

in Hb A_{1c} assays and to be useless for clinical diagnostic purposes (1–4), these 2 examples show that considering labile Hb A_{1c} values when validating Hb A_{1c} results has potential utility for monitoring conformity with preanalytical procedures and for ensuring the global quality of laboratory performance. We propose, however, that the estimated labile Hb A_{1c} value be used only as a tool in interpreting Hb A_{1c} results. We recommend that labile Hb A_{1c} values should not be used separately, because the analyzers that separate labile Hb A_{1c} from carbamylated Hb and Hb A_{1c} are not calibrated for quantifying these subcomponents. Moreover, any problem that could alter the interpretation of labile Hb A_{1c} must be taken into account. For example, extended storage of samples before analysis could cause red cells to consume glucose, which could lead to the dissociation of labile Hb A_{1c}.

We believe that each Hb chromatogram must be studied carefully. Useful information may be obtained from all Hb fractions (5). Optimal use of Hb A_{1c} values in clinical practice may be achieved only when both physicians (i.e., those ordering the test) and medical biologists (those performing the assays and interpreting the results) fully exert their respective responsibilities.

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Emmanuelle Corbé-Guillard
Stéphane Jaisson
Cécile Pileire
Philippe Gillery*

*Laboratory of Paediatric Biology
 and Research
 American Memorial Hospital
 University Hospital of Reims and
 Laboratory of Biochemistry and
 Molecular Biology
 UMR CNRS/URCA no. 6237
 Faculty of Medicine
 Reims, France*

* Address correspondence to this author at:

Laboratoire de Biologie et de
 Recherche Pédiatriques
 American Memorial Hospital
 CHU de Reims
 47 rue Cognacq-Jay
 F-51092 Reims Cedex, France
 Fax +33-3-26-78-38-82
 E-mail pgillery@chu-reims.fr

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Urinary Catalytic Iron in Patients with Type 2 Diabetes without Microalbuminuria—a Substudy of the ACCORD Trial

To the Editor:

The presence of microalbuminuria in patients with diabetes mellitus (DM)¹ is associated with a 3- to 5-fold higher risk of cardiovascular mortality than in patients with DM without microalbuminuria and a risk of progression to end-stage kidney disease that is 10-fold higher. Predicting and/or preventing the development of albuminuria have substantial potential to improve outcomes and reduce costs in patients with DM.

Catalytic iron, also known as labile iron, consists of chemical forms that can participate in redox cycling. This property makes catalytic iron potentially hazardous because it can participate in the generation of powerful oxidant species, such as hydroxyl radicals and/or reactive iron–oxygen complexes such as the ferryl or perferryl ion (1, 2). Animal models of glomerular disease exhibit increased urinary catalytic iron, and administration of an iron chelator reduces concentrations and protects against proteinuria (3). These findings suggest a pathogenetic role for catalytic iron. We postulated that the concentrations of catalytic iron may be higher in patients with DM, and if that is so, higher concentrations could precede the occurrence of proteinuria.

We conducted an ancillary study in a subgroup of 9 clinical centers (in 2 networks from the southeastern and western regions of the US) of the NIH-sponsored ACCORD (Action to Control Car-

¹ Nonstandard abbreviations: DM, diabetes mellitus; ACCORD, Action to Control Cardiovascular Risk in Diabetes.

Table 1. Clinical characteristics of patients with and without increased urinary catalytic iron.^a

	Without increased iron (n = 231)	With increased iron (n = 276)	P
Urinary catalytic iron, nmol/mg	83.3 (90.5)	623.6 (405.6)	<0.0001
Urinary isoprostane, pmol/mmol	111.1 (156.4)	101.4 (235.0)	0.76
Age, years	64.5 (5.6)	64.5 (5.8)	0.94
Males/female sex, n	123/108	140/136	0.57
Approximate DM duration, years	13.1 (7.5)	12.8 (7.4)	0.63
UALB/Ucreat, ^b μg/mg	26.2 (128.3)	18.3 (32.9)	0.37
Plasma creatinine, mg/dL	0.8 (0.2)	0.8 (0.2)	0.46
Hemoglobin A _{1c} , %	8.2 (1.1)	8.2 (1.1)	0.47
Systolic BP, mmHg	136.6 (14.8)	135.6 (16.7)	0.5
Diastolic BP, mmHg	76.9 (9.5)	76.3 (10.1)	0.49
Total cholesterol, mg/dL	191.8 (44.4)	197.1 (42.8)	0.18
LDL cholesterol, mg/dL	110.7 (35.0)	114.7 (37.2)	0.22
HDL cholesterol, mg/dL	42.8 (11.1)	43.5 (12.2)	0.49
BDI/Ucreat, nmol/mg	95.6 (72.1)	846.3 (593.4)	<0.0001

^a Data are presented as the mean (SD) except for the sex data.
^b UALB/Ucreat, urine microalbumin–urine creatinine ratio; BP, blood pressure; BDI/Ucreat, urine bleomycin-detectable iron–urine creatinine ratio.

diovascular Risk in Diabetes) Trial (4) to determine the prevalence of increased catalytic iron in participants with nonpathologic renal function and the absence of microalbuminuria (<30 μg/mg creatinine) at baseline. We had 507 urine samples available and suitable for the analysis of catalytic iron, as well as complete clinical data. Of these samples, we randomly selected 167 samples for additional measurement of urinary isoprostane, a marker of oxidative stress.

