Combining HbA_{1c} and glycated albumin improves detection of dysglycaemia in mixed-ancestry South Africans

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Summary

Background Combining HbA_{1c} with glycated albumin (GA) may improve detection of dysglycaemia. As BMI correlates positively with HbA_{1c} and negatively with GA, HbA_{1c} may be more effective in obese and GA in nonobese individuals.

Methods To relate these findings to Africans, we assessed in 1274 South Africans living in CapeTown (male 26%; age 48±16y; BMI 28.7 kg/m^2 (range 15.6−73.8); obesity 39.9% and no prior diabetes history) the: (1) correlation of BMI with HbA_{1c} and GA, (2) ability of HbA_{1c} and GA separately and jointly, to detect OGTT-diagnosed dysglycaemia (diabetes plus prediabetes). Data collection took place between 2014 and 2016 in the City of Cape Town. Dysglycaemia was diagnosed by glucose criteria for the OGTT. Youden index was used to optimize diagnostic thresholds for HbA_{1c} and GA.

Findings Normal glucose tolerance, prediabetes and diabetes occurred in 76%, 17% and 7%, respectively. BMI positively correlated with HbA_{1c} [r = 0·34 [95%CI: 0·29,0·39)] and negatively with GA [-0·08 (0·13,0·03)]. For HbA_{1c} the optimal threshold by Youden-index for dysglycaemia diagnosis was: 6·0% (95%CI: 5·8,6·2) and for GA: 13·44% (12·72,14·71). In the nonobese, obese and total cohort, HbA_{1c}-alone detected: 51% (42−60), 72% (65,78), 63% (57,68), respectively; GA-alone detected 55% (52% (46,63), 52% (44, 59) and 53% (47,53), respectively; whereas: HbA_{1c}+GA detected: 69% (60,76), 82% (75,87) and 76% (71, 81). Therefore, for the total cohort detection of dysglycaemia HbA_{1c}-alone vs HbA_{1c}+GA detected 63% (57,68) vs 76% (71,81).

Interpretation The opposite correlations of HbA_{1c} and GA with BMI have now been demonstrated in an African-based population. Improving detection of dysglycaemia by combining HbA_{1c} and GA has important implications for diabetes risk screening.

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Keywords: Dysglycaemia; HbA_{1c}; Glycated albumin; Screening; Africa

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Research in context

Evidence before this study

We searched PubMed-Medline on 24 January 2022, without date and language restrictions using a combination of keywords relating to HbA_{1c}, glycated albumin (GA), adiposity, screening/diagnosis, and diabetes mellitus/dysglycaemia. We found few studies reporting on the comparative association HbA_{1c} and GA with body mass index (BMI), their comparative performance to diagnose diabetes/dysglycaemia or to predict diabetes control and related complications, with or without accounting for the effect of BMI. These studies were mostly from Asia and northern America, with none originating from Africa.

Added value of this study

We demonstrated for the first time in an African population the existence of both the positive correlation between BMI and HbA_{1c} and the negative correlation between BMI and GA. This observation contributes to why we and others, observed that HbA_{1c} performs better as a diagnostic test in the obese and GA in the non-obese. Hence, combining these two non-fasting markers of glycemia improves detection of dysglycaemia across BMI categories.

Implications of all evidence available

As Africa is experiencing the most rapid rise in the world of diabetes and the highest proportion of people living with diabetes who are undiagnosed, this study suggests an approach that can be operationalized and incorporated into existing screening programs for diabetes in the African setting, namely combining HbA_{1c} with GA. Reliable screening approaches are urgently needed particularly for the large segment of non-obese young adult African who are currently less prioritized for diabetes risk screening where implemented.

Introduction

The worldwide increase in diabetes disproportionately affects sub-Saharan Africa (SSA) where the International Diabetes Federation (IDF) has predicted that the number of adults aged 19–79 years with diabetes will increase by 134% from 24 million in 2021 to 55 million by 2045.[1] These growing numbers will be fueled by the progression over time of the many adults with non-diabetic range dysglycaemia (prediabetes) to diabetes. Diabetes in Africa is increasingly common in the segments of the population previously assumed to be at low risk of the condition. These include young adults and non-obese Africans,[2–5] in whom diabetes occurrence is likely driven by beta-cell failure.[6,7] These segments of the populations are not prioritized for diabetes screening where implemented, and accordingly, are likely driving at least in part the burden of undiagnosed diabetes in Africa.[8] Indeed, the proportion of undiagnosed diabetes is highest in Africa where about 54% of adults living with diabetes are unaware of their condition.[1] Therefore, strategies to contain diabetes in SSA should be two-pronged and include actions to prevent the development of diabetes in those without the disease, as well as efforts to bring to the medical attention people with diabetes to allow the implementation of interventions to mitigate the risks associated with the condition. For both undertakings, appropriate diabetes risk screening is the reasonable entry point,[9] including in non-obese and young adults who receive little attention in existing screening programs.

