



# Detection of Abnormal Glucose Tolerance in Africans Is Improved by Combining A1C With Fasting Glucose: The Africans in America Study

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## OBJECTIVE

Abnormal glucose tolerance is rising in sub-Saharan Africa. Hemoglobin A<sub>1c</sub> by itself and in combination with fasting plasma glucose (FPG) is used to diagnose abnormal glucose tolerance. The diagnostic ability of A1C in Africans with heterozygous variant hemoglobin, such as sickle cell trait or hemoglobin C trait, has not been rigorously evaluated. In U.S.-based Africans, we determined by hemoglobin status the sensitivities of 1) FPG  $\geq 5.6$  mmol/L, 2) A1C  $\geq 5.7\%$  (39 mmol/mol), and 3) FPG combined with A1C (FPG  $\geq 5.6$  mmol/L and/or A1C  $\geq 5.7\%$  [39 mmol/mol]) for the detection of abnormal glucose tolerance.

## RESEARCH DESIGN AND METHODS

An oral glucose tolerance test (OGTT) was performed in 216 African immigrants (68% male, age  $37 \pm 10$  years [mean  $\pm$  SD], range 20–64 years). Abnormal glucose tolerance was defined as 2-h glucose  $\geq 7.8$  mmol/L.

## RESULTS

Variant hemoglobin was identified in 21% (46 of 216). Abnormal glucose tolerance occurred in 33% (72 of 216). When determining abnormal glucose tolerance from the OGTT (2-h glucose  $\geq 7.8$  mmol/L), sensitivities of FPG for the total, normal, and variant hemoglobin groups were 32%, 32%, and 33%, respectively. Sensitivities for A1C were 53%, 54%, and 47%. For FPG and A1C combined, sensitivities were 64%, 63%, and 67%. Sensitivities for FPG and A1C and the combination did not vary by hemoglobin status (all  $P > 0.6$ ). For the entire cohort, sensitivity was higher for A1C than FPG and for both tests combined than for either test alone (all  $P$  values  $\leq 0.01$ ).

## CONCLUSIONS

No significant difference in sensitivity of A1C by variant hemoglobin status was detected. For the diagnosis of abnormal glucose tolerance in Africans, the sensitivity of A1C combined with FPG is significantly superior to either test alone.

The International Diabetes Federation predicts that by the year 2035, 41.5 million sub-Saharan Africans will have diabetes and 66 million will have prediabetes (1). This represents a 109% increase in the prevalence of diabetes and is the highest anticipated increase in the world (1).

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Hemoglobin A<sub>1c</sub> (A1C), a glycosylated form of hemoglobin A, is now widely used by itself or in combination with fasting plasma glucose (FPG) for the diagnosis of abnormal glucose tolerance, a summary term for diabetes and prediabetes (2–4).

Normally, hemoglobin A represents >90% of the hemoglobin in red blood cells. Yet, in heterozygous variant hemoglobin conditions, such as HbAS (i.e., sickle cell trait) and HbAC (i.e., hemoglobin C trait), hemoglobin A represents <60% of red blood cell hemoglobin (5,6). The diagnostic ability of A1C in individuals with heterozygous variant hemoglobin has not been carefully evaluated.

Sickle cell trait occurs in 10–40% of people from equatorial Africa, with the highest rates occurring in areas where malaria is endemic (7). Sickle cell trait is most common in people of African descent but also occurs at high rates in the Middle East and central India (6). Overall, sickle cell trait occurs in 6–8% of African Americans and in 10% of African Caribbeans (8–10). Hemoglobin C trait occurs in 2% of African Americans but is much more common in West Africa, where rates as high as 15% have been reported (5,9). Therefore, if variant hemoglobin interferes with the efficacy of A1C as a diagnostic test, the effect would be felt throughout the African diaspora, the Middle East, and India, but magnified in Africa.

