, while still common

among patients treated with dialysis, ESA treatment has become less common among those not on dialysis since randomized trials have shown no cardiovascular benefit and potential harm with ESAs (31–34). Relatively few participants in our study were anemic, so further research on biomarker performance as a surrogate of glycemia is needed in this subgroup.

Unexpectedly, we did observe some evidence of bias in HbA_{1c} among participants with albuminuria. In exploratory analyses (data not shown), the association was no longer significant when additionally adjusted for eGFR. Together with the observation that participants with albuminuria tended to have lower eGFR, we speculate that the observed result may partially reflect differences in HbA_{1c} across levels of eGFR. This surprising result should be evaluated in future studies to be confirmed.

Although they have been proposed as superior alternatives to HbA_{1c} in this study, glycated albumin and fructosamine performed no better than HbA_{1c} with respect to CGM mean glucose either overall or in participants with CKD. In particular, several participant characteristics significantly biased the observed serum concentrations of glycated albumin and fructosamine compared with those predicted by mean CGM glucose. Glycated albumin and fructosamine were lower than expected for participants with albuminuria, lower serum iron concentration, lower transferrin saturation, younger age, and higher BMI. We speculate that people with these characteristics may have higher albumin turnover, allowing less time for albumin glycation. Albumin turnover is known to be increased with heavy urinary albumin loss (35). Absent albuminuria, catabolism is the most important mechanism of albumin clearance, but clinical determinants of catabolism are not well described (36). Higher BMI has been previously associated with lower distributions of glycated albumin and fructosamine concentration (37), and our data suggest this is not attributable to lower glycemic exposure.

The differences between the observed and predicted biomarkers were more variable overall for glycated albumin and fructosamine than for HbA_{1c}, and for all markers, this difference showed significantly greater variability in participants

Figure 2—Glycemic markers and mean CGM blood glucose and difference in observed and predicted biomarker and eGFR values. Predicted biomarker values were derived from the linear regression of the biomarker on mean CGM glucose.

with lower GFR than in those with normal GFR. A 2018 study by Jung et al. (38) of cross-sectional associations of these markers of glycemia across categories of eGFR in 1,665 Atherosclerosis Risk in Communities Study participants with diagnosed diabetes age >65 years found similar correlations of glycated albumin, fructosamine, and HbA_{1c} with fasting glucose in those with eGFR <60 mL/min/1.73 m², with worse correlation among those with the lowest eGFR. However, the study compared these markers with a single fasting blood glucose, not mean CGM glucose. Among a Japanese cohort of 41 patients treated with dialysis and 56 patients without kidney disease, Hayashi et al. (39) found that unlike HbA_{1c}, glycated albumin estimated mean CGM glucose correctly on

average but was more variable than HbA_{1c}. Further studies using CGM are needed to fully understand the utility of these biomarkers in the dialysis population.

Our study had several notable strengths. A state-of-the-art CGM system was able to comprehensively characterize glycemia over an average of 11 days per participant. The sample size was the largest to date for a study of glycemic biomarkers compared with CGM. We studied participants across a wide range of eGFR (6–100 mL/min/1.73 m²), including 80 participants with eGFR <60 mL/min/1.73 m² who represent 14% of all U.S. adults with diabetes (40). The biomarkers that we evaluated were all measured in a specialized NGSP diabetes testing laboratory. However, we do recognize some

limitations as well. As a cross-sectional study, biomarkers were not linked with either clinical or patient-reported outcomes. The sample size, while relatively large, did not permit definitive evaluation of biomarkers within pertinent subgroups, such as participants with eGFR <30 mL/min/1.73 m², participants with anemia, or race/ethnicity. There may be combinations of factors, such as those related to eGFR, uACR, anemia, and hypoalbuminemia, for which HbA_{1c} performs more poorly; we were unable to investigate these subgroups further because of limited sample size. Not all participants had >10 days of analyzable CGM data recommended by consensus guidelines (41).