We used the bleomycin-detectable iron assay to measure catalytic iron in urine (5). F_{2t}-isoprostane–isoprostane concentrations were measured at the University of Colorado with the liquid chromatography–tandem mass spectrometry assay (6). We ln-transformed data for the following outcome variables: the concentration ratios of urinary bleomycin-detectable iron to creatinine and of urinary microalbumin to creatinine. Logistic regression models

fitted to determine predictors of abnormal urinary iron and isoprostane concentrations included age, sex, race, weight, body mass index, LDL, and HDL as covariates. SAS software (version 9.1; SAS Institute) was used to analyze the data.

We used the NCCLS (now CLSI) guidelines (7) to establish a reference interval for the ratio of urinary bleomycin-detectable iron to creatinine [95% CI, 140.9–253.2 nmol/mg (15.9–28.6 nmol/μmol); mean, 197.0 nmol/mg (22.3 nmol/μmol)] with a population of 100 healthy people from New Orleans (50 men and 50 women; mean age, 37.4 years; mean body mass index, 22.9 kg/m²) (5). None of the patients had DM, hepatitis C, or hemochromatosis. This group of healthy individuals served as an external reference population (control group) for the ACCORD study participants.

Participants from the ACCORD population with a urinary catalytic iron value >253.2 nmol/mg

(>28.6 nmol/μmol) were classified as having increased iron, in accordance with the data from the external referent population. Use of this threshold indicated that 276 (54.4%) of the ACCORD participants had increased values for urinary catalytic iron. Table 1 summarizes the characteristics of the participants with and without increased catalytic iron so defined. None of the baseline characteristics examined were associated with increased urinary catalytic iron.

In our study, 21% (35 of 167) of the participants had increased urinary isoprostane (>120 pmol/mmol creatinine). There was no relationship between the ratio of urinary bleomycin-detectable iron to creatinine and the urinary isoprostane concentration, either in the entire cohort (*r* = −0.12; *P* = 0.12) or in the patients with higher urinary isoprostane concentrations.

In this cross-sectional study, we observed increased urinary catalytic iron concentrations in 54% of the participants with DM without mi-

croalbuminuria and with a non-pathologic renal function. Our study has limitations. The ACCORD trial enrolled participants with DM and an additional cardiovascular risk factor. Participants were in different stages of DM. None of the traditional risk factors (highly prevalent in this population) were associated with increased urinary catalytic iron in participants without microalbuminuria. Thus, if urinary catalytic iron is associated with future cardiovascular or renal events, that association is likely to be independent of the covariates we examined. Urinary catalytic iron has potential to be useful as an independent predictor of risk of nephropathy and cardiovascular events.

This cross-sectional study represents an initial step in the development of a biomarker of risk prediction—the demonstration that urinary catalytic iron is abnormal in a population with DM and cardiovascular risk factors but without increased urinary albumin. Because catalytic iron potentially provides a direct target for a therapeutic intervention, we recommend that longitudinal studies be conducted to examine the potential role of urinary catalytic iron in identifying patients at risk for incident renal and cardiovascular events.

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Tina Thethi²
Mohan Rajapurkar³
Patrick Walker⁴
Roberta McDuffie²
David C. Goff, Jr.⁵
Jeffrey Probstfield⁶
C. Lillian Yau⁷
Sudhir Shah⁸
Uwe Christians⁹
John Buse¹⁰
Vivian Fonseca^{2*}

² Tulane University Health Sciences Center
New Orleans, LA

³ Muljibhai Patel Society for
Research in Nephro-Urology
Nadiad, India

⁴ Nephropathology Associates
Little Rock, AR

⁵ Wake Forest University School of Medicine
Winston-Salem, NC

⁶ University of Washington School of
Public Health
Seattle, WA

⁷ School of Public Health and
Tropical Medicine
Tulane University
New Orleans, LA

⁸ University of Arkansas for
Medical Sciences College of Medicine
Little Rock, AR

⁹ Department of Anesthesiology
University of Colorado at Denver and
Health Sciences Center
Denver, CO

¹⁰ University of North Carolina
Health Sciences
Chapel Hill, NC

*Address correspondence to this author at:
1430 Tulane Ave., SL-53
New Orleans, LA 70112
Fax 504-988-6271
E-mail vfonseca@tulane.edu

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Red Cell Transfusion Decreases Hemoglobin A_{1c} in Patients with Diabetes

To the Editor:

Hemoglobin A_{1c} (Hb A_{1c})¹ is a mainstay of diabetes diagnosis and management that allows clinicians to estimate the recent mean blood glucose concentration of a patient. Glycation of hemoglobin is an irreversible, nonenzymatic process that depends on the glucose concentration in red blood cells (RBCs), and Hb A_{1c} represents the integrated glucose concentration in RBCs over their life span.

