Published in final edited form as:
J Diabetes. 2010 June ; 2(2): 118–124. doi:10.1111/j.1753-0407.2010.00070.x.

Measurement of HbA1c from stored whole blood samples in the Atherosclerosis Risk in Communities study

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Abstract

Background—The aims of the present study were to demonstrate the reliability of HbA1c measurements across two time periods and to compare these measurements with HbA1c distribution in the general US population.

Methods—HbA1c was measured in 14 069 whole blood samples in the Atherosclerosis Risk in Communities (ARIC) study using different HPLC instruments during two time periods, namely 2003–2004 and 2007–2008. At the time of measurement, samples had been in storage at –70°C for 14–18 years. To assess differences in values, HbA1c measurements were repeated in 383 samples at both periods. Indirect comparisons were made by comparing our measurements against those from a nationally representative study.

Results—The coefficients of variation for quality control samples were 1.8% (n = 89) in 2003–2004 and 1.4% (n = 259) in 2007–2008. The correlation between measurements at the two time points was high (r = 0.99), but with a slight bias: 0.29% points higher in 2007–2008 versus 2003–2004 (n = 383; $P < 0.0001$). The comparison yielded the following Deming regression equation: $y_{(2007–2008)} = 0.073+1.034x_{(2003–2004)}$. After alignment using this equation, the distribution of HbA1c in the ARIC study was similar to that in the national study using fresh samples.

Conclusions—Measurements of HbA1c from samples stored for 14–18 years are highly reliable when using state-of-the-art HPLC instruments, but with some bias introduced over time. The HbA1c data now available in the ARIC study should be invaluable for investigations into the clinical utility of HbA1c as a diagnostic test for diabetes.

Keywords

diabetes; epidemiology; glycated hemoglobin; HbA1c; stored samples; the Atherosclerosis Risk in Communities (ARIC) study; the National Health and Nutrition Examination Survey (NHANES)

Introduction

Levels of HbA1c are central to the clinical management of diabetes and are an integrated measure of endogenous glucose over the previous 2–3 months. The American Diabetes Association has recently revised clinical practice guidelines to recommend the use of HbA1c as a diagnostic test for Type 2 diabetes [1, 2]. In the present study, we determined the HbA1c

values from over 14 000 stored whole blood samples from the Atherosclerosis Risk in Communities (ARIC) study during two separate time periods. At the time of measurement, these samples had been in storage at –70°C for 14–18 years.

The aims of the present study were to: (i) demonstrate the reliability of HbA1c measurements across the two time periods from whole blood samples stored for almost 20 years; (ii) compare the distribution of our measurements in the ARIC study to HbA1c measurements in nationally representative samples of the US population; and (iii) document the methods used in the ARIC HbA1c Ancillary Study.

Methods

Study populations

The ARIC study—The ARIC study is a large, community-based study of over 15 000 people who attended four clinical examinations three years apart: Visit 1, 1987–1989; Visit 2, 1990–1992; Visit 3, 1993–1995; and Visit 4, 1996–1998. Rigorous assessment of the health of each participant, including a comprehensive physical examination, medical history, interview, and measurement of traditional and novel risk factors for cardiovascular disease, diabetes, kidney disease, and other important health outcomes, was conducted using standardized protocols at each visit. HbA1c was not measured in all participants as part of the original protocol.

However, whole blood samples were obtained from all participants at the second examination (1990–1992) and stored at –70°C. We undertook an ancillary study in 2003–2004 to measure HbA1c on a subsample (approximately 5000) of the stored whole blood samples from Visit 2. Several years later, in 2007–2008, we obtained additional funding to measure HbA1c on all remaining specimens. As a result of this work, HbA1c measurements are now available for all participants who attended the second ARIC examination and who had stored whole blood available (n = 14 069), the vast majority of whom did not have diabetes.

The ARIC study was approved by institutional review boards at all participating centers and informed consent was obtained from all participants.

The National Health and Nutrition Examination Surveys—The National Health and Nutrition Examination Surveys (NHANES) are cross-sectional, multistage, stratified, clustered probability samples of the US civilian, non-institutionalized population conducted by the National Center for Health Statistics (NCHS), a branch of the Centers for Disease Control 3›4. Subjects participated in an interview and an extensive physical examination, performed at a mobile examination center, which included blood sampling. Here, we analyze data from the third NHANES (NHANES III), conducted in 1988–1994, and the most recent data available from the continuous NHANES, initiated in 1999 and released in 2-year intervals (NHANES 1999–2006).

