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Concordance between fasting plasma glucose and HbA_{1c} in the diagnosis of diabetes in black South African adults: a cross-sectional study

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ABSTRACT

Objectives We investigated concordance between haemoglobin A1c (HbA_1c)-defined diabetes and fasting plasma glucose (FPG)-defined diabetes in a black South African population with a high prevalence of obesity.

Design Cross-sectional study.

Setting Rural South African population-based cohort.

Participants 765 black individuals aged 40–70 years and with no history of diabetes.

Primary and secondary outcome measures The primary outcome measure was concordance between HbA_{1c}-defined diabetes and FPG-defined diabetes. Secondary outcome measures were differences in anthropometric characteristics, fat distribution and insulin resistance (measured using Homoeostatic Model Assessment of Insulin Resistance (HOMA-IR)) between those with concordant and discordant HbA_{1c}/FPG classifications and predictors of HbA_{1c} variance.

Results The prevalence of HbA_{1c}-defined diabetes was four times the prevalence of FPG-defined diabetes (17.5% vs 4.2%). Classification was discordant in 15.7% of participants, with 111 individuals (14.5%) having HbA_{1c}-only diabetes (kappa 0.23; 95% CI 0.14 to 0.31). Median body mass index, waist and hip circumference, waist-to-hip ratio, subcutaneous adipose tissue and HOMA-IR in participants with HbA_{1c}-only diabetes were similar to those in participants who were normoglycaemic by both biomarkers and significantly lower than in participants with diabetes by both biomarkers ($p<0.05$). HOMA-IR and fat distribution explained additional HbA_{1c} variance beyond glucose and age only in women.

Conclusions Concordance was poor between HbA_{1c} and FPG in diagnosis of diabetes in black South Africans, and participants with HbA_{1c}-only diabetes phenotypically resembled normoglycaemic participants. Further work is necessary to determine which of these parameters better predicts diabetes-related morbidities in this population and whether a population-specific HbA_{1c} threshold is necessary.

Strengths and limitations of this study

- In contrast to the few previous studies of the association between fasting glucose and haemoglobin A1c (HbA_{1c}) in sub-Saharan African populations, this study compares adipose tissue distribution and markers of insulin resistance between individuals with diabetes defined by different biomarkers.
- This study was population based and conducted in a rural, underserved population, reflecting the majority of sub-Saharan Africa that resides in rural communities.
- Two hour glucose tolerance tests were not performed and the contribution of postprandial glucose to HbA_{1c} variability could not be assessed.

INTRODUCTION

Sub-Saharan Africa is projected to experience a 140% increase in the prevalence of diabetes mellitus by 2045[1] and accurate, comparable prevalence estimates will be essential to planning and monitoring by public health authorities. The WHO guidelines for the diagnosis of diabetes,[2] which inform the approach in many sub-Saharan African countries, include haemoglobin A_{1c} (HbA_{1c})≥6.5% (48 mmol/mol) as a diagnostic criterion for diabetes. Diagnosis based on HbA_{1c} is attractive because it provides an integrated assessment of glycaemic status over the preceding 3 months and has low analytical variability, but the extent to which this single threshold may be adopted in all sub-Saharan African populations is questionable. Existing data suggest that in individuals of African descent, HbA_{1c} may be higher for any given degree of glycaemia than in individuals of European descent.[3–5] Beyond this, there is intracontinental variation in the prevalence of conditions which may affect red blood cells such as anaemia and haemoglobinopathies.[6 7] Unlike black populations from West Africa or the largely West African-descent African-American and

Afro-Caribbean populations, haemoglobinopathies such as sickle cell disease are rare in South Africa.[7] Regional evaluation of the appropriateness of the internationally recommended HbA_{1c} criterion within different areas of sub-Saharan Africa is, therefore, necessary.

Previous studies comparing diabetes prevalence using different biomarkers have revealed significant heterogeneity. In a meta-analysis of 96 population-based studies, HbA_{1c}-based prevalence was lower than fasting plasma glucose (FPG)-based prevalence in 42.8% of age-sex-survey groups, higher in 41.6% and similar in 15.6%.[8] Interpreting this result in the context of sub-Saharan Africa more broadly and South Africa in particular is difficult, however, as a single study from a mixed ancestry sub-Saharan African population[9] was included. This study may not be representative of South African populations with less genetic admixture.

We investigated the concordance between diabetes defined by two commonly used tests, namely FPG and HbA_{1c}, in a black South African population with high background rates of obesity[10] and therefore at higher risk for dysglycaemia. We hypothesised that the prevalence of diabetes would differ by biomarker and performed analyses to investigate what factors, in addition to FPG, predicted HbA_{1c} overall and in analyses stratified by sex.

METHODS

Study setting and sample

This work was nested in two studies: Health and Ageing in Africa-a Longitudinal Study in an INDEPTH community (HAALSI)[11] and the Africa Wits-INDEPTH partnership for Genomic Studies (AWI-Gen),[12] which jointly recruited participants from the Agincourt Health and Socio-Demographic Surveillance System (HDSS). The Agincourt HDSS comprises 450 km^2 and approximately 120 000 people living in 31 research villages and is located 500 km northeast of Johannesburg in rural Mpumalanga, South Africa.[13] The HDSS is managed by the MRC/Wits Rural Public Health and Heath Transitions Research Unit (Agincourt), which annually enumerates the entire population of the HDSS to capture all vital events, that is, births, deaths and migrations, which ensures robust denominators.