The ultimate aim of diabetes risk screening is to uncover both components of dysglycaemia, specifically prediabetes and diabetes.[9] For this purpose, the combination of a fasting glucose and 2 h blood glucose measurements during an oral glucose tolerance test (OGTT) remains the reference standard.[9] Alternative glucose-based tests for dysglycaemia include fasting glucose alone and random blood glucose. The challenges of performing an OGTT in routine settings and issues relating to the preanalytical stability and day-to-day variability of glucose-based tests, have fueled efforts to develop non-glucose-based tests to diagnose dysglycaemia.[10,11] In this regard, glycated haemoglobin (HbA_{1c}) which is the reference standard test to monitor diabetes control, has been promoted in the last decade as diagnostic test for dysglycaemia.[12] However, the relationship of HbA_{1c} with blood glucose is affected by many factors, some of which are race/ethnic or setting specific, resulting in variable performance of HbA_{1c} to diagnose dysglycaemia in diverse populations and settings.[13,14] Furthermore, there is increasing awareness that HbA_{1c} is less effective for screening in non-obese individuals,[15–17] which could make HbA_{1c} a sub-optimal test for dysglycaemia screening in a large proportion of the African population. Glycated albumin (GA) is a ketoamine formed through binding of albumin and glucose by a non-enzymatic glycation reaction. GA reflects short-term average glucose levels (14–21 days) in view of the short half-life of serum albumin, and therefore represents a potential alternative biomarker to monitor glycemic control, particularly in the presence of conditions that make HbA_{1c} measurement unreliable.[11,18] The diagnostic utility of GA for dysglycaemia is also increasingly investigated,[19] with data from Asia suggesting that GA is more effective in non-obese individuals.[17,20] Other suggested diagnostic markers of dysglycaemia include fructosamine.[11]

The few available studies in African populations both within Africa and in the global north have provided mixed results on the performance of HbA_{1c}, GA, and fructosamine to diagnose dysglycaemia, with suggestions that none of these tests taken separately matches fasting glucose alone, and that their combination in

parallel does not necessarily enhance their diagnostic performance.[10,15,21] According to the available evidence, GA is more effective than fructosamine for dysglycaemia diagnosis across a broad range of clinical settings,[22] due to a better reproducibility of GA. Emerging data suggest that accounting for the diverging associations of adiposity with HbA_{1c} and GA could uncover segments of the populations in which, combining the two biomarkers will enhance diabetes risk screening. This has been demonstrated in African-born Blacks in the US where, adding GA to HbA_{1c} resulted in improved detection of dysglycaemia in non-obese participants.[21] However, both the relationship of adiposity with non-glucose-based biomarkers of dysglycaemia, and how this relationship affects the diagnostic performance of those biomarkers for dysglycaemia, have not been fully investigated in the African setting. Clarifying these issues have relevance, considering the urgent need for tests with optimal diagnostic accuracy for dysglycaemia in non-obese Africans.

Therefore, we assessed the correlation of HbA_{1c} and GA with BMI and determined the performance of HbA_{1c} and GA separately and jointly, to detect OGTT-diagnosed dysglycaemia in a large sample of mixed ancestry South Africans in Cape Town.

Methods

Study design, and population

This study uses data from the Cape Town Vascular and Metabolic Health (VMH) cohort, which is an extension of the Cape Town Bellville South study. Both are described in detail elsewhere.[10,23] The cross-sectional data used were collected between 2014 and 2016, through a population-based survey in the Township of Bellville South in Cape Town. The population is predominantly of mixed-ancestry or coloured (76%) followed by Black Africans (18.5%) and Caucasian and Asians comprising only 1.5% of the total. The study was approved by the Research Ethics Committees of the Cape Peninsula University of Technology (CPUT) and Stellenbosch University (respectively, NHREC: REC - 230 408 – 014 and N14/01/003), and conducted in compliance with the code of ethics of the World Medical Association (Declaration of Helsinki). Included participants voluntarily signed a written consent and permission to conduct the study was also obtained from relevant authorities including the city and community authorities.