The protocols for conduct of NHANES were approved by the NCHS Institutional Review Board and informed consent was obtained from all participants.

Measurement of HbA1c in the ARIC study

Blood samples were collected from participants in the ARIC study as part of the original study protocol in 1990–1992. The whole blood aliquot was frozen at –70°C and shipped to the Central Chemistry Laboratory, University of Minnesota, and placed in long-term storage. Measurements of HbA1c on 4918 samples were conducted in 2003–2004 at the University of Minnesota (by M.W.S.) using the Tosoh 2.2 Plus HPLC instrument (Tosoh

Bioscience, South San Francisco, CA, USA). Measurements on the remaining 9151 specimens were conducted in 2007–2008 in the same laboratory using the Tosoh G7 HPLC instrument. These instruments are certified by the National Glycohemoglobin Standardization Program (NGSP), led by the Diabetes Diagnostic Laboratory at the University of Missouri-Columbia, and are the same instruments used in the Diabetes Control and Complications Trial (DCCT) follow-up study, the Epidemiology of Diabetes Interventions and Complications (EDIC). The University of Minnesota, which has sustained the DCCT/EDIC Central Biochemistry Laboratory, has maintained consistency of the HbA1c assays with the backup DCCT/EDIC Laboratory (the NGSP laboratory at the University of Missouri-Columbia) for over 20 years by adjusting nominal values for calibrators to long-term quality control samples in Missouri [5]. As part of our work in 2007–2008, we re-analyzed a convenience sample of specimens that had been analyzed previously for HbA1c in 2003–2004 using the Tosoh 2.2 instrument. Laboratory personnel who were masked to sample identifiers selected these samples pseudorandomly. These repeat measurements from the same samples at these two time periods form the basis of our internal comparison of ARIC measurements in the present paper.

Measurement of HbA1c in NHANES

NHANES III (1988–1994)—The HbA1c measurements in NHANES III were performed in 1988–1994 at the Diabetes Diagnostic Laboratory, University of Missouri-Columbia, using the Diamat HPLC assay (Bio-Rad Laboratories, Hercules, CA, USA). All measurements were standardized against the DCCT reference method. The analytic coefficients of variation (CV) for this assay ranged from 1.1% to 3.1%. Because variant hemoglobins can interfere with HbA1c measurement using the Diamat assay, samples suspected of containing abnormal hemoglobin were then analyzed by affinity chromatography. Detailed information on data collection and laboratory procedures in NHANES III are available elsewhere [6,7].

NHANES 1999–2004 and 2005–2006—The HbA1c measurements for NHANES 1999–2004 were also performed by the Diabetes Diagnostic Laboratory, University of Missouri-Columbia, on Primus CLC330 and Primus CLC385 instruments (Primus, Kansas City, MO, USA).3 HbA1c measurements in the NHANES 2005–2006 were performed at the University of Minnesota (by M.W.S.) using the Tosoh A1c 2.2 Plus HPLC instrument (Tosoh Bioscience) 8. A crossover study was performed by the NCHS to compare the NHANES 2003–2004 (University of Missouri-Columbia) HbA1c measurements with those conducted using the Tosoh 2.2 instrument in 2005–2006 (University of Minnesota). A Deming regression analysis yielded the following equation: $y_{(\text{Primus})} = 0.4892+0.9277_{(\text{Tosoh 2.2})}$, $n = 207$, $r = 0.980$.[8] Thus, because of this documented difference, we considered the HbA1c results from the 1999–2004 and 2005–2006 surveys separately.

All HbA1c measurements in the present study (ARIC and NHANES) were conducted using NGSP-certified methods and standardized to the method used by the DCCT (see Table 1 for a summary of the HbA1c assays).

Statistical analysis

To assess the reliability of the ARIC measurements, we compared 383 repeated measurements of HbA1c from stored whole blood specimens conducted in the same samples in 2003–2004 (Tosoh 2.2) and again in 2007–2008 (Tosoh G7) at the University of Minnesota. We conducted a paired *t*-test and displayed differences between the methods (time-periods) graphically using scatter and Bland-Altman plots. We compared the relationship between these measurements using Deming regression, which accounts for error in both the dependent and independent variables [9].

Because we observed a systematic difference between the measurements at the two time periods, we evaluated the impact of this difference on the prevalence of undiagnosed diabetes defined by an HbA1c cut-off point of ≥6.5%.[1,2] We compared the prevalence of diabetes in the ARIC population with alignment of HbA1c values to the Tosoh 2.2 in 2003–2004 and then to the Tosoh G7 in 2007–2008. These analyses were conducted after excluding participants with a history of diabetes or a history of using diabetes medication.