Both HAALSI and AWI-Gen have been described in detail previously.[11 14] In brief, 6281 individuals of the 12 875 people ≥40 years and resident in the HDSS who met eligibility criteria were randomly selected to participate in HAALSI and 5059 were enrolled in the study cohort. A random sample of 3,273 HAALSI participants, stratified by age, were invited to enrol in the AWI-Gen cohort. A total of 2486 individuals enrolled in in AWI-Gen and samples for 1497 of these individuals were randomly selected for HbA_{1c} analysis.

The sampling strategy for this analysis is shown in online supplemental figure S1. HAALSI/AWI-Gen cohort members were eligible for inclusion in this analysis if they were aged 40–70 years, reported never having been diagnosed with diabetes by a healthcare practitioner and had valid results for HbA_{1c}, FPG and study covariates in the dataset. Individuals≥70 years were excluded from the analysis as these individuals completed a limited study protocol and did not attend clinic visits as outlined below.

Patient and public involvement

Prior to the initiation of the HAALSI and AWI-Gen studies, an extensive process of community engagement was led by Dr Rhian Twine, head of the Agincourt Office of Public Engagement. This included meetings with the Community Advisory Group, nominated by Community Development Forums and civic and traditional leadership structures to discuss planned research activities. Feedback on the results of this study will be included in the annual feedback of study results to villagers and community leaders.

Data collection

Data collection occurred at household and clinic visits which took place between November 2014 and August 2016.

Household visits

Sociodemographic and health status data were obtained from participants during household visits as previously described.[11] Capillary blood samples and dried blood spots were collected.

Clinic visits

Participants were subsequently evaluated at a single central facility (median 160 days between household and clinic visits) where weight, height, waist circumference (WC) and hip circumference (HC) were measured using standard procedures and visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured with ultrasound as previously described.[14] In brief, VAT was measured as the thickness of the fat pad between the peritoneum and anterior spine at end expiration and SAT as the distance between the skin and the outer edge of the linea alba. Venous blood samples were collected at the clinic visit after an overnight fast.

Sample processing

Sample collection and processing occurred at the same location, which facilitated immediate sample processing. Samples for FPG and insulin were collected in potassium oxalate/sodium fluoride and clot activator tubes, respectively, and centrifuged immediately after collection, with storage of the resulting supernatant at −80°C until analysis. Two millilitres of whole blood were collected in an EDTA tube for HbA_{1c} determination and frozen under similar conditions until analysis.

Sample analyses

Capillary blood samples were tested for haemoglobin at point of collection (Haemocue Hb 201+analyser; Haemocue, Sweden). Whole blood was analysed for HbA_{1c} using high-performance liquid chromatography on the

National Glycohaemoglobin Standardisation Programme-traceable Bio-Rad D-10 (Bio-Rad Laboratories, USA) with a reportable range of 3.8%–18.5% (18–179 mmol/mol) and coefficient of variation (CV) <1.3%. Plasma was analysed for glucose using colorimetric methods on the Randox Plus clinical chemistry analyser (Randox, UK) with a range of 0.36–35 mmol/L and CV <2.3%. Serum insulin assays were performed on the Immulite 1000 chemistry analysis system (Siemens, Germany), using a solid-phase, enzyme-labelled chemiluminescent immunometric assay (range 2–300 µIU/mL; CV <8%).

Dried blood spots were analysed for HIV serostatus using the Vironostika Uniform 11 (Biomeriuex, France) screening assay. Positive tests were confirmed with Roche Elecsys (Roche,USA).

Definition of variables

Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Individuals were classified as HIV positive if they reported being previously diagnosed with HIV or tested positive on screening and subsequent confirmatory tests, HIV negative if they reported previously having tested negative or tested negative on screening and indeterminate if they were unaware of their status and declined a screening test; antiretroviral therapy use was self-reported.

Individuals were classified as having diabetes by FPG criteria if FPG was ≥7.0 mmol/L and by HbA_{1c} criteria if HbA_{1c} was ≥6.5% (48 mmol/mol).[2 15 16] Insulin resistance was estimated using the Homoeostatic Model Assessment of Insulin Resistance (HOMA-IR), calculated as fasting glucose (mmol/L) × fasting insulin (mU/mL)/22.5.[17]

Statistical analyses

Continuous variables were described using medians and IQR as several of our variables, including the key variables of FPG and HbA_{1c}, were not normally distributed; categorical variables were described using percentages. Concordance between FPG and HbA_{1c} classifications was determined using Cohen's kappa statistic and was designated as negative by both biomarkers, HbA_{1c}-only diabetes, FPG-only diabetes or diabetes by both biomarkers. As several of our variables were not normally distributed, the non-parametric Mann-Whitney U test was used to compare continuous variables between groups stratified by sex, while the Kruskal-Wallis test was used to compare continuous variables between groups stratified by concordance classification. Post hoc Dunn's tests were used to compare continuous variables between concordance classification pairs if the overall test was significant. χ^2 and Fisher's exact tests were used to compare categorical variables between groups.