Interviews and physical examination

Interviews and physical examinations were conducted by trained fieldworkers at a research clinic located within the study suburb. Fieldworkers went door-to-door in the community to distribute fliers to raise awareness of the study and invite potentially eligible participants to take part in the study. Those who volunteered for the study where then scheduled for an appointment at our research clinic for further procedures. A day before the scheduled appointment, fieldworkers contacted participants to remind them to fast overnight and confirm the pick-up location. At the clinic, data were collected on demographics, medical histories, ongoing treatments, and habits including smoking using a questionnaire on a password-protected personal digital assistant (PDA). Physical examination involved data collection on blood pressure (BP) using a semi-automatic device (Omron M6 comfort-preformed cuff BP Monitor) and following the World Health Organisation (WHO) guidelines.[24] BP was measured on the right arm in sitting position and at rest for at least 10 min. The lowest systolic BP (SBP) of three consecutive measures and the corresponding diastolic BP (DBP) were used in all analyses. Body weight (to the nearest 0.1 kg) was measured with the subject in light clothing and without shoes, using an Omron body fat meter HBF-511 digital bathroom scale. Height to the nearest centimeter was measured with a stadiometer, with subjects standing on a flat surface. Body mass index (BMI) was calculated as weight per square meter (kg/m^2). Waist circumference was measured with a non-elastic tape at the level of the narrowest part of the torso, as seen from the anterior view. Anthropometric measurements were performed three times and their average used for analysis. Blood samples were collected from all participants after an overnight fast, and two hours after a 75 g OGTT following the WHO recommendations.[25]

Biochemical analysis

GA was determined with the quantLab® Glycated Albumin assay (Werfen™, Italy, Ref 0,018,256,640) on a Roche Cobas 6000 analyser (Roche Diagnostics, Manheim, Germany). In this assay, the concentration of GA is determined with an enzymatic method and the concentration of albumin is determined separately with the Bromocresol purple method. GA is expressed as a percentage of total albumin and the equation includes an inter-method arithmetic factor for comparability between this method and results obtained by high performance liquid chromatography (HPLC).[26] This method was validated for use on the Cobas® 6000 analyzer (Roche Diagnostics®) according to the CLSI EP15-A3 protocol.[27] The within-assay CV was 2.2% and within-laboratory CV was 2.3% (bias 0.88%) for the low concentration control sample (target mean 15.7%) and a within-assay CV of 1.3% and a within-laboratory CV of 1.4% (bias 0.36%) for the high concentration control sample (target mean 37.4%). The total error observed for high and low concentration control samples were 4.72% and 2.62% respectively.

Other biochemical parameters were analysed at an ISO 15,189-accredited Pathology practice (PathCare, Reference Laboratory, Cape Town, South Africa). Plasma glucose and HbA_{1c} were measured, respectively, by enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa) and NGSP-certified HPLC (Biorad Variant Turbo, BioRad, South Africa). Insulin was determined by a paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). High-density lipoprotein cholesterol (HDL-C) was by enzymatic immunoinhibition, triglycerides by glycerol phosphate oxidase-peroxidase and low-density lipoprotein cholesterol (LDL-C) by enzymatic selective protection – End Point (Beckman AU, Beckman Coulter, South Africa). Total protein and albumin were determined by immunoturbidimetry on an ABX Pentra 400 (Horiba Medical, USA).

Classification of glucose tolerance status, insulin resistance and adiposity

OGTT glucose values were used as recommended by WHO[28] to classify the glucose tolerance status of participants as: 1) normal glucose tolerance (FPG<6.1 mmol/l & 2 h glucose <7.8 mmol/l); 2) prediabetes including impaired fasting glycaemia (IGT, i.e. 6.1≤FPG<7.0 mmol/l), impaired glucose tolerance (IGT, i.e. 7.8<2 h glucose<11.1 mmol/l) and the combination of both; and 3) screen-detected diabetes (FPG≥7.0 mmol/l and/or 2 h glucose≥11.1 mmol/l). Participants were classified according to their BMI as normal weight (BMI<25 kg/m^2), overweight (25 kg/m^2≤BMI<30 kg/m^2) and obese (BMI≥30 kg/m^2). Finally, waist circumference (WC) ≥90 cm was used to define abdominal obesity (high WC) in both men and women, in line with previous report from this population.[29]