For decades, the 2-h oral glucose tolerance test (OGTT) has been the reference method for the diagnosis of abnormal glucose tolerance (11). Yet due to cost, time, and patient inconvenience, conducting an OGTT is often infeasible for patient care or population-based studies (12). FPG has been used as an inexpensive alternative to the OGTT, but FPG is also associated with challenges, including the requirement for an 8-h fast (12). Progress in the standardization and accuracy of the measurement of A1C led to the adoption in 2010 of A1C as a diagnostic test for abnormal glucose tolerance (2,13). The advantage of A1C over FPG and OGTT is that it can be drawn any time of day and does not require fasting. However, with widespread use of A1C as a diagnostic test, there has been concern about whether A1C was sufficiently sensitive to be used as a stand-alone test (4,14–16). To improve detection of abnormal

glucose tolerance, some investigators have suggested combining A1C with FPG (4).

Data on the ability of FPG and A1C alone and together to detect abnormal glucose tolerance in Africans are sparse. Therefore, our goal was to determine the ability of 1) FPG, 2) A1C, and 3) FPG combined with A1C to identify abnormal glucose tolerance in African immigrants with normal and heterozygous variant hemoglobin.

## RESEARCH DESIGN AND METHODS

The participants were 216 African immigrants (68% male, age  $37 \pm 10$  years [mean  $\pm$  SD], range 20–64 years; BMI  $27.6 \pm 4.6$  kg/m<sup>2</sup>, range 18.2–41.2 kg/m<sup>2</sup>) enrolled in the Africans in America cohort. All participants were born in equatorial Africa. The African region of birth was 53% West, 28% Central, and 19% East Africa (Supplementary Table 1 provides frequency distribution for country of birth). As described previously, recruitment was achieved by newspaper advertisements, flyers, and the National Institutes of Health (NIH) website (17–19).

At enrollment, participants self-identified as healthy and denied any history of diabetes or diabetic symptoms, including polyuria, polydipsia, or weight loss. The study was approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board. All participants gave informed written consent.

Three outpatient visits were held at the NIH Clinical Research Center in Bethesda, MD. At visit 1, a medical history, physical examination, urinalysis, and electrocardiogram were performed. Blood samples were taken to document an absence of anemia and kidney, liver, and thyroid dysfunction. Complete blood counts were assessed in all participants, and absolute reticulocyte count, percentage reticulocyte, iron, transferrin, and ferritin levels were also determined in 96 consecutively enrolled participants.

For visits 2 and 3, participants came to the Clinical Center after a 12-h fast. At visit 2, tests performed in all participants were A1C, FPG, and a 2-h glucose obtained during a standard OGTT using 75 g dextrose (Trutol 75; Custom Laboratories, Baltimore, MD) and waist circumference. Visceral adipose tissue volume was measured in 209 of 216 participants

by abdominal computed tomography (CT) scan at L2–3 (17).

Glucose tolerance status was defined based on American Diabetes Association criteria for 2-h glucose obtained during the OGTT (20); specifically, normal glucose tolerance: 2-h glucose <7.8 mmol/L; prediabetes: 2-h glucose  $\geq$ 7.8 mmol/L but <11.1 mmol/L; and diabetes: 2-h glucose  $\geq$ 11.1 mmol/L. Abnormal glucose tolerance (a summary term for prediabetes and diabetes combined) was defined as 2-h glucose  $\geq$ 7.8 mmol/L.

At visit 3, an insulin-modified frequently sampled intravenous glucose tolerance test (IM-FSIGT) was performed. Intravenous catheters were placed in both antecubital veins. After baseline blood samples were obtained, dextrose (0.3 g/kg) was administered intravenously. Insulin was infused for 5 min starting at 20 min ( $4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). As described previously, blood samples for glucose and insulin concentrations were obtained at 32 time points between baseline and 180 min (21). The insulin sensitivity index (*S*<sub>i</sub>) was determined by entering the glucose and insulin concentrations into the minimal model (MinMOD Millennium v.6.02) (22). The acute insulin response to glucose (AIR<sub>g</sub>), a measure of  $\beta$ -cell function, was calculated as the area under the insulin curve between 0 and 10 min for the insulin concentration above basal (22).

