# A1C Underestimates Glycemia in HIV Infection

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**OBJECTIVE** — The objective of this study was to determine the relationship between A1C and glycemia in HIV infection.

**RESEARCH DESIGN AND METHODS** — We completed a prospective cross-sectional study of 100 HIV-infected adults with type 2 diabetes (77%) or fasting hyperglycemia (23%) with measured glucose, A1C, mean corpuscular volume (MCV), and fructosamine. A total of 200 HIV-uninfected type 2 diabetic subjects matched for key demographic characteristics served as control subjects.

**RESULTS** — Relative to the control subjects, A1C underestimated glucose by 29 ± 4 mg/dl in the HIV-infected subjects. Current nucleoside reverse transcriptase inhibitors (NRTIs), higher MCV and hemoglobin, and lower HIV RNA and haptoglobin were associated with greater A1C-glucose discordance. However, only MCV and current NTRI use, in particular abacavir, remained significant predictors in multivariate analyses. Fructosamine more closely reflected glycemia in the HIV-infected subjects.

**CONCLUSIONS** — A1C underestimates glycemia in HIV-infected patients and is related to NRTI use. Use of abacavir and increased MCV were key correlates in multivariate analyses. Fructosamine may be more appropriate in this setting.

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Diabetes is increasingly recognized in HIV. Current guidelines for the management of diabetes in HIV are similar to diabetes guidelines for the general population (1). However, a previously published case series and a retrospective study noted elevated glucose values relative to A1C levels in HIV-infected subjects (2,3). The authors of both the case series and the retrospective study speculated that hemolysis may have contributed to the inaccuracy of A1C in this setting. Therefore, we sought to prospectively characterize the relationship between A1C and glucose levels in this population in relation to hematologic and metabolic indicators.

## RESEARCH DESIGN AND METHODS

— A total of 100 HIV-infected adults with type 2 diabetes or hyperglycemia (fasting glucose ≥100 and <126 mg/dl) participated between March 2007 and March 2008. Patients who were pregnant or had hemoglobinopathy, recent changes in antiretroviral (ARV) or diabetes therapy, anemia, opportunistic infection, renal disease, creatinine >1.8 mg/dl, a recent blood transfusion, or corticosteroid use were excluded. All participants gave written informed consent.

Sex-, race-, and age-matched HIV-uninfected type 2 diabetic control subjects ($n$ = 200) were obtained from MedStar Health's electronic medical record system. Except for HIV factors, exclusion criteria were the same. Random serum glucose and A1C measurements were obtained. This study was approved by the National Institute of Allergy and Infectious Diseases and MedStar institutional review boards.

Measurements included fasting and nonfasting plasma and serum glucose, fructosamine and A1C, and haptoglobin, albumin, HIV viral load, and CD4 counts. Glucose was determined on a Synchron LX20 (Beckman Coulter, Fullerton, CA), A1C was determined by ion-exchange high-performance liquid chromatography (HPLC) on a Variant Hemoglobin Testing System (Bio-Rad, Hercules, CA), and fructosamine was determined by Esoterix laboratory services (Calabasa Hills, CA). Analyses for control subjects were completed at MedStar laboratories, and A1C was determined by HPLC on a Tosoh HLC-723 GHbG7 (Tosoh Bioscience, Rivoli, Italy) at Washington Hospital Center. There is documented good agreement between Tosoh HLC-723 and Bio-Rad Variant systems (4–6).

### Statistical analysis

Group comparisons were made using Student's $t$ tests and $\chi^2$ analyses. Random serum glucose and A1C values in control subjects were used to generate a reference regression equation. The following equation was applied to the measured A1C in the HIV sample in order to calculate the predicted glucose.

A1C-glucose discordance =
mean of one fasting and one random glucose
– predicted glucose for the measured A1C

Regression analyses were performed to identify variables associated with A1C-glucose discordance. Significant variables on univariate analyses were entered into forward stepwise multivariate regressions. Current ARV use was coded by drug class; separate analyses were performed with individual nucleoside reverse transcriptase inhibitors (NRTIs). All analyses were repeated utilizing the reference equation of A1C and glucose for the external validation (7).

Fructosamine-glucose discordance (measured-predicted glucose) was calculated using a reference equation (8). Two-tailed $\alpha < 0.05$ was used for statistical

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**Table 1—*Demographic and clinical characteristics of study participants***

| | HIV-infected | Control | *P* |
|---|---|---|---|
| *n* | 100 | 200 | |
| Age (years) | 52 ± 1 | 52 ± 1 | 0.6 |
| Sex (% male/female) | 70/30 | 70/30 | 0.9 |
| Race/ethnicity (%) | | | 0.1 |
| African American | 65 | 65.5 | |
| Caucasian | 25 | 27.5 | |
| Hispanic | 7 | 7 | |
| Asian/other | 3 | 0 | |
| Duration of HIV (years) | 13.5 ± 0.7 | — | |
| CD4 (cells/ml) | 561 ± 25 | — | |
| HIV viral load <50 copies/ml (%) | 61 | — | |
| Current ARV therapy (%) | 78 | — | |
| NRTI | 78 | — | |
| Protease inhibitor | 42 | — | |
| Non-NRTI | 38 | — | |
| Type 2 diabetes (%) | 77 | 100 | |
| Diabetes duration (years) | 6.8 ± 0.6 | — | |
| Current insulin use (%) | 30 | 29 | 0.9 |
| BMI (kg/m$^2$) | 30.6 ± 0.8 | 35.2 ± 0.6 | <0.0001 |
| A1C (%)* | 6.7 ± 0.2 | 8.1 ± 0.1 | <0.0001 |
| Mean plasma glucose (mg/dl) | 156 ± 5 | — | |
| Serum glucose (mg/dl)* | 176 ± 7 | 161 ± 6 | 0.09 |
| Mean fructosamine (μmol/l) | 275 ± 6 | — | |
| Hemoglobin (g/dl) | 13.9 ± 0.1 | — | |
| MCV (fL) | 96 ± 1 | — | |
| Haptoglobin (mg/dl) | 111 ± 8 | — | |
| Albumin (g/dl) | 3.8 ± 0.05 | — | |
| A1C-glucose discordance (mg/dl) | 29 ± 4 | — | |
| Current NRTI | | | |
| Yes ($n$ = 78) | 35 ± 5† | — | |
| No ($n$ = 22) | 6 ± 5† | — | |
| Current abacavir | | | |
| Yes ($n$ = 24) | 56 ± 11† | — | |
| No ($n$ = 76) | 20 ± 3† | — | |

