# Glycated hemoglobin concentrations of red blood cells minimally increase during storage under standard blood banking conditions

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**BACKGROUND:** Few and inconsistent data exist describing the effect of storage duration on glycated hemoglobin (HbA1c) concentrations of red blood cells (RBCs), impeding interpretation of HbA1c values in transfused diabetic patients. Hence the aim of this study was to evaluate to what extent HbA1c concentrations of RBCs change during the maximum allowed storage period of 42 days.

**STUDY DESIGN AND METHODS:** Blood was drawn from 16 volunteers, leukofiltered, and stored under standard blood banking conditions. HbA1c concentrations of RBCs were measured on Days 1 and 42 of storage using three different validated devices (ion-exchange high-performance liquid chromatography Method A1 and A2, turbidimetric immunoassay Method B).

**RESULTS:** Mean HbA1c concentrations of RBCs on Day 1 were 5.3 ± 0.3% (Method A1), 5.4 ± 0.4% (Method A2), and 5.1 ± 0.4% (Method B). HbA1c concentrations increased to 5.6 ± 0.3% (A1, $p < 0.0001$), 5.7 ± 0.3% (A2, $p = 0.004$), and 5.5 ± 0.4% (B, $p < 0.0001$) on Day 42, respectively, corresponding to a 1.06-fold increase across all methods. Glucose concentrations in the storage solution of RBCs decreased from 495 ± 27 to 225 ± 55 mg/dL ($p < 0.0001$), confirming that stored RBCs were metabolically active.

**CONCLUSION:** These results suggest a significant, albeit minor, and most likely clinically insignificant increase in HbA1c concentrations during storage of RBCs for 42 days.

Determination of glycated hemoglobin (HbA1c) concentrations is recommended to diagnose and monitor the treatment of diabetes mellitus.[1,2] HbA1c is continuously generated over the life span of a red blood cell (RBC) via nonenzymatic glycation. Via this posttranslational process, (D-)glucose is covalently bound to a protein, in this case a minor hemoglobin. The rate of nonenzymatic glycation mainly depends on glucose concentration and RBC turnover.[3,4] Consequently, several endogenous and exogenous factors can

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alter HbA1c test results and thereby mask the true glycemic state of a patient. The most relevant factors for reduced or elevated HbA1c concentrations are anemia, due to its effects on RBC life span, and transfusion of RBCs, respectively.[2,5]

High glucose concentrations are required in storage solutions of RBCs to provide energy for the metabolically active RBCs. To our knowledge, only one retrospective study has evaluated the effects of RBC transfusions on HbA1c concentrations in patients,[6] and no consensus has been reached about whether RBC transfusions elevate or reduce HbA1c concentrations. Moreover, reports on temporal evolution of HbA1c concentrations during storage of RBCs provide contradictory data.[6–10] This lack of data and evidence has prompted some authors to conclude that HbA1c values of patients having received transfusions must be considered uninterpretable.[5]

The aim of this study was to assess HbA1c concentrations of RBCs during the maximum allowed storage period of 42 days. In this report, we present the results of a single-arm study comparing HbA1c concentrations between Day 1 and Day 42 of storage measured by using three different standardized validated devices.

## MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Medical University of Vienna (1043/2015) and registered at http://clinicaltrials.gov (NCT02639780). Sixteen healthy volunteers donated 1 RBC unit after providing written informed consent. Blood was leukofiltered (log 4 leukocyte reduction), processed according to standard protocols of transfusion services of the Austrian Red Cross (Vienna, Austria), and stored in a saline-adenine-glucose-mannitol solution under standard blood banking conditions at 4°C for 42 days. Samples were drawn aseptically from storage bags on Day 1 and Day 42, and HbA1c as well as glucose concentrations were measured.

HbA1c was measured independently by three different standardized validated devices based on two biochemical methods. First (referred to as Method A1), HbA1c was assessed using a Bio-Rad D-100 (Bio-Rad Laboratories). This method is based on ion-exchange high-performance liquid chromatography (HPLC) and represents the standard method to determine HbA1c at our institution. Second (referred to as Method A2), HbA1c was measured using a Bio-Rad D-10 (Bio-Rad Laboratories), an alternative HPLC-based platform. Third (referred to as Method B), HbA1c was measured using a turbidimetric immunoassay on an Advia 1800 (Siemens Healthcare Diagnostics). All three methods were applied according to manufacturers' instructions.

Glucose concentrations in the storage solution of RBCs were measured with a Glucose HK Gen.3 kit on a Roche/Hitachi cobas c System (Roche Diagnostics).