Thirty-nine participants did not undergo an IM-FSIGT. Twenty-three people were unable to return for a third visit. The remaining 13 were found during their OGTT at visit 2 to have an elevated 2-h glucose consistent with the diagnosis of diabetes and per protocol did not proceed to the IM-FSIGT. Ten of these 13 people agreed to return for a second OGTT. The median interval between the two OGTTs was 8 days.

## Analytic Measures

Hemoglobin, hematocrit, mean corpuscular volume (MCV), absolute reticulocyte count, and percentage reticulocyte count were measured in EDTA-anticoagulated whole blood using a Sysmex XE-5000 analyzer (Chicago, IL). Glucose, total bilirubin, direct bilirubin, liver enzymes, blood urea nitrogen, creatinine, vitamin B<sub>12</sub>, and folate were measured in serum using the Vista analyzer (Siemens Healthcare, Malvern, PA). Insulin was measured in serum on the Cobas 6000

instrument (Roche Diagnostics, Indianapolis, IN). Iron, transferrin, and ferritin were analyzed in serum on the Immulite XP and analyzer (Siemens Healthcare), and urinary microalbumin was measured using the Dimension Xpand (Siemens Healthcare).

### A1C by High-Performance Liquid Chromatography

A1C values were determined in all 216 participants by high-performance liquid chromatography (HPLC) using three different National Glycohemoglobin Standardization Program (NGSP)-certified instruments that were made by the same manufacturer (Bio-Rad Laboratories, Hercules, CA) and used the same HPLC technology. Samples from the first 33 consecutively enrolled participants were analyzed with the Bio-Rad Classic Variant system. A1C in the next 157 individuals was measured by the Bio-Rad Variant II instrument and in the remaining 26 consecutively enrolled persons was measured using a D10 instrument. The correlation ( $R^2$ ) between the Bio-Rad Classic Variant and Bio-Rad Variant II instruments was 0.9921, and the  $R^2$  between the Bio-Rad II and D10 instruments was 0.9934. For low controls, the coefficient of variation (CV) was <3%, and for high controls, the CV was <2%. Neither HbAS nor HbAC interferes with the A1C measurement on the Bio-Rad instruments used in this study (23). The presence of variant hemoglobin was determined by retention time on HPLC.

### A1C by Boronate Affinity Chromatography

To confirm that the chromatographic peaks identified as A1C on HPLC were not misinterpreted in individuals with HbAC or HbAS, whole blood samples in 90 consecutively enrolled Africans were analyzed for A1C by the boronate affinity chromatography method on the Premier Hb9210 analyzer (Trinity Biotech, Bray, Ireland). The reagents and the instrument, which is NGSP-certified, were provided by Trinity Biotech. The method measures total glycated hemoglobin and values are converted to A1C. The CVs were 1.1%, 1.3%, and 1.6% at A1C values of 5.4%, 6.4%, and 9.3%, respectively.

### Hemoglobin Electrophoresis

To validate the detection of hemoglobin variants by HPLC, we used a definitive

method to identify variant hemoglobins, namely hemoglobin electrophoresis. Hemoglobin electrophoresis was performed in 75 consecutively enrolled participants. An additional seven participants were included in this analysis because they had undergone hemoglobin electrophoresis at another time, with results available in the NIH database. Altogether, hemoglobin electrophoresis results were available from 82 participants.

Hemoglobin electrophoresis was performed in cellulose acetate (pH 8.4–8.6) and citrate agar (pH 6.0–6.3) to identify variant hemoglobin proteins S and C, using a Helena Zip-Zone electrophoresis instrument (Helena Laboratories, Beaumont, TX). Identities of hemoglobin proteins were confirmed by comparison with known samples of HbA, HbS, and HbC.