The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: HOMA-IR= [fasting insulin concentration (mIU/L) x fasting plasma glucose (mmol/ L]/ 22.5.[30]

Statistical analysis

The R statistical software (The R Foundation for Statistical Computing Platform) version 4.0.3 (2020–10–10) was used for all data analysis. Results are reported as count (and percentages), mean (and standard deviation) and median (25th-75th percentiles). Baseline characteristics were compared across glucose tolerance subgroups using chi square test, analysis of the variance and Kruskal-Wallis test. The covariance estimation of multivariate *t* distribution was used to calculate the correlation between pairs on continuous measures. This provides some degree of robustness to outliers without giving a high breakdown point. The significance of the difference between two dependent correlation coefficients sharing one variable was assessed using Williams' test.[31] The *'robcor'* package was used to assess the effect of controlling for age on partial correlations of BMI with HbA_{1c} and GA.[32] The area under the receiver operating characteristic curve (AUC) was used to assess and compare the ability of continuous markers of glucose homeostasis to predict the presence of OGTT-diagnosed abnormal glucose tolerance.[33] The *AROC.sp* command of the '*ROCnReg*' package was used to compute the semi-parametric covariate-adjusted ROC curve.[34,35] In order to assess the diagnostic utility of the markers on the same footing, the J-point of Youden was used to derive the optimal cut-off points for the diagnosis of different OGTT-based categories of abnormal glucose tolerance. The performance of markers to diagnosed OGTT-defined abnormal glucose tolerance at these cut-offs was then assessed by computing the following performance measures and accompanying 95%CI: sensitivity, specificity, Youden's Index, positive predictive value (PPV), negative predictive value (NPV), accuracy, diagnostic odd ratio (DOR), number needed to diagnose (NND), likelihood ratio of the positive test (LR+) and likelihood ratio of a negative test (LR-). In secondary analyses, we tested the performance of markers at recommended/published thresholds for dysglycaemia which were 5.7% for HbA_{1c} and 14% for GA.[19] We also tested the performance of the data-specific 75th percentile (Q3) as diagnostic threshold for HbA_{1c} (6.0%) and GA (13.85%). The diagnostic performance of the combination of markers at their data-specific optimal thresholds (Boolean combinations) was also assessed under the scenario of parallel testing and the assumption of a positive result from any of the tests being equivalent to a positive screening for abnormal glucose tolerance. The predictive effect of the linear combination of HbA_{1c} and GA was further assessed in logistic regressions, with comparison of three models: covariates only (age, sex and BMI), HbA_{1c} and GA alone, and the combination of HbA_{1c}, GA and covariates. AUC comparison was based on non-parametric methods.[33] P-values <0.05 were used to characterise statistically significant results. This study is reported according to the Standards for Reporting of Diagnostic Accuracy Studies (STARD).[36]

Role of the funding source: The funder had no involvement in the study. APK and TEM had full access to the data and APK took the decision to submit for publication.

Results

General characteristics of the sample

The starting sample included 1518 participants, of which four were removed for missing data on GA. Another 22 participants lack data on HbA_{1c} whereas three had missing data on fasting glucose. Of the remaining 1489 participants, 193 were previously diagnosed with diabetes, whereas two had missing OGTT values. Therefore, the

final analytic sample included 1274 (74.2% female) participants of whom 492 (38.6%) were normal weight, 273 (21.4%) were overweight and 509 (39.9%) were obese (Supplemental Fig. 1). Furthermore, 616 (48.3%) had abdominal obesity (waist circumference≥90 cm). The BMI ranged from 15.6 to 73.8 kg/m^2 (Figure 1, Panel A). The mean age was 47.8 years and expected differences in the distribution of cardiometabolic risk profile across BMI categories were observed (Table 1). Mean HDL-cholesterol decreased whereas mean age, blood pressure, waist and hip circumference, and other lipid variables increased across increasing BMI categories (all $p<0.001$).

Mean fasting-, 2 h glucose and HbA_{1c} consistently increased across increasing BMI categories (all $p<0.001$), whereas mean GA was mostly similar ($p = 0.351$). The distribution of glucose tolerance status in the overall sample was 75.6% for normo-tolerance, 17.3% for prediabetes and 7.1% for newly diagnosed diabetes. Across increasing BMI categories, the proportion of normo-tolerant decreased from 85.8% among normal weight to 64.4% among obese, whereas the proportion of prediabetes increased from 10.8% to 24.6% and that for diabetes from 3.4% to 11.0%; $p<0.001$ for the difference in the distribution (Table 1). By waist circumference categories, the distribution of glucose tolerance status was 86.0% (normo-tolerant), 10.9% (prediabetes) and 3.0% (diabetes) among participants with WC<90 cm; and 64.4%, 24.2% and 11.4% among those with WC≥90 cm ($p<0.001$ for the difference in the distribution across WC categories). In cross-classification 95.1% (468/492) of the normal weight participants had normal WC, 52.7% (152/273) overweight participants had normal WC, while 92.5% (471/509)) of the obese participants had abdominal obesity.