Statistical analysis was performed using GraphPad Prism v.7.02 (GraphPad Software, Inc.). Data distribution was analyzed visually (histogram) and by using the D'Agostino-Pearson omnibus normality test. Changes of HbA1c and glucose values between Day 1 and Day 42 were analyzed using a paired t test. Differences of HbA1c concentrations at baseline between the different methods were assessed using a one-way analysis of variance and the Tukey multiple comparison test. Correlations of HbA1c concentrations between methods were calculated according to Pearson. Normally distributed data are reported as means ± standard deviation, and all other data as median and range. Differences are reported as mean differences and 95% confidence intervals (CIs). All HbA1c concentration changes are reported as absolute percentage points unless otherwise indicated.

## RESULTS

In total, blood was drawn from 16 donors, eight men and eight women, with an average age of 47 years (Table 1 provides more demographic information). All donors fulfilled the blood donor criteria of the Austrian Red Cross.

Mean HbA1c concentrations on Day 1 were 5.3 ± 0.3% (Method A1, Fig. 1A), 5.4 ± 0.4% (Method A2, Fig. 1B), and 5.1 ± 0.4% (Method B, Fig. 1C). All HbA1c values were below the generally accepted limits for nondiabetic patients (<6.5%).[11] Only the results between Method A2 and B were statistically different; however, they were of borderline or no clinical significance (mean difference 0.4%, 95% CI, 0.03-0.7, p = 0.028). Correlations of HbA1c concentrations between methods are depicted in Fig. S1 (available as supporting

**TABLE 1. Demographic data and blood cell count of volunteers***

| Characteristic | Value |
|---|---|
| Age, y, mean (IQR) | 47 (29-60) |
| Gender | |
| Female, n (%) | 8 (50) |
| Male, n (%) | 8 (50) |
| ABO blood group | |
| A, n (%) | 10 (63) |
| B, n (%) | 0 (0) |
| O, n (%) | 6 (37) |
| Rhesus blood group | |
| Positive, n (%) | 11 (69) |
| Negative, n (%) | 5 (31) |
| Blood cell count | |
| RBC ($\times 10^{-6}$ μL) | 5.0 ± 0.5 |
| Hemoglobin (g/dL) | 15.4 ± 1.3 |
| WBC ($\times 10^{-3}$ μL) | 7.1 ± 2.4 |
| Neutrophils ($\times 10^{-3}$ μL) | 4.2 ± 1.7 |
| Lymphocytes ($\times 10^{-3}$ μL) | 2.1 ± 0.6 |
| Monocytes ($\times 10^{-3}$ μL) | 0.5 ± 0.2 |
| Eosinophils ($\times 10^{-3}$ μL) | 0.2 ± 0.1 |
| Basophils ($\times 10^{-3}$ μL) | 0.1 ± 0.1 |

*All data are presented as mean ± SD unless otherwise indicated.
IQR = interquartile range.

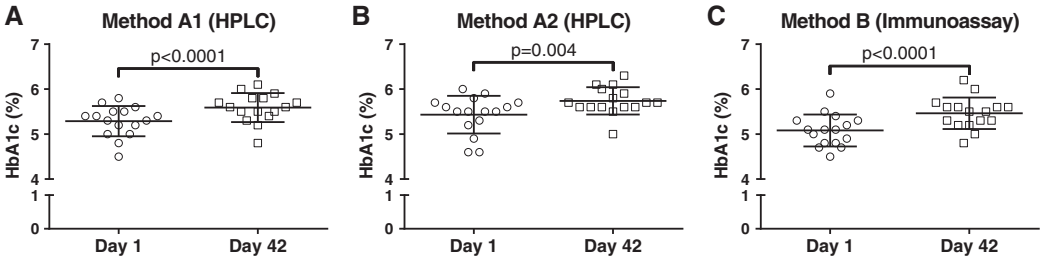


**Fig. 1. HbA1c concentrations at Day 1 and Day 42. Method A1 (Panel A) = ion-exchange HPLC on a Bio-Rad D-100; Method A2 (Panel B) = HPLC on a Bio-Rad D-10; Method B (Panel C) = turbidimetric immunoassay on an Advia 1800. Data are depicted as mean ± SD.**

information in the online version of this paper). Mean HbA1c concentrations increased to 5.6 ± 0.3% (Method A1, $p < 0.0001$, Fig. 1A), 5.7 ± 0.3% (Method A2, $p = 0.004$, Fig. 1B), and 5.5 ± 0.4% (Method B, $p < 0.0001$, Fig. 1C) by Day 42. Thus mean absolute changes in HbA1c concentrations from Day 1 until Day 42 were 0.3 ± 0.1% (95% CI, 0.27-0.33, $p < 0.0001$), 0.3 ± 0.4% (95% CI, 0.1-0.5, $p = 0.004$), and 0.4 ± 0.1% (95% CI, 0.3-0.5, $p < 0.0001$) for Methods A1, A2, and B, respectively. The mean change in HbA1c concentrations across all three methods was 0.3 ± 0.1% (95% CI, 0.3-0.4, $p < 0.0001$), corresponding to a 1.06-fold increase over a period of 41 days. Mean changes in HbA1c concentrations did not differ between male and female donors (data not shown).