### Statistics

Unless stated otherwise, data are presented as mean  $\pm$  SD. The Student  $t$  test and  $\chi^2$  test were used to compare the characteristics of patients with normal and variant hemoglobin. Correlation between A1C by boronate affinity chromatography, the reference method, and HPLC was determined by the Lin concordance correlation coefficient and the agreement by using the Bland-Altman method.

Abnormal glucose tolerance was defined as 2-h glucose  $\geq$ 7.8 mmol/L. Logistic regression was conducted to determine whether variant hemoglobin had an independent effect on the ability of the A1C or FPG to detect abnormal glucose tolerance. Sensitivities and specificities were calculated for FPG  $\geq$ 5.6 mmol/L and A1C  $\geq$ 5.7% (39 mmol/mol) separately and then when both tests were combined for the entire cohort and by variant hemoglobin status. Then,  $\chi^2$  tests were performed to compare sensitivity and specificity of FPG, A1C, and the combined tests by variant hemoglobin status. When the cohort was examined as a single group ( $n = 216$ ), the McNemar test for matched pairs was used to compare sensitivities of FPG to A1C and to both tests combined (i.e., sensitivity of A1C in the whole cohort vs. sensitivity of FPG in the whole cohort).  $P$  values of  $\leq$ 0.05 were considered significant. Analyses were performed with Stata 13.0 software (StataCorp LP, College Station, TX).

## RESULTS

Demographic and metabolic characteristics of the cohort are presented in Table 1. There was no difference in age, body size, visceral adiposity, liver or kidney function, or social and economic factors by variant hemoglobin status (Table 1).

### Hemoglobin Evaluation

On the basis of the HPLC analyses, variant hemoglobin was detected in 21% (46 of 216) of participants. By region of origin, 27% of the West, 20% of the Central, and 8% of the East Africans had variant hemoglobin. Only 14% of Africans were aware of their variant hemoglobin status at enrollment.

Hemoglobin electrophoresis performed in 82 participants confirmed that HPLC correctly distinguished between normal and variant hemoglobin in all cases. Of the 19 individuals identified by HPLC as having variant hemoglobin, electrophoresis determined 17 had HbAS trait and 2 had HbC trait.

Overall, hemoglobin levels were  $140 \pm 13$  g/L and did not vary by group (Table 1). Reticulocyte absolute and percent reticulocyte did not vary hemoglobin status. There was no evidence of iron, vitamin B<sub>12</sub>, or folate deficiency in either group. However, variant hemoglobin status was associated with lower MCV and higher total and direct bilirubin levels (Table 1).

### Comparison of A1C Levels by Boronate Affinity Chromatography and HPLC

Of the 90 participants who had A1C determined by both boronate affinity chromatography and HPLC, 24% (22 of 90) had variant hemoglobin. The Pearson correlation coefficients for A1C between the two methods was 0.98 in the normal hemoglobin group and was 0.84 in the variant hemoglobin group (both  $P < 0.001$ ). The Lin concordance for the normal and variant hemoglobin groups were 0.96 and 0.72 (both  $P < 0.001$ ), respectively. The average differences between boronate affinity chromatography and HPLC for the normal and variant hemoglobin groups were  $-0.12 \pm 0.21$  and  $-0.19 \pm 0.24$ , respectively (Fig. 1). The Bland-Altman limits of agreement were  $-0.54, 0.29$ , and  $-0.65, 0.27$ , respectively (Fig. 1).