In conclusion, our results suggest that HbA_{1c} is no more variable and less biased than glycated albumin and fructosamine in patients with type 2 diabetes and eGFR <60 mL/min/1.73 m² not treated with dialysis, supporting guideline recommendations to measure HbA_{1c} to monitor long-term trends in glycemia in this population. However, direct measurements of blood glucose, such as those obtained using CGM, are necessary to capture week-to-week and shorter-term variability, which these markers of glycemia do not reflect.

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Table 4—Covariate determinants of bias of glycemic markers

	HbA _{1c}		Glycated albumin		Fructosamine	
	% difference (95% CI)	P value	% difference (95% CI)	P value	% difference (95% CI)	P value
Age (per 10 year increment)	−1.6 (−3.4, 0.2)	0.08	5.6 (2.1, 9.3)	0.002	4.9 (2.0, 8.0)	0.0009
Male sex	−2.3 (−6.1, 1.6)	0.24	1.2 (−5.2, 8.0)	0.72	4.8 (−0.5, 10.4)	0.08
Race/ethnicity						
Black	−4.7 (−13.4, 4.8)	0.32	−3.6 (−15.6, 10.1)	0.59	−2.0 (−11.3, 8.2)	0.69
Other	−2.9 (−9.1, 3.7)	0.38	2.1 (−5.4, 10.2)	0.6	−0.5 (−8.6, 8.4)	0.91
BMI (per 5 kg/m ² increment)	−0.8 (−2.5, 1.0)	0.38	−4.0 (−6.6, −1.3)	0.004	−4.0 (−6.0, −1.9)	0.0002
eGFR (per 15 mL/min/1.73 m ² decrement)	−1.1 (−2.3, 0.2)	0.09	0.6 (−1.2, 2.5)	0.5	0.8 (−1.1, 2.7)	0.41
eGFR <30 mL/min/1.73 m ²	−4.0 (−9.3, 1.6)	0.15	−2.4 (−10.3, 6.2)	0.57	−3.6 (−10.7, 4.1)	0.35
uACR (per doubling)	−0.2 (−0.9, 0.4)	0.43	−0.7 (−1.8, 0.3)	0.18	−1.0 (−2.0, 0.0)	0.047
uACR >1,000 mg/g Cr	−5.5 (−10.6, −0.1)	0.045	−11.0 (−17.5, −3.9)	0.003	−13.2 (−19.6, −6.3)	0.0003
Hemoglobin (per g/dL increment)	1.1 (−0.3, 2.4)	0.13	−1.5 (−3.3, 0.3)	0.11	−0.3 (−2.3, 1.7)	0.75
Serum albumin (per 0.5 g/dL increment)	0.5 (−2.3, 3.2)	0.75	3.7 (−0.1, 7.7)	0.06	8.3 (5.8, 10.9)	<0.0001
Serum iron (per 25 µg/dL increment)	0.6 (−1.4, 2.7)	0.55	4.1 (0.5, 7.7)	0.03	4.8 (2.2, 7.4)	0.0003
Transferrin saturation (per 10% increment)	−0.2 (−2.1, 1.8)	0.86	3.6 (0.6, 6.7)	0.02	3.0 (0.8, 5.3)	0.007

Estimates are derived from linear regression of the log-transformed biomarker on the listed covariate, adjusted for mean CGM glucose. Difference entries are the percent difference in the alternative marker of glycemia per difference in the listed covariate when additionally adjusting for mean CGM glucose. Analyses exclude one participant with an implausible HbA_{1c}-to-mean CGM glucose relationship. Cr, creatinine.

manuscript for intellectual content. A.D. acquired data and critically reviewed the manuscript for intellectual content. D.L.T. and I.B.H. interpreted data, conceived and designed the study, and critically reviewed the manuscript for intellectual content. I.H.d.B. conceived and designed the study, analyzed and interpreted data, and wrote the manuscript. L.R.Z. and I.H.d.B. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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