The association between FPG (both FPG and FPG^2 terms were included, given the quadratic relationship between fasting glucose and HbA_{1c}[18]) and HbA_{1c} was explored in age-adjusted linear regression models which were sequentially adjusted for potential confounders. Confounders were included if they were associated with HbA_{1c} on univariate regression analysis (p<0.2) or if previous research suggested a possible relationship with HbA_{1c} and were grouped as medical history (previous diagnosis of tuberculosis, HIV status and haemoglobin), anthropometrics (BMI, WC, HC and waist-to-hip ratio), markers of insulin resistance (HOMA-IR) and indices of fat distribution (VAT and SAT). WC, HC and waist-to-hip ratio proved to be multicollinear (variance inflation factor greater than 5) and WC and HC were then excluded from the model, leaving BMI and waist-to-hip ratio in the anthropometrics grouping. HIV status was categorised as positive, negative or indeterminate and HOMA-IR was categorised into two strata: (1) incalculable due to undetectable insulin or below the median of available HOMA-IR values and (2) above the median of available HOMA-IR values. Likelihood ratio testing was performed to evaluate the statistical significance of additional variables in the model.

Observations were excluded from the analysis if data were missing for FPG, HbA_{1c} or any of the study covariates. Sensitivity analyses were performed for our primary outcome of concordance between FPG and HbA_{1c} in all individuals with both FPG and HbA_{1c}, regardless of whether covariate data were missing. We also performed a sensitivity analysis to explore the effect of antiretroviral drugs on HbA_{1c} variability in which HIV status, categorised as HIV negative, HIV positive not taking antiretroviral therapy and HIV positive taking antiretroviral therapy, was included in the medical history confounder.

Non-normal continuous variables were log transformed prior to linear regression analyses to improve normality. Values of p<0.05 were considered statistically significant. Analyses were performed using STATA V.14.2 (StataCorp).

An abstract presenting a similar analysis of these data was accepted for presentation at the 2020 conference of the Endocrine Society and published in a supplement of the Journal of the Endocrine Society.[19]

RESULTS

Determination of analytic sample

The determination of the analytic sample is illustrated in online supplemental figure S1. Of the 1497 individuals whose samples were randomly selected for HbA_{1c} analysis, 100 (6.7%) reported having previously been diagnosed with diabetes and were excluded from the analytic sample. Of the remaining 1397 participants, 1121 were aged 40–70 years and of these, 954 had available data on both FPG and HbA_{1c}. One hundred and fifty four individuals were missing valid data on FPG, 12 were missing valid data on HbA_{1c} and one individual was missing both.

One hundred and eighty-nine participants were excluded due to missing data for one or more covariates. The most frequently missing covariates were visceral fat, which was missing in 12% of participants and subcutaneous fat which was missing in 9% of participants. Participants excluded due to missing covariate data did not

Table 1 Clinical and demographic characteristics of the study sample

	Overall (n=765)	Women (n=384)	Men (n=381)	P value (women vs men)
Age (years)	55 (48, 62)	55 (49, 62)	55 (48, 62)	0.77
BMI (kg/m^2)	26.2 (22.4, 31.7)	29.6 (25.4, 34.3)	23.8 (20.7, 27.4)	<0.01
Waist circumference (cm)	92 (82, 103)	98 (86, 108)	87 (79, 97)	<0.01
Hip circumference (cm)	101 (93, 110)	107 (99, 115)	96 (90, 102)	<0.01
Waist-to-hip ratio	0.91 (0.86, 0.96)	0.91 (0.85, 0.96)	0.91 (0.87, 0.97)	0.29
Family history of diabetes	115 (15.0)	66 (17.2)	49 (12.9)	0.20
Previous history of tuberculosis	70 (9.2)	30 (7.8)	40 (10.5)	0.20
HIV positive	148 (19.4)	66 (17.2)	82 (21.5)	0.29
Haemoglobin (g/L)	12.9 (11.7, 14.1)	12.3 (11.1, 13.2)	13.7 (12.5, 14.8)	<0.01
Fasting glucose (mmol/L)	4.8 (4.4, 5.4)	4.8 (4.4, 5.3)	4.9 (4.4, 5.4)	0.19
HbA_{1c} (%)	5.5 (5.1, 6.2)	5.5 (5.1, 6.3)	5.5 (5.1, 6.1)	0.26
HbA_{1c} (mmol/mol)	37 (32, 44)	37 (32, 45)	37 (32, 43)	0.26
Fasting insulin (µIU/mL)*	6.3 (3.6, 11.4)	6.3 (3.7, 11.3)	6.2 (3.5, 11.7)	0.82
HOMA-IR*	1.3 (0.8,2.6)	1.3 (0.8,2.5)	1.4 (0.8,2.7)	0.79
Visceral fat (cm)	6.3 (5.0, 7.9)	6.3 (4.7, 7.9)	6.4 (5.2, 8.0)	0.06
Subcutaneous fat (cm)	1.6 (1.0, 2.3)	2.2 (1.5, 2.8)	1.1 (0.8, 1.6)	<0.01
Diabetes (fasting glucose criteria)	32 (4.2)	17 (4.4)	15 (3.9)	0.74
Diabetes (HbA_{1c} criteria)	134 (17.5)	79 (20.6)	55 (14.4)	0.03