**Table 1—Metabolic and demographic characteristics**

Parameter	Total group N = 216*	Normal Hb n = 170	Variant Hb n = 46	P value†
Male (%)	68	69	63	0.41
Age (years)	37 ± 10	37 ± 10	37 ± 10	0.72
BMI (kg/m <sup>2</sup> )	27.6 ± 4.6	27.8 ± 4.8	26.8 ± 3.7	0.23
Waist circumference (cm)	90 ± 12	91 ± 12	88 ± 10	0.22
Visceral adipose tissue (cm <sup>3</sup> ) (n = 209)	95 ± 71	96 ± 70	93 ± 73	0.80
Hemoglobin (g/L)	140 ± 13	140 ± 13	141 ± 11	0.89
Hematocrit (%)	41.9 ± 3.3	42.1 ± 3.4	40.9 ± 2.8	0.03
MCV (fl)	84.3 ± 5.4	85.1 ± 5.3	81.1 ± 4.8	<0.01
Reticulocyte absolute (×10 <sup>3</sup> /μL) (n = 96)	63.5 ± 22.6	63.4 ± 23.8	64.1 ± 18.5	0.90
Reticulocyte (%) (n = 96)	1.26 ± 0.48	1.27 ± 0.51	1.26 ± 0.36	0.97
Iron (μmol/L) (n = 96)	15 ± 5	15 ± 5	16 ± 4	0.40
Transferrin (g/L) (n = 96)	2.45 ± 0.35	2.47 ± 0.36	2.41 ± 0.30	0.51
Saturation (%) (n = 96)	25 ± 9	25 ± 9	27 ± 8	0.34
Ferritin (pmol/L) (n = 96)	252 ± 207	252 ± 213	252 ± 189	0.98
Vitamin B <sub>12</sub> (pmol/mL)	545 ± 271	546 ± 278	539 ± 244	0.87
Folate (nmol/L)	30.8 ± 11.6	30.6 ± 11.3	31.5 ± 12.9	0.65
Bilirubin total (μmol/L)	10.4 ± 5.8	10.1 ± 5.1	12.1 ± 7.4	0.03
Bilirubin direct (μmol/L)	2.4 ± 1.2	2.4 ± 1.2	2.7 ± 1.2	0.04
Alanine aminotransferase (units/L)	33 ± 16	33 ± 16	32 ± 14	0.77
Aspartate aminotransferase (units/L)	23 ± 14	23 ± 15	21 ± 7	0.33
Blood urea nitrogen (mmol/L)	4.3 ± 1.1	4.3 ± 1.1	3.9 ± 0.7	0.46
Creatinine (μmol/L)	80 ± 17	80 ± 17	78 ± 14	0.47
eGFR (mL/min/1.73 m <sup>2</sup> )‡	110 ± 19	110 ± 20	109 ± 16	0.83
A1C (%)	5.5 ± 0.7	5.5 ± 0.7	5.4 ± 0.6	0.17
A1C (mmol/mol)	37 ± 7.7	37 ± 7.7	36 ± 6.6	
Abnormal glucose tolerance (%)§	33	34	33	0.91
Diabetes (%)	6	5	11	0.12
Prediabetes (%)¶	27	30	24	0.46
Fasting glucose (mmol/L)	5.1 ± 0.7	5.1 ± 0.8	5.0 ± 0.6	0.90
2-h glucose (mmol/L)	7.3 ± 2.2	7.3 ± 2.2	7.3 ± 2.3	0.97
S <sub>i</sub> ([mU/L] <sup>-1</sup> min <sup>-1</sup> ) (n = 174)	4.25 ± 2.77	4.25 ± 2.60	4.25 ± 3.35	0.99
AI <sub>Rg</sub> (mU L <sup>-1</sup> min) (n = 174)	670 ± 435	678 ± 450	642 ± 384	0.66
Less than 1 drink/week (%)	75	78	67	0.15
Smoker (%)	5	6	0	0.21
Married (%)	44	44	46	0.85
College graduate (%)	67	66	70	0.69
Median income (\$)	45,000	40,000	50,000	0.08
Health insurance (%)	65	64	70	0.49

eGFR, estimated glomerular filtration rate. \*Unless noted otherwise, results available for all 216 participants and presented as mean ± SD. †Comparison by unpaired *t* tests for continuous variables and  $\chi^2$  for categorical variables. ‡Based on the Modification of Diet in Renal Disease Study Equation. §Defined by 2-h glucose  $\geq 7.8$  mmol/L. || Defined by 2-h glucose  $\geq 11.1$  mmol/L. ¶ Defined by 2-h glucose  $\geq 7.8$  mmol/L and  $< 11.1$  mmol/L.