We also compared the distribution of HbA1c measurements we obtained in ARIC with that in the NHANES III, 1999–2004, and 2005–2006 surveys to assess indirect agreement with a similar general population of adults after alignment of all ARIC values to the 2007–2008 measurements on the Tosoh G7. We limited these analyses to comparable NHANES and ARIC participants: Black or White race/ethnicity only, aged 48–58 years, and without a history of diabetes or the use of glucose-lowering medication. We overlaid the distribution of HbA1c values from NHANES III (weighted to the 1990 US Census population) with the distribution in ARIC (unweighted data) using a kernel density smoother. We calculated means, medians, and 5th, 25th, 75th, and 95th percentiles overall and stratified by race/ethnicity to compare the distribution of HbA1c in NHANES III and ARIC, and calculated the percent difference (NHANES III minus ARIC). Analyses of NHANES were performed incorporating the sampling weights (2- and 6-year combined weights) using StataSE Version 10.0 (StataCorp, College Station, TX, USA) and R (Version 2; Free Software Foundation, Boston, MA, USA) to obtain nationally representative estimates from these surveys [10].

Results

Adequate sample was available and valid HbA1c measurements were obtained from 14 069 (98%) of the 14 348 ARIC participants who attended Visit 2 and 99.9% of all people who provided a blood sample (n = 14 082). In 2007–2008, we re-analyzed 383 of specimens that had been analyzed previously for HbA1c in 2003–2004 using the Tosoh 2.2 instrument (Table 2). Pearson's correlation between the measurements at the two time points was high (r = 0.99), but with a slight bias: 0.29% higher HbA1c in 2007–2008 (Tosoh G7) compared with 2003–2004 (Tosoh 2.2; n = 383 duplicate measurements; $P < 0.0001$. The intraclass correlation (ICC) for the paired measurements was 0.98 (95% confidence interval (CI) 0.97–0.99) and a within-sample CV of 3.9% (95% CI 3.6%–4.2%) [11]. The high correlation between measurements and systematic bias is shown in Figs 1 and 2. Comparison of the two measurements yielded the following Deming regression equation: $y_{(2007–2008\ \text{Tosoh G7})} = 0.073+1.034x_{(2003–2004\ \text{Tosoh 2.2})}$.

We compared the prevalence of undiagnosed diabetes using HbA1c values aligned to the different periods of measurement. With alignment of all ARIC HbA1c values to the 2003–2004 measurements obtained on the Tosoh 2.2 instrument, the prevalence of undiagnosed diabetes (HbA1c ≥6.5%) was 2.7%. After realignment to the 2007–2008 values obtained on the Tosoh G7 instrument, the prevalence of undiagnosed diabetes was 4.5%, a >60% increase in prevalence.

We used NHANES data to assess the comparability of our measurements in ARIC to nationally representative estimates of HbA1c in the general US population. Owing to systematic differences between 2003–2004 (Tosoh 2.2) and 2007–2008 (Tosoh G7), the above Deming regression equation was used to align the 2003–2004 ARIC measurements to the 2007–2008 (Tosoh G7) results. After calibration, ARIC measurements were similar to nationally representative data from a comparable population of adult participants in the NHANES (III, 1999–2004, and 2005–2006). Stratified results are shown in Table 3. The overall mean and median HbA1c levels in ARIC were 5.5% and 5.4%, respectively, similar to the mean and median values in NHANES III (5.4% and 5.4%, respectively) NHANES

1999–2004 (5.5% and 5.4%, respectively), and NHANES 2005–2006 (5.5% and 5.4%, respectively). Blacks and older people had consistently higher HbA1c values compared with Whites, but these differences were consistent across studies. Additional stratified analyses comparing the 5th, 25th, 75th, and 95th percentiles were similar (data not shown). NHANES III, conducted in 1988–1994, is the most contemporaneous to the ARIC HbA1c population (1990–1992). We compared the distribution of HbA1c in NHANES III and ARIC among participants who were aged 48–68 years, Black or White, and with no history of diabetes using overlayed kernal density plots (Fig. 3). These plots reveal a slightly more variable (wider) distribution among Blacks in NHANES III compared with ARIC, but almost completely overlapping distributions in Whites. Black participants in ARIC were only enrolled at the Jackson, Mississippi, and Forsyth County, North Carolina field sites. Thus, differences in the distribution of HbA1c comparing Blacks in ARIC with those in NHANES III may be the result of geographic differences and other related population characteristics. Nonetheless, overall measures of central tendency and the distributions of HbA1c in ARIC and NHANES surveys were similar, suggesting the comparability of ARIC measurements conducted using a modern state-of-the-art HPLC instrument to nationally representative data from the same era.