Data are means ± SEM. *Nonfasting values only. †Difference between current use (yes vs. no) significantly different at $P < 0.0001$ for NRTI and $P = 0.003$ for abacavir.

significance. All values are presented as means ± SEM unless indicated. Data were analyzed using SAS JMP Version 7.0 (SAS, Cary, NC).

**RESULTS** — Clinical characteristics of the HIV-infected and control subjects are presented in Table 1. For the HIV-uninfected control subjects with diabetes, the following regression equation was generated:

$$\text{Glucose (mg/dl)} = 25.32(\text{A1C}) - 43.57 \ (r^2 = 0.39, P < 0.0001)$$

For HIV-infected subjects, the following equation was generated:

$$\text{Glucose (mg/dl)} = 22.56(\text{A1C}) + 3.63 \ (r^2 = 0.46, P < 0.0001)$$

Relative to control subjects, A1C underestimated glucose by 29 ± 4 mg/dl. Current NRTI use ($P < 0.001$), mean corpuscular volume (MCV) ($P = 0.0001$), hemoglobin ($P = 0.04$), albumin ($P = 0.03$), low HIV RNA ($P = 0.02$), and low haptoglobin ($P = 0.01$) were associated with A1C-glucose discordance. In multivariate analysis, only MCV remained significant ($P = 0.002$). When individual NRTIs were assessed, current abacavir ($P = 0.001$), lamivudine ($P = 0.02$), and zidovudine ($P = 0.03$) use were associated with A1C-glucose discordance. In multivariate analysis, abacavir use ($P = 0.0004$) and MCV ($P = 0.001$) were independent predictors of A1C-glucose discordance. When A1C-glucose discordance was calculated using a published reference equation (7), the major findings in this study were unchanged.

For fructosamine, the mean measured-predicted glucose was −2.8 ± 5.0 mg/dl. There were no significant associations between fructosamine-glucose discordance and ARV use, CD4 count, viral load, or hematologic indexes.

There were inverse correlations between A1C and MCV ($r = -0.37$, $P = 0.0002$) and haptoglobin and MCV ($r = -0.39$, $P < 0.0001$). There was a significant correlation between haptoglobin and HIV viral load ($r = 0.29$, $P = 0.004$), whereas lower haptoglobin was associated with NRTI use ($P = 0.005$). Abacavir use was not associated with MCV or haptoglobin levels.

**CONCLUSIONS** — In this first prospective study evaluating the accuracy of A1C in HIV-infected patients with diabetes, we found that glucose levels were significantly higher than anticipated by A1C levels when compared with HIV-uninfected control subjects and established reference values (7). Use of NRTIs was strongly associated with this discordance. Fructosamine more accurately predicted glucose levels and was not affected by use of ARVs.

Though earlier reports suggested that hemolysis played a role in the described discordance (2,3), neither study directly evaluated haptoglobin relative to A1C-glucose discordance. In this study, haptoglobin was not an independent predictor of A1C-glucose discordance. Thus, hemolysis seems an unlikely cause.

Macrocytosis is a well-documented effect of the thymidine analogs (9,10). Increased MCV, driven largely by use of NRTIs, is an important factor in the relationship between A1C and glycemia. Prior investigation shows that MCV decreases in parallel to increases in A1C and correlates with cell age (11,12). Similarly, we identified a strong relationship between A1C and MCV.

Additionally, we found that abacavir was associated with A1C-glucose discordance. Abacavir is not regarded as hemotoxic (13), and there was no relationship between abacavir and macrocytosis. In multivariate analyses, abacavir use remained a predictor of A1C-glucose discordance independent of MCV and cumulative exposure to other NRTIs. Further investigation is needed to evaluate potential mechanisms underlying these observations.

Fructosamine reliably predicted glucose in our cohort and was not influenced by MCV or current abacavir or NRTI use.

Fructosamine may be an appropriate substitute for A1C in the setting of HIV and macrocytosis.

One limitation is that we did not obtain multiple glucose determinations but rather one fasting and one random determination. We believe this is mitigated by the large number of patients in our cohort. We used contemporary HIV-uninfected control subjects matched on key demographic characteristics. We were also able to replicate all of our key findings using an established reference equation (7).

Our study suggests that A1C may not be accurate for assessing glycemia among HIV-infected patients on NRTI-based therapy, especially those with macrocytosis or those on abacavir. Fructosamine may be an appropriate alternative for assessment of glycemia in this context.

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