### Glucose Metabolism

Neither A1C nor prevalence of altered glucose tolerance differed by hemoglobin status (Table 1). In addition, there were no significant differences in any parameter related to glucose metabolism, including fasting glucose, 2-h glucose, insulin resistance determined from S<sub>i</sub>, and  $\beta$ -cell function assessed by AI<sub>Rg</sub>.

### Glucose Pattern During the OGTT

In the entire cohort, the prevalence of abnormal glucose tolerance was 33% (72 of 216). Diabetes was present in 6% (13 of 216) and prediabetes in 27% (59 of 216) (Table 1).

Of the 13 people with diabetes, 10 underwent a second OGTT (Supplementary Table 2 and Supplementary Fig. 1).

On the repeat OGTT, seven individuals again met the 2-h glucose criterion for diabetes ( $\geq 11.1$  mmol/L). However, three individuals on repeat OGTT transitioned from the category of diabetes to prediabetes because they had 2-h glucose  $< 11.1$  mmol/L, specifically, 11 mmol/L, 10.9 mmol/L, and 10.2 mmol/L.

### Sensitivity and Specificity for FPG and A1C

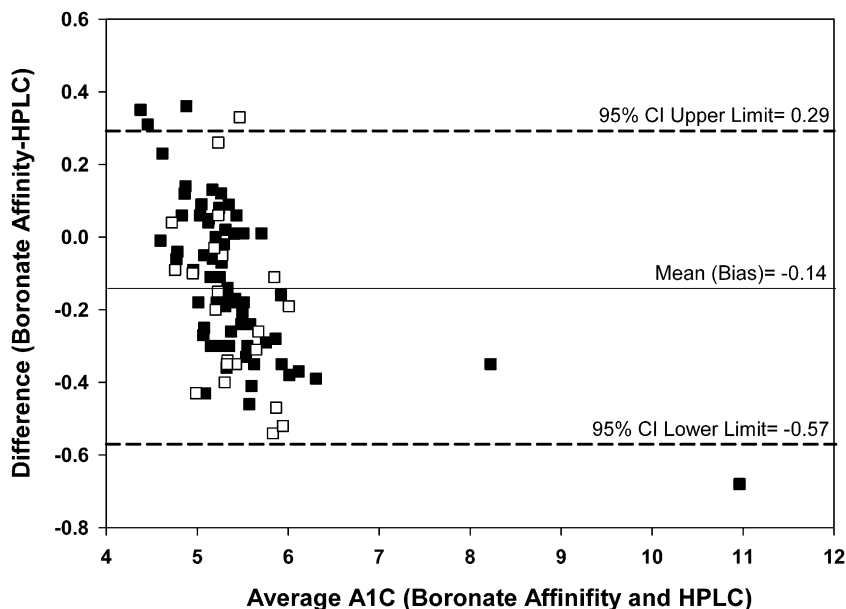
Sensitivity and specificity for the entire cohort and according to hemoglobin status are provided in Table 2. When FPG was used as the screening test for the entire cohort, the sensitivity was 32% (23 of 72). When A1C alone was used, the sensitivity was 53% (38 of 72). The sensitivity of A1C alone was significantly greater than for FPG ( $P = 0.01$ ) (Table 2).

In addition, the sensitivity for the combined tests was significantly greater than for either test alone (both  $P \leq 0.01$ ). This occurred because 20 people were identified by both screening tests, but 8 people (11%) identified by FPG were not detected by A1C, and 18 people (25%) identified by A1C were not detected by FPG. Hence, combining the two tests increased the sensitivity to 64% (46 of 72).