Correlations between indices of glucose homeostasis

In the total sample, the correlation coefficients (95% confidence intervals) ranged from 0.54 (0.50 to 0.58) for fasting vs. 2 h glucose to 0.27 (0.22 to 0.32) for fasting glucose vs. GA. For each marker (except GA), the lowest correlation coefficient was always recorded with GA. Furthermore, the correlation of HbA_{1c} with GA was stronger [0.36 (0.31 to 0.40)] than that of fasting glucose [0.27 (0.22 to 0.32)] or 2 h glucose [0.28 (0.23 to 0.33)] with GA (Supplemental Table 2). In analyses stratified by BMI categories the pattern of differences in correlation coefficients between pairs of markers was broadly similar. However, for each given pair of markers, correlation coefficients systematically strengthened from normal weight to obese. For instance, the correlation of HbA_{1c} vs GA was 0.29 (0.21 to 0.37) in normal weight, 0.43 (0.33 to 0.52) in overweight and 0.47 (0.40 to 0.53) in the obese (Supplemental Table 1). The correlation coefficients in analyses stratified by WC categories are also shown in Supplemental Table 2, with effect sizes and patterns across biomarkers among participants with abdominal obesity (WC≥90 cm) mostly similar to those observed in those with general obesity, while effect sizes and patterns in participants without abdominal obesity (WC<90 cm) were in line with those seen in the normal weight group or in the combined normal weight and overweight group.

In the overall sample, the correlation of BMI with non-glucose-based markers was: BMI vs HbA_{1c} 0.34 (0.29 to 0.39) and BMI vs. GA: −0.080 (−0.13 to −0.03). Equivalent results for waist circumference were 0.37 (0.32 to 0.42) and −0.045 (−0.10 to 0.01). The patterns of these correlations were broadly similar across BMI categories, although estimates were not always significant. By glucose tolerance status, correlations of BMI with HbA_{1c} and GA were always stronger in non-diabetic individuals than in newly diagnosed diabetes: 0.33 (0.28 to 0.38) and 0.22 (0.01 to 0.41) for BMI vs. HbA_{1c}, with accompanying figures being −0.11 (−0.17 to 0.06) and 0.0004 (−0.20 to 0.22) for BMI vs. GA (Figure 1, Panels B & C). Controlling for age had no effect on the correlation of BMI with HbA_{1c} and GA.

Discrimination of dysglycaemia

The C-statistic (95% confidence interval) for the prediction of OGTT-diagnosed diabetes by markers of glucose homeostasis in the overall sample was: fasting glucose 0.940 (0.910−0.967), 2 h glucose 0.960 (0.929 −0.990), HbA_{1c} 0.899 (0.855−0.944) and GA 0.842 (0.787−0.896); with non-significant difference between fasting and 2 h glucose ($p = 0.381$), fasting glucose and HbA_{1c} ($p = 0.081$), and HbA_{1c} and GA ($p = 0.050$). Withing BMI categories, GA performed less well than HbA_{1c} in normal weight and overweight participants (both $p<0.033$) whereas both markers had similar performance among obese participants ($p = 0.402$); Table 2.

For the prediction of dysglycaemia (the combination of diabetes and prediabetes), 2 h glucose always outperformed all other markers as expected, whereas fasting glucose performed equally with HbA_{1c} and GA in overweight, but outperformed them in normal-weight and obese; and HbA_{1c} did better than GA only in the overall sample and among obese participants (Table 2). The continuous predictive ability of HbA_{1c} and GA for dysglycaemia is shown in Figure 2. There was no indication of perfect diagnostic cut-off.

The prediction of diabetes and dysglycaemia within WC categories is also summarized in Table 2, with again estimates of c-statistics and patterns of differences across markers in participants with abdominal obesity and those without, mirroring respectively those observed in participants with general obesity, and in normal weight participant or the combined normal weight and overweight group. Due to these consistent similarities, no further analyses by WC categories were conducted.