Glucose concentrations in the storage solution of RBCs were supraphysiologic on Day 1 (495 ± 27 mg/dL). To confirm that the stored RBCs were metabolically active, glucose concentrations were measured again on Day 42. Glucose concentrations decreased by more than 50% to 225 ± 55 mg/dL ($p < 0.0001$).

## DISCUSSION

In this study, we demonstrated a significant but minor increase of HbA1c concentrations from Day 1 through Day 42 in RBCs stored under standard blood banking conditions.

Our data are in agreement with an early study by Zeller and colleagues, in which blood was drawn from 10 fasted volunteers and subsequently stored for 35 days.[10] The authors reported HbA1c concentrations, measured by HPLC, of 6.0% on Day 1 and 6.6% on Day 35, although the difference was not significant. Apart from the fact that baseline HbA1c concentrations suggest impaired glucose metabolism in at least some subjects of the study population, the reported increase of HbA1c is comparable with our observed absolute increase of 0.3%. In addition, Spencer and colleagues reported HbA1c concentrations of 5.4% and 5.7% in two unused RBC units on Day 42 of storage.[6] These results parallel our data, where the mean HbA1c across all methods was 5.6 ± 0.3% on Day 42 of storage.

In contrast, Szelenyi and colleagues, who drew blood from five healthy donors and subsequently stored the whole blood for 3 weeks in acid citrate-phosphate preservatives at 4°C, reported an increase of the HbA1c of 2.0 ± 0.5%, based on a colorimetric method.[8] Although the mean baseline HbA1c (5.7%) is similar to our results, the authors reported a significantly larger increase during storage. These diverging results might be influenced by the method used to determine HbA1c concentrations, storage conditions, and because whole blood is an entirely different product compared with the leukofiltered RBCs used in our study. Interestingly, D'Alessandro and colleagues, who stored leukodepleted RBC units collected from 10 healthy volunteers for 42 days, reported a statistically and potentially clinically significant change of the HbA1c from 4.1 ± 0.3% to 10.9 ± 0.3%.[7] This drastic increase might be explained by the method used to determine HbA1c concentrations because quantification of HbA1c by means of MALDI-TOF mass spectrometry–based analysis is not a standard method of measuring HbA1c. Furthermore, differences in leukoreduction, manufacturing, and storage conditions, as well as varying anticoagulants or additive solutions, might contribute to these differences.

Clinically, the question of whether HbA1c concentrations are altered in (diabetic) patients receiving RBC transfusions remains unclear because no prospective studies have investigated this issue. The only study to date by Spencer and colleagues is a retrospective analysis of patients transfused with RBCs.[6] Among the 45 patients analyzed, 31 (69%) had a decrease in HbA1c concentrations after transfusion, 21 of whom had a pretransfusion HbA1c 7% or higher. Among the other 14 patients in whom HbA1c increased or remained unchanged, 12 had a pretransfusion HbA1c of 6.5% or lower. This suggests that transfusion of RBCs lowers HbA1c concentrations in diabetic patients, especially those with poor glycemic control. Our findings add to this field of research by demonstrating that storage duration of RBCs only has marginal effects on HbA1c concentrations in RBCs. Extrapolating from our results, a diabetic patient with a pretransfusion HbA1c of 8.0% receiving 1 RBC unit with an HbA1c of 5.6% would show a posttransfusion HbA1c of 7.8%. This calculation assumes a dilutional

ratio of the transfused blood to the patient's blood of 1:10 in an adult man. The reduction of HbA1c would be even greater with increasing severity of the initial anemia and number of RBC units transfused.

In contrast, transfusion of RBCs from donors with markedly elevated HbA1c concentrations[12] could also increase HbA1c concentrations in RBC recipients. Moreover, RBCs obtained from donors with impaired glucose metabolism could result in augmented formation of advanced glycation end products.[13] Because these proteins have been associated with an increased generation of reactive oxygen species,[14] elevated concentrations might induce adverse effects in susceptible recipients.

Taken together, our data suggest that HbA1c concentrations are significantly higher in RBC units after 42 days of storage, although this increase might not be clinically relevant. Carefully extrapolating these data infers that HbA1c concentrations most likely decrease when diabetic patients with elevated HbA1c values receive RBC transfusions. The data generated in this study might facilitate the interpretation of HbA1c concentrations in transfused diabetic patients.

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#### CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** Glycated hemoglobin (HbA1c) correlation between methods A1 vs. A2 (Panel A), A1 vs. B (Panel B), and A2 vs. B (Panel C). Method A1 = ion-exchange high-performance liquid chromatography (HPLC) on a Bio-Rad D-100. Method A2 = HPLC on a Bio-Rad D-10. Method B = turbidimetric immunoassay on an Advia 1800. Green dots represent HbA1c values measured on Day 1; red dots represent HbA1c values measured on Day 42. Correlations were calculated using the Pearson coefficient.