Data are expressed as median (IQR) or n (%).
*A total of 107 women and 172 men had insulin levels below the limit of detection (<2 µIU/mL); HOMA-IR, therefore, could not be calculated.
BMI, body mass index; HbA_{1c}, haemoglobin A1c; HOMA-IR, Homoeostatic Model Assessment of Insulin Resistance.

differ from the included participants by age (p=0.172), sex (p=0.807) or median FPG (p=0.770). Median HbA_{1c} was slightly lower in participants who were excluded (5.4% (36 mmol/mol) vs 5.5% (37 mmol/mol); p=0.026). The baseline characteristics of individuals excluded from the study are shown in online supplemental table S1.

Seven hundred and sixty-five participants were included in the final analysis.

Characteristics of analytical sample

The characteristics of the sample are shown in table 1. The median age was 55 years (IQR 48–62) and, as expected given the sampling strategy, half the sample was male. Women had greater general obesity (BMI 29.6 vs 23.8 kg/m^2; p<0.01) and regional obesity (WC 98 vs 87 cm, p<0.01; HC 107 vs 96 cm, p<0.01). Direct assessments of body fat distribution revealed higher SAT in women (2.2 vs 1.1 cm, p<0.01), but no statistically significant difference in VAT (6.3 vs 6.4 cm; p=0.06). Diabetes prevalence defined by HbA_{1c} was four times higher than by FPG (17.5% vs 4.2%), with this several-fold increase in prevalence evident in both women (20.6% vs 4.4%) and men (14.4% vs 3.9%).

Concordance between FPG classification and HbA_{1c} classification

In 84.3% of cases, glycaemic status classification by FPG and HbA_{1c} was the same with 81.3% of individuals being classified as normoglycaemic by both measures and 3% classified as having diabetes (table 2). Classification discordance was largely due to having diabetes by HbA_{1c} but normoglycaemia by FPG, with 111 individuals (14.5%) in this category. Nine (1.2%) individuals were normoglycaemic by HbA_{1c} but had diabetes by FPG. The overall Cohen's kappa statistic was 0.23. Using FPG-diagnosed diabetes as the standard, HbA_{1c} had a sensitivity of 71.9% and specificity of 84.9%.

In women, HbA_{1c} and FPG classifications were concordant in 82.8% of individuals (78.9% were normoglycaemic and 3.9% were classified as having diabetes), while 16.7% of women had diabetes defined only by HbA_{1c} and 0.5% had diabetes defined only by FPG. Concordance was similar in men, with 83.7% having normoglycaemia by both HbA_{1c} and FPG and 2.1% having diabetes by both measures; HbA_{1c}-only diabetes was present in 12.3% of men and FPG-only diabetes in 1.8%. Kappa statistics were 0.26 and 0.18 for women and men, respectively.

Concordance between HbA_{1c} and FPG classifications was similar in sensitivity analyses which included all 954 participants with valid FPG and HbA_{1c} data (online supplemental table S2).

Phenotypic comparison by concordance classification

Phenotypic differences were evident between classification groups (figure 1 and online supplemental table S3). No significant differences existed between those with HbA_{1c}-only diabetes and normoglycaemia, but there

Table 2 Agreement in diabetes classification by fasting glucose and HbA_{1c} in study participants

		Normoglycaemia n (%)	Diabetes n (%)	Total N (%)	Kappa statistic (95% CI)
	HbA_{1c} (overall)				
Fasting glucose (overall)	Normoglycaemia	622 (81.3)	111 (14.5)	733 (95.8)	
	Diabetes	9 (1.2)	23 (3.0)	32 (4.2)	
	Total	631 (82.5)	134 (17.5)	765 (100)	0.23 (0.14 to 0.31)
	HbA_{1c} (women)				
Fasting glucose (women)	Normoglycaemia	303 (78.9)	64 (16.7)	367 (95.6)	
	Diabetes	2 (0.5)	15 (3.9)	17 (4.4)	
	Total	305 (79.4)	79 (20.6)	384 (100)	0.26 (0.15 to 0.37)
	HbA_{1c} (men)				
Fasting glucose (men)	Normoglycaemia	319 (83.7)	47 (12.3)	366 (96.0)	
	Diabetes	7 (1.8)	8 (2.1)	15 (3.9)	
	Total	326 (85.6)	55 (14.4)	381 (100)	0.18 (0.05 to 0.31)

HbA_{1c}, haemoglobin A1c.

were significant differences in obesity and insulin resistance indices between those with HbA_{1c}-only diabetes and diabetes by both biomarkers. Median BMI in those with HbA_{1c}-only diabetes was 26.0 (22.7–32.8) kg/m^2 vs 26.0 (22.1–31.2) kg/m^2 (p=0.301) in those who were normoglycaemic and 31.6 (28.6–35.0) kg/m^2 (p=0.003) in those who had diabetes by both measures. Significant differences were also evident in other anthropometric measures. In those with HbA_{1c}-only diabetes, WC was 93 (83–106) cm vs 91 (81–102) cm (normoglycaemia) (p=0.204) vs 103 (98–115) cm (diabetes by both) (p=0.001), while HC was 102 (95–112) cm (HbA_{1c}-only) vs 100 (93–110) cm (normoglycaemia) (p=0.093) vs 109 (101–114) cm (diabetes by both) (p=0.033). Waist-to-hip ratio was 0.91 (0.86–0.96) cm (HbA_{1c} only) vs 0.91 (0.86–0.96) cm (normoglycaemia) (p=0.967) vs 0.97 (0.92–1.02) cm (diabetes by both) (p=0.001).