Next, diagnostic sensitivities for FPG, A1C, and the combined tests were compared according to variant hemoglobin status. By logistic regression, no difference in sensitivity was detected by variant hemoglobin status. Specifically when FPG and variant hemoglobin were both entered into the model, the effect of variant hemoglobin on 2-h glucose was not significant (OR 0.91 [95% CI 0.42, 1.96]). When A1C and variant hemoglobin were both entered into the model, the effect of variant hemoglobin on 2-h glucose was again not significant (OR 1.07 [95% CI 0.52, 2.18]). Further, FPG sensitivities for the normal and variant hemoglobin groups were 32% vs. 33% ( $P = 0.90$ ); the sensitivities for A1C were 54% vs. 47% ( $P = 0.59$ ); and the sensitivities for FPG and A1C combined were 63% and 67% ( $P = 0.80$ ), respectively.

### CONCLUSIONS

Effective screening programs that identify asymptomatic Africans with early disease are an essential step toward slowing or reversing the diabetes epidemic. Therefore, we undertook the



**Figure 1**—Bland-Altman plot for agreement between A1C determined by boronate affinity method and HPLC. The x-axis presents the mean of the two determinations and the y-axis the difference. The ■ represent the 68 participants with normal hemoglobin. The □ represent the 22 participants with variant hemoglobin.

first investigation to determine the sensitivity of FPG, A1C, and FPG and A1C combined in an asymptomatic African cohort stratified by heterozygous variant hemoglobin status. We had four major findings. First, sensitivity of A1C as a diagnostic test in all Africans was 53%. Second, sensitivity of A1C as a diagnostic test did not vary by variant hemoglobin status. Third, sensitivity of FPG as a single diagnostic test was 32%, which was significantly lower than for A1C. Fourth, due to the added value of combined tests, A1C combined with FPG had a sensitivity of 64%, which was significantly higher than for either test alone.

As anticipated, the sensitivity of FPG was not influenced by hemoglobin status. Yet, FPG as a single diagnostic test for the detection of abnormal glucose tolerance had a sensitivity of only 32%. However, combining FPG with A1C significantly increased sensitivity for the

diagnosis of abnormal glucose tolerance to 64%; therefore, the cost and inconvenience of combining these two tests must be weighed against the benefits of improved detection of abnormal glucose tolerance in all Africans.

With a sensitivity of 32%, FPG performed less effectively than A1C as a diagnostic test in Africans. In white Americans, FPG is many times more sensitive than A1C in the detection of altered glucose tolerance (4). Therefore, we need to re-evaluate this observation in a larger sample to rule out random variability or the effect of a relatively small sample size. Nonetheless, the superiority of FPG over A1C is less certain in African Americans (4). In fact, the National Health and Nutrition Examination Survey 1999–2002 revealed that even though the prevalence of both prediabetes and diabetes is higher in African Americans than in whites, African

Americans are less likely to have fasting hyperglycemia (24). Lower than expected levels of fasting glucose in African Americans may be explained by lower hepatic fat in African Americans than in whites (25). Data on hepatic fat in Africans are not available, but it is likely that Africans are similar to African Americans and have low hepatic fat and lower than expected levels of fasting glucose in the early asymptomatic phase of abnormal glucose tolerance.

We did not detect a statistically significant difference between the diagnostic sensitivity of A1C in the variant hemoglobin group compared with the normal hemoglobin group. This was an important issue to address. There are two mechanistic reasons why variant hemoglobin could have affected the efficacy of A1C as a diagnostic test. First, glycated hemoglobin S and C do not appear as A1C, which is the product of glycation of the N-terminal valine of the  $\beta$ -chain of hemoglobin A. Therefore, variant hemoglobin reduces the amount of HbA available to serve as the substrate for conversion to A1C. Second, in the presence of HbAS trait and HbAC trait, there may be increased red blood cell turnover, leading to decreased formation of A1C. Older data with small cohorts, suggest that the red blood cell life span in individuals with variant hemoglobin is closer to 90 than 120 days (26,27). Indeed, the lower MCV and higher bilirubin levels we observed in the variant hemoglobin group are consistent with increased red blood cell turnover. Nevertheless, additional more modern studies of red blood cell turnover in individuals with normal and variant hemoglobin are necessary to verify this potential mechanism.