Discussion

These data demonstrate high reliability of HbA1c measurements from stored whole blood samples conducted using the Tosoh 2.2 and G7 HPLC analyzers at two different time points, but suggest a systematic difference in the measurements at these two times. These results are analogous with those of previous studies showing the impact of calibration differences in serum creatinine concentrations and the need for correction to ensure alignment across time periods [12,13]. By aligning our HbA1c values to those obtained in 2007–2008 using a state-of-the-art Tosoh G7 HPLC instrument, the distribution was nearly identical to measurements obtained from fresh samples in a contemporaneous general population. In general, our data support the success of national efforts to standardize HbA1c measurements and suggest the reproducibility of results in separate large epidemiologic studies conducted in different laboratories. Previous studies have documented the stability of HbA1c in stored whole blood specimens[14–16]. Previously, we demonstrated the feasibility of measuring HbA1c from a subset of stored whole blood specimens in ARIC by comparing the 2003–2004 measurements to measurements from a case-control study conducted in 1990–1992 using a subset of the same samples [17, 18]. This previous study demonstrated high reliability of the 2003–2004 measurements, but with a systematic bias: measurements conducted in 1990–1992 using the Bio-Rad Diamat instrument were significantly and consistently lower by 0.35% HbA1c [17]. Overall, the data from the present study demonstrate high reliability (ICC = 0.98, $r = 0.99$) and little bias when comparing measurements using modern, state-of-the-art Tosoh HPLC instruments. The small but consistent bias could have resulted from method difference, differences in assay standardization over time, or a possible “storage effect” (e.g. breakdown products coeluting with the HbA1c peak). However, the distribution of the Tosoh G7 measurements in ARIC participants conducted in 2007–2008 is highly consistent with HbA1c measurements in the nationally representative NHANES populations. To account for the small but significant difference in the ARIC measurements across the two time periods, the Deming regression equation will be used to directly calibrate to the Tosoh 2.2 HbA1c measurements in 2003–2004 to those obtained using the Tosoh G7 instrument in 2007–2008 in the ARIC study.

As the most commonly used measure to assess glucose control among persons with diabetes, extensive HbA1c data are available from studies of diabetes. In contrast, relatively few population-based studies of non-diabetic people have routinely conducted measurements of HbA1c.

In conclusion, the present study demonstrates the reliability of HbA1c measurements from stored whole blood samples in ARIC stored for 14–18 years and the comparability of these measurements to other large epidemiologic studies with measurements traceable to DCCT/EDIC, including NHANES. Despite the systematic difference observed when comparing the HbA1c measurements from these two time periods, the high reliability indicates that the ranking of people would be unchanged and relative measures of association (e.g. odds ratios, relative risks) would not be appreciably affected. Alignment to the most recent measurements obtained using the Tosoh G7 instrument ensured similarity to HbA1c measurements from fresh samples in a comparable general population. We anticipate that subsequent analyses of this large community-based study of middle-aged adults with over two decades of follow-up for clinical outcomes will be invaluable for the investigation into the clinical utility of HbA1c as a diagnostic test for diabetes.

Acknowledgments

This research was supported by NIH/NIDDK grant R21 DK080294. E.S. was supported by NIH/NIDDK grant K01 DK076595. The ARIC study is performed as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022. The authors thank the staff and participants of the ARIC study for their important contributions. The authors also thank Dr Randie LITTLE (Associate Research Professor, Co-Director Diabetes Diagnostic Laboratory, University of Missouri) for her helpful feedback on preliminary analyses of these data.

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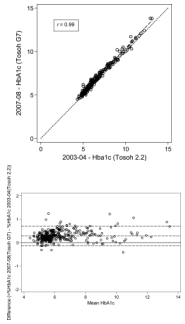

Figure 1.
Comparison of 2003–2004 and 2007–2008 HbA1c (%) measurements performed using the Tosoh 2.2 and Tosoh G7 HPLC instruments (Tosoh Bioscience, South San Francisco, CA, USA), respectively (n = 383).