Similar patterns were also seen in other characteristics with median SAT in HbA_{1c}- only diabetes of 1.7 (1.2–2.3) cm vs 1.5 (0.9–2.3) cm (normoglycaemia) (p=0.467) vs 2.5 (1.7–3.5) cm (diabetes by both) (p=0.001) and median HOMA-IR of 1.5 (0.8–2.6) (HbA_{1c}-only) vs 1.3 (0.8–2.3) (normoglycaemia) (p=0.192) vs 3.3 (2.5–6.8) (diabetes by both) (p<0.001).

Factors explaining HbA_{1c} variance

FPG and age explained 14.8% of the variance in HbA_{1c} in women, compared with 11.4% of the variance in men (table 3). In women, significantly greater variance in HbA_{1c} was explained with the addition of either of HOMA-IR or indices of fat distribution to the model. The greatest increase was, however, seen with the inclusion of both sets of variables in the same model (19.9%, likelihood ratio test p<0.001). In men, these factors did not explain additional variance.

Medical history (including HIV status categorised as positive, negative or indeterminate, previous history of tuberculosis and haemoglobin) did not explain a significant degree of variance in HbA_{1c} over that explained by the base model. In sensitivity analyses in which HIV status was categorised as HIV negative, HIV positive and not taking antiretroviral medication and HIV positive taking antiretroviral medication, previous medical history explained 15.2% of HbA_{1c} variance in women (likelihood ratio test p=0.22) and 12% in men (likelihood ratio test p=0.184).

DISCUSSION

In this rural black South African population with a high prevalence of obesity, concordance between FPG and HbA_{1c} in the diagnosis of diabetes was poor. Individuals with diabetes defined by HbA_{1c} alone had anthropometric measures, fat distribution and measures of insulin resistance that more closely resembled those in individuals who were normoglycaemic by both biomarkers; in contrast, they were significantly different from those with diabetes by both biomarkers. Sex differences were