In the past, some methods provided spurious A1C results in the presence of variant hemoglobin (28). Yet, improvements in HPLC methods have eliminated interference from HbAS and HbAC

**Table 2—Sensitivities and specificities for abnormal glucose tolerance\***

Group	N	Sensitivity			Specificity		
		FPG	A1C	Combined†	FPG	A1C	Combined
Whole cohort	216	32 (23/72)	53‡ (38/72)	64§ (46/72)	95 (137/144)	75 (108/144)	74 (106/144)
Normal Hb	170	32 (18/57)	54 (31/57)	63 (36/57)	95 (107/113)	74 (84/113)	73 (83/113)
Variant Hb	46	33 (5/15)	47 (7/15)	67 (10/15)	97 (30/31)	81 (25/31)	77 (24/31)

Data are presented as % (n/N). \*Abnormal glucose tolerance defined by elevated 2-h glucose ( $\geq 7.8$  mmol/L). †FPG and A1C combined. ‡According to the McNemar test, different from FPG,  $P \leq 0.01$ . §According to the McNemar test, different from FPG and from A1C, both  $P \leq 0.01$ .

(28,29). Nevertheless, we measured A1C by boronate affinity chromatography in 90 participants in our study, including 22 (24%) with variant hemoglobin. We selected boronate affinity chromatography because the common hemoglobin variants have little or no effect on A1C measurement by this technique. We verified this with the Premier Hb9210 HbA<sub>1c</sub> analyzer, which revealed that neither HbAC nor HbAS interfered with A1C measurement (30).

A challenge associated with this investigation is the reliance on a single OGTT as the reference standard (4,14–16). However, epidemiological investigations evaluating the diagnostic efficacy of A1C also used a single OGTT as the standard (4,14–16). In addition, all of the landmark lifestyle intervention studies have used a single OGTT to document at regular intervals whether there has been progression to diabetes (31–33). Yet, it is known that there is biological variation in the OGTT, particularly early in the development of abnormal glucose tolerance (12). Therefore, some vacillation between normal glucose tolerance and prediabetes as well as between prediabetes and diabetes should be anticipated.

The focus of this investigation was the combined epidemic of diabetes and prediabetes. This decision was made because cardiovascular disease, the leading cause of death in people with diabetes, becomes established in the prediabetic phase (34).

This type of intensive, resource-rich investigation of glucose tolerance by OGTT, A1C by both HPLC and boronate affinity chromatography, hemoglobin status by electrophoresis, insulin resistance by the minimal model,  $\beta$ -cell secretion measured by the AIRg, and visceral adiposity by CT scan, has not been performed in Africa. Certainly, the prevalence of abnormal glucose tolerance might differ in African immigrants living in the United States and Africans living in the country of their birth, but the ability of tests to detect abnormal glucose tolerance are likely to be independent of the country of residence.

The three main limitations of our investigation are, first, the small number of people with both variant hemoglobin and abnormal glucose tolerance; second, the analyses are based on single

determinations of A1C, FPG, and 2-h glucose (i.e., a single OGTT); and third, the cross-sectional design precludes us from making judgments on efficacy, particularly in the prevention of complications.

In sub-Saharan Africa where millions are at risk for abnormal glucose tolerance and the prevalence of variant hemoglobin is high, it is essential that tools used to determine health statistics and treatment options perform optimally. Using tests recommended by the American Diabetes Association and International Diabetes Federation, we have shown in Africans that A1C as a single diagnostic test is superior to FPG, but A1C combined with FPG has a higher sensitivity for abnormal glucose tolerance than either test alone. In addition, with NGSP-certified assays we confirmed that A1C is accurately measured in the presence of variant hemoglobin. Overall, until better diagnostic modalities for abnormal glucose tolerance are identified and validated in Africans, A1C combined with FPG should help identify millions of Africans with abnormal glucose tolerance.

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