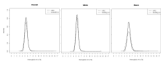

Figure 2.
Bland-Altman plot of the difference in HbA1c (%) between values determined using the Tosoh G7 (Tosoh Bioscience, South San Francisco, CA, USA) minus those obtained using the Tosoh 2.2 compared against the mean. The solid line is the zero (reference) line, whereas the dashed lines represent the mean and ±1.96SD.

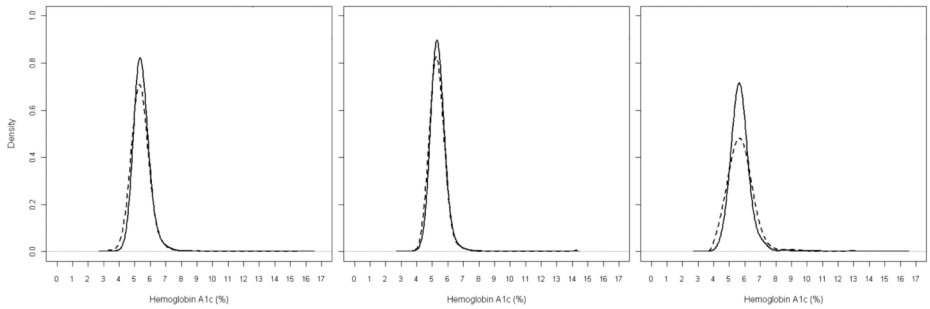

Figure 3.

Distributions of HbA1c in the Atherosclerosis Risk in Communities (ARIC) study (recalibrated; —) and the Third National Health and Nutrition Examination Survey (NHANES III; ------) overall (a), as well as in non-Hispanic Black (c) and White (b) people, without a history of diabetes and aged 48–58 years.

Table 1

Summary of HbA1c assays

Study	Specimen	Instrument
ARIC (2003–2004)	Stored whole blood	Tosoh 2.2 Plus HPLC (Tosoh Bioscience, South San Francisco, CA, USA)
ARIC (2007–2008)	Stored whole blood	Tosoh G7 HPLC
NHANES III (1988–94)	Fresh whole blood	Diamat HPLC (Bio-Rad Laboratories, Hercules, CA, USA)
NHANES 1999–2004	Fresh whole blood	Primus CLC330 and CLC385 (Primus, Kansas City, MO, USA)
NHANES 2005–2006	Fresh whole blood	Tosoh 2.2 Plus HPLC

ARIC, Atherosclerosis Risk in Communities; NHANES, National Health and Nutrition Examination Survey.

Table 2

Summary statistics comparing 2003–2004 and 2007–2008 HbA1c measurements (n = 383 pairs)

Mean (±SD) 2003–2004 (Tosoh 2.2) HbA1c (%)	6.16 ± 1.47
Mean (±SD) 2007–2008 (Tosoh G7) HbA1c (%)	6.45 ± 1.52
Mean (95% CI) difference (2007–2008 minus 2003–2004 HbA1c)	0.29 (0.26, 0.31)
P value*	<0.0001

The Tosoh 2.2 and G7 instruments are manufactured by Tosoh Bioscience (South San Francisco, CA, USA).

CI, confidence interval.

* Paired *t*-test of the hypothesis that the means are equal.

Table 3

Mean and median HbA1c (%) levels in non-Hispanic White and non-Hispanic Black participants without diagnosed diabetes or glucose-lowering medications, aged 48–68 years, in the Atherosclerosis Risk in Communities (ARIC) study and National Health and Nutrition Examination Survey (NHANES)

	Overall	Age (years)		Black	White	Black		White	
		48–<58	58–68			48–<58 years	58–68 years	48–<58 years	58–68 years
ARIC (recalibrated; *n* = 12 497)									
Mean	5.5	5.5	5.6	5.8	5.5	5.8	5.9	5.4	5.5
Median	5.4	5.4	5.5	5.7	5.4	5.8	5.8	5.3	5.4
NHANES III (*n* = 2741)									
Mean	5.4	5.4	5.5	5.8	5.4	5.7	5.9	5.3	5.5
Median	5.4	5.3	5.4	5.7	5.4	5.7	5.7	5.3	5.4
NHANES 1999–2004 (*n* = 2248)									
Mean	5.5	5.4	5.5	5.7	5.4	5.6	5.8	5.4	5.5
Median	5.4	5.4	5.4	5.5	5.4	5.5	5.7	5.3	5.4
NHANES 2005–2006 (*n* = 864)									
Mean	5.5	5.4	5.6	5.7	5.4	5.7	5.8	5.4	5.5
Median	5.4	5.4	5.4	5.6	5.4	5.6	5.8	5.3	5.4