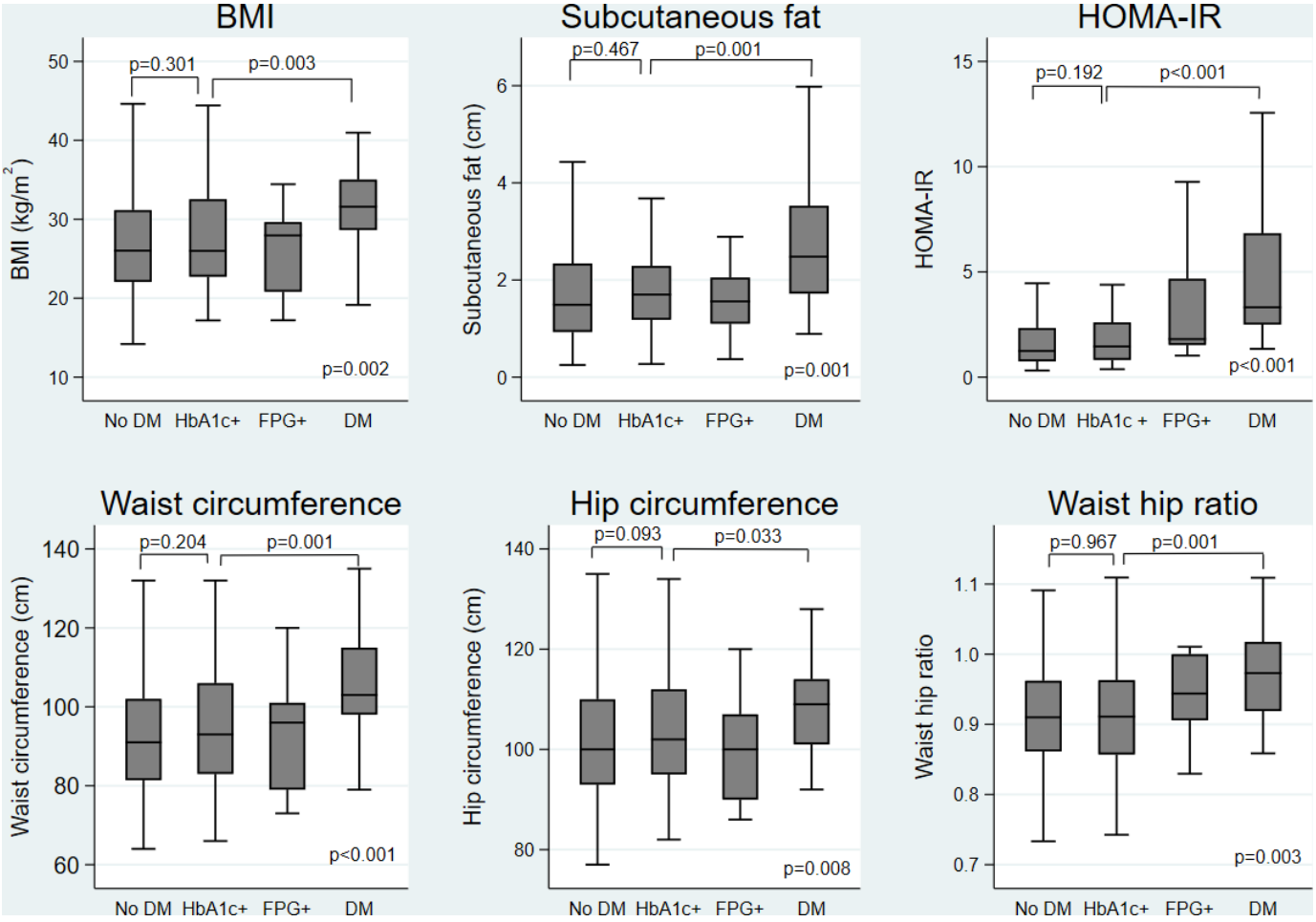

Figure 1 Comparison of selected anthropometric, insulin resistance and fat distribution indices by concordance classification. DM, diabetes mellitus by both HbA_{1c} and fasting glucose criteriaa; FPG+, diabetes by fasting glucose criteria only; HOMA-IR, Homoeostatic Model Assessment of Insulin Resistance; HbA_{1c}+, diabetes by HbA_{1c} criteria only; No DM, no DM by either biomarker. BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, haemoglobin A1c.

also evident in the degree to which insulin resistance and indices of fat distribution explained variance in HbA_{1c}.

Our study has several strengths. We rigorously collected standardised data and used internationally standard laboratory techniques in a South African environment. While studies in similar environments are frequently restricted to more easily accessible urban, clinical populations, our work was community-based and conducted in an underserved, rural population. This is particularly important as approximately 60% of sub-Saharan Africa still lives in rural areas.[20] We also collected extensive phenotypic data on our participants, and therefore, unlike previous studies, we were able to investigate associations between HbA_{1c} and adipose tissue distribution and measures of

Table 3 Analysis of the effects of sequential adjustment on HbA_{1c} variance by sex

	Women		Men	
	Adjusted R^2	P value of LR test	Adjusted R^2	P value of LR test
Base model-glucose, glucose^2, age	0.148	–	0.114	–
Model 1-base model plus history of TB, HIV status, Hb	0.154	0.142	0.116	0.281
Model 2-base model plus anthropometrics (BMI, WHR)	0.148	0.327	0.113	0.437
Model 3-base model plus visceral fat, subcutaneous fat	0.163	0.011	0.120	0.083
Model 4-base model plus HOMA-IR category	0.187	<0.001	0.112	0.496
Model 5-base model plus visceral fat, subcutaneous fat, HOMA-IR category	0.198	<0.001	0.121	0.094

BMI, body mass index; Hb, haemoglobin; HbA_{1c}, haemoglobin A1c; HOMA-IR, Homoeostatic Model Assessment of Insulin Resistance ; LR, likelihood ratio; TB, tuberculosis; WHR, waist-to-hip ratio.

insulin resistance. Our study does have limitations which merit discussion. We excluded individuals who reported a previous diagnosis of diabetes, but given limited health literacy, individuals may have been unaware of diabetes diagnoses and/or treatment and may, therefore, have been inadvertently included in our analysis. Diabetes medications, however, would affect both FPG and HbA_{1c} though possibly to varying degrees. We did not perform 2-hour oral glucose tests and so could not evaluate the contribution of postprandial glucose to HbA_{1c} variability.

To our knowledge, only two other population-based studies have specifically investigated the relationship between laboratory-based FPG and HbA_{1c} in diagnosing diabetes in black sub-Saharan African individuals, although concordance was not specifically determined in these studies. While there are similarities in participant ethnicity between our work and these studies, there are key differences that distinguish our research. Hird *et al*[21] found that the age-standardised prevalence of diabetes in 1190 urban Black South Africans was similar using FPG and HbA_{1c} (11.9% vs 13.1%), with HbA_{1c} having a sensitivity of 74.1% and specificity of 98.1% in detecting FPG-defined diabetes. However, participants in that study were younger than in ours, with a median age of 39.7 years. Data from a study conducted in 3645 urban and rural Malawians (median age 33 years) revealed an HbA_{1c}-based prevalence of 7.3% compared with an FPG-based prevalence of 1.7%. HbA_{1c} had a sensitivity of 78.7% and specificity of 94.0% to detect FPG-diagnosed diabetes.[22] The high HbA_{1c} specificity in both of these studies relative to our study may be partly attributable to the age-dependent relationship between HbA_{1c} and FPG, with HbA_{1c} increasing in older people independent of glycaemia.[23] A second important difference is the lower BMI (median 22.6 kg/m^2) in the Malawian study, given higher BMI is also associated with higher HbA_{1c} independent of glycaemia.[24] Consequently, while previous studies in black sub-Saharan African populations have suggested comparable performance characteristics between venous HbA_{1c} and FPG in the diagnosis of diabetes, our study suggests that this may not be the case in a key demographic at high risk of developing diabetes, namely older adults with higher BMIs. Performance characteristics of HbA_{1c} and FPG may, however, be different in individuals who are not overweight or obese.