RBC transfusion can complicate the interpretation of Hb A_{1c} values in diabetic patients because it introduces hemoglobin molecules exposed to glucose concentrations that may have been different from the glucose concentrations in the diabetic transfusion recipient. The potential effect of transfusion on Hb A_{1c} has been recognized for some time, but opinions on the direction of the effect are contradictory. Data from the older literature (1–3) suggest that the high concentration of glucose in RBC storage medium promotes glycation and causes Hb A_{1c} values to increase over time, which would predict that Hb A_{1c} might increase in transfused patients. This

concept has been stated in a recent review article (4) and on consumer Web sites, such as Lab Tests Online (<http://labtestsonline.org/understanding/analytes/a1c/test.html>); however, a recent case in which a pathology resident was contacted to explain a patient's Hb A_{1c} value decreasing from 7.4% to 5.4% in 3 days after the patient received 3 units of RBCs suggested that transfusion may lower Hb A_{1c} values in diabetic patients. Indeed, the majority of blood donors are not diabetic, and donor RBCs would dilute the increased Hb A_{1c} value (>6.5%) in a diabetic patient. To our knowledge, no study has used contemporary Hb A_{1c} methods to examine the effect of RBC storage conditions on Hb A_{1c} or the overall effect of RBC transfusion on Hb A_{1c} in patients.

To investigate these questions, we used an immunoassay (Siemens Dimension RxL) to measure Hb A_{1c} in 2 unused RBC units stored in additive storage medium (AS-1) under standard blood bank conditions. These 2 units were unacceptable for transfusion because they had been out of the control of the blood bank for >30 min. The Hb A_{1c} values for samples obtained from the units on their expiration date (42 days after collection) were 5.4% (B Rh-positive) and 5.7% (O Rh-positive), which are within our reference interval (<6.0%). The glucose concentrations in the blood units at this time were 36.9 mmol/L (665 mg/dL) and 32.5 mmol/L (586 mg/dL), confirming the supraphysiological glucose content of the RBC storage medium. We observed minimal increases in Hb A_{1c} values in the units after additional incubation at room temperature for 9 days (5.7% and 5.9%) and at 37 °C for another 14 days (6.0% and 6.0%). This finding strongly suggests that glycation of hemoglobin in stored RBC units is negligible despite the

high glucose concentrations in stored RBC units.

To determine if RBC transfusion has a measurable effect on Hb A_{1c} in patients, we retrospectively queried an institutional review board–approved database of deidentified patient data from our hospital for patients who received RBC transfusions and had Hb A_{1c} measurements performed within 28 days before and 14 days after they received at least 1 RBC transfusion. We allowed a maximum of 7 days between the first and last transfusions for patients who received multiple transfusions; therefore, the maximum time between the pre- and posttransfusion Hb A_{1c} measurements was 49 days. Fig. 1 shows the change in Hb A_{1c} after transfusion for 45 patients who met the inclusion criteria. The Hb A_{1c} decreased in 31 (69%) of the patients overall and in all 21 patients whose pretransfusion Hb A_{1c} measurement was ≥7%. Of the 14 patients whose Hb A_{1c} value increased or remained unchanged, 12 had a pretransfusion Hb A_{1c} value of <6.5%; thus, one would not expect the transfusion of RBCs with a typical Hb A_{1c} value to have a large effect. The mean decrease in Hb A_{1c} across all patients was 0.829%, which is statistically significant ($P = 0.00124$, 2-sided paired t -test). The mean decrease for the 21 patients with pretransfusion values ≥7% was 1.97% Hb A_{1c}.

These results suggest that RBC transfusion will reduce the Hb A_{1c} concentration in diabetic patients. We did not design this study to fully characterize this phenomenon, but it does show that appreciable glycation does not occur during typical blood bank storage. Thus, we would expect decreased Hb A_{1c} values after transfusion to be most pronounced in patients who receive large transfusion volumes and/or who have a high pretransfusion Hb A_{1c}, because of dilution with RBCs containing typical amounts of Hb A_{1c}. Indeed, pa-

¹ Nonstandard abbreviations: Hb A_{1c}, hemoglobin A_{1c}; RBC, red blood cell.