While data on concordance in black sub-Saharan African populations are limited, evidence from other black populations, primarily of Western African descent, does suggest that existing HbA_{1c} and FPG criteria may have limited agreement. In 939 individuals in Barbados, while there was no difference in diabetes prevalence using HbA_{1c} or FPG (4.9% vs 3.5%), concordance was limited with a kappa statistic of 0.39.[25] Agreement was higher than in our study, with the glycaemic status classification by FPG and HbA_{1c} being the same in 93.8% of cases, with a further 3.8% having diabetes by HbA_{1c} and normoglycaemia by FPG, and 2.3% having normoglycaemia by HbA_{1c} and diabetes by FPG. Adults≥25 years were included in this study, with 42% of the sample ≤45 years. Another study suggests that existing HbA_{1c} criteria may more frequently classify African-Americans as having diabetes. In a US population aged 70–79 years, the prevalence of HbA_{1c}-diagnosed diabetes in African-Americans was 5.7% compared with a prevalence of 3.5% using FPG criteria, in contrast with a prevalence in the entire sample, including Whites, of 3.1% (HbA_{1c}) vs 2.7% (FPG).[26]

Our finding that those with HbA_{1c}-only diabetes were more comparable to those who were normoglycaemic by both biomarkers than to those who had diabetes by both biomarkers suggests that the HbA_{1c} elevation is not merely indicative of worsened glucose tolerance and individuals further along the dysglycaemia continuum. Indeed, indices of insulin resistance and fat distribution which may indirectly reflect glucose tolerance explained significantly more variance in HbA_{1c} only in our female participants and this was still limited to 20% of the overall variance. Further, the limited degree of HbA_{1c} variance explained by FPG supports existing evidence that non-glycaemic factors are important contributors to HbA_{1c} in this population. Similar findings have been reported in other population groups, with data in Finnish men without diabetes suggesting that indices of insulin sensitivity explained little additional HbA_{1c} variance over the 12% explained by age, FPG and C reactive protein.[27] Glycaemic factors, defined as preprandial glucose, postprandial glucose and glycaemic variability calculated from continuous glucose monitoring, along with age, sex, BMI and ethnicity explained 35% of HbA_{1c} variance in adults without diabetes, of which half was explained by the non-glycaemic variables.[28] The importance of non-glycaemic variables in the determination of HbA_{1c} is further supported by the association of non-glycaemic loci with HbA_{1c},[29–31] but these associations require further investigation in individuals across different sub-Saharan African regions, given the extensive genetic variation on the continent.

Our study shows a high degree of discordance between venous HbA_{1c} and FPG in, to our knowledge, one of the first such studies in a black population in rural South Africa. Furthermore, our phenotypic data suggest that the current HbA_{1c} threshold overdiagnoses diabetes in this population. Our findings highlight that elevated HbA_{1c} may reflect factors other than hyperglycaemia and further research, including genetic studies, is necessary to understand other determinants of HbA_{1c} in this population. Given the anticipated increase in the prevalence of diabetes in this region, additional longitudinal work is essential to determine which of these biomarkers better predicts diabetes-related morbidities and whether population-specific HbA_{1c} thresholds are necessary when diagnosing diabetes in this population. In the interim, clinicians in these environments should be cautious in diagnosing diabetes based solely on an HbA_{1c}≥6.5% (48 mmol/mol).

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REFERENCES

1 Saeedi P, Petersohn I, Salpea P, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019;157:107843.
2 World Health Organisation. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus Geneva. 2011. Report No.: WHO/NMH/CHP/CPM/11.1.
3 Menke A, Rust KF, Savage PJ, *et al*. Hemoglobin A1c, fasting plasma glucose, and 2-hour plasma glucose distributions in U.S. population subgroups: NHANES 2005–2010. *Ann Epidemiol* 2014;24:83–9.
4 Davidson MB, Schriger DL. Effect of age and race/ethnicity on HbA_{1c} levels in people without known diabetes mellitus: implications for the diagnosis of diabetes. *Diabetes Res Clin Pract* 2010;87:415–21.
5 Ziemer DC, Kolm P, Weintraub WS, *et al*. Glucose-Independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. *Ann Intern Med* 2010;152:770–7.
6 Kassebaum NJ, Jasrasaria R, Naghavi M, *et al*. A systematic analysis of global anemia burden from 1990 to 2010. *Blood* 2014;123:615–24.
7 Krause A, Wainstein T, Essop FB, *et al*. Testing for haemoglobinopathies in Johannesburg, South Africa: a 30-year review. *S Afr Med J* 2013;103:989–93.
8 NCD Risk Factor Collaboration (NCD-RisC). Effects of diabetes definition on global surveillance of diabetes prevalence and diagnosis: a pooled analysis of 96 population-based studies with 331,288 participants. *Lancet Diabetes Endocrinol* 2015;3:624–37.
9 Zemlin AE, Matsha TE, Hassan MS, *et al*. HbA1c of 6.5% to diagnose diabetes mellitus -- does it work for us? -- the Bellville South Africa study. *PLoS One* 2011;6:e22558.
10 Gaziano TA, Abrahams-Gessel S, Gomez-Olive FX, *et al*. Cardiometabolic risk in a population of older adults with multiple co-morbidities in rural South Africa: the HAALSI (health and aging in Africa: longitudinal studies of indepth communities) study. *BMC Public Health* 2017;17:206.
11 Gómez-Olivé FX, Montana L, Wagner RG, *et al*. Cohort profile: health and ageing in Africa: a longitudinal study of an indepth community in South Africa (HAALSI). *Int J Epidemiol* 2018;47:689–90.
12 Ramsay M, Crowther N, Tambo E, *et al*. H3Africa AWI-Gen collaborative centre: a resource to study the interplay between

genomic and environmental risk factors for cardiometabolic diseases in four sub-Saharan African countries. *Glob Health Epidemiol Genom* 2016;1:e20.
13 Kahn K, Collinson MA, Gómez-Olivé FX, *et al*. Profile: Agincourt health and socio-demographic surveillance system. *Int J Epidemiol* 2012;41:988–1001.
14 Ali SA, Soo C, Agongo G, *et al*. Genomic and environmental risk factors for cardiometabolic diseases in Africa: methods used for phase 1 of the AWI-Gen population cross-sectional study. *Glob Health Action* 2018;11:1507133.
15 World Health Organisation. Definition and diagnosis of diabetes mellitus and intemediate hyperglycaemia: report of a WHO/IDF consultation; 2006.
16 American Diabetes Association. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020;43:S14–31.
17 Matthews DR, Hosker JP, Rudenski AS, *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.
18 Bazo-Alvarez JC, Quispe R, Pillay TD, *et al*. Glycated haemoglobin (HbA_{1c}) and fasting plasma glucose relationships in sea-level and high-altitude settings. *Diabet Med* 2017;34:804–12.
19 Wade AN, Crowther N, Gomez-Olive FX, *et al*. SUN-616 poor diagnostic concordance between fasting plasma glucose and glycosylated hemoglobin in a black South African population. *J Endocr Soc* 2020;4:A637–8.
20 The World bank. Rural population (% of total population). Available: https://data.worldbank.org/indicator/SP.RUR.TOTL.ZS?locations=ZG [Accessed May 2020].
21 Hird TR, Pirie FJ, Esterhuizen TM, *et al*. Burden of diabetes and first evidence for the utility of HbA1c for diagnosis and detection of diabetes in urban black South Africans: the Durban diabetes study. *PLoS One* 2016;11:e0161966.
22 Rathod SD, Crampin AC, Musicha C, *et al*. Glycated haemoglobin A_{1c} (HbA_{1c}) for detection of diabetes mellitus and impaired fasting glucose in Malawi: a diagnostic accuracy study. *BMJ Open* 2018;8:e020972.
23 Pani LN, Korenda L, Meigs JB, *et al*. Effect of aging on A_{1c} levels in individuals without diabetes: evidence from the Framingham offspring study and the National health and nutrition examination survey 2001-2004. *Diabetes Care* 2008;31:1991–6.
24 Selvin E, Zhu H, Brancati FL. Elevated A_{1c} in adults without a history of diabetes in the U.S. *Diabetes Care* 2009;32:828–33.
25 Unwin N, Howitt C, Rose AM, *et al*. Prevalence and phenotype of diabetes and prediabetes using fasting glucose vs HbA_{1c} in a Caribbean population. *J Glob Health* 2017;7:020407.
26 Lipska KJ, De Rekeneire N, Van Ness PH, *et al*. Identifying dysglycemic states in older adults: implications of the emerging use of hemoglobin A1c. *J Clin Endocrinol Metab* 2010;95:5289–95.
27 Fizelova M, Stancakova A, Lorenzo C, *et al*. Glycated hemoglobin levels are mostly dependent on nonglycemic parameters in 9398 Finnish men without diabetes. *J Clin Endocrinol Metab* 2015;100:1989–96.
28 Færch K, Alssema M, Mela DJ, *et al*. Relative contributions of preprandial and postprandial glucose exposures, glycemic variability, and non-glycemic factors to HbA_{1c} in individuals with and without diabetes. *Nutr Diabetes* 2018;8:38.
29 Hachiya T, Komaki S, Hasegawa Y, *et al*. Genome-wide meta-analysis in Japanese populations identifies novel variants at the TMC6-TMC8 and SIX3-SIX2 loci associated with HbA_{1c}. *Sci Rep* 2017;7:16147.
30 Chen P, Takeuchi F, Lee J-Y, *et al*. Multiple nonglycemic genomic loci are newly associated with blood level of glycated hemoglobin in East Asians. *Diabetes* 2014;63:2551–62.
31 Soranzo N, Sanna S, Wheeler E, *et al*. Common variants at 10 genomic loci influence hemoglobin A_{1c} levels via glycemic and nonglycemic pathways. *Diabetes* 2010;59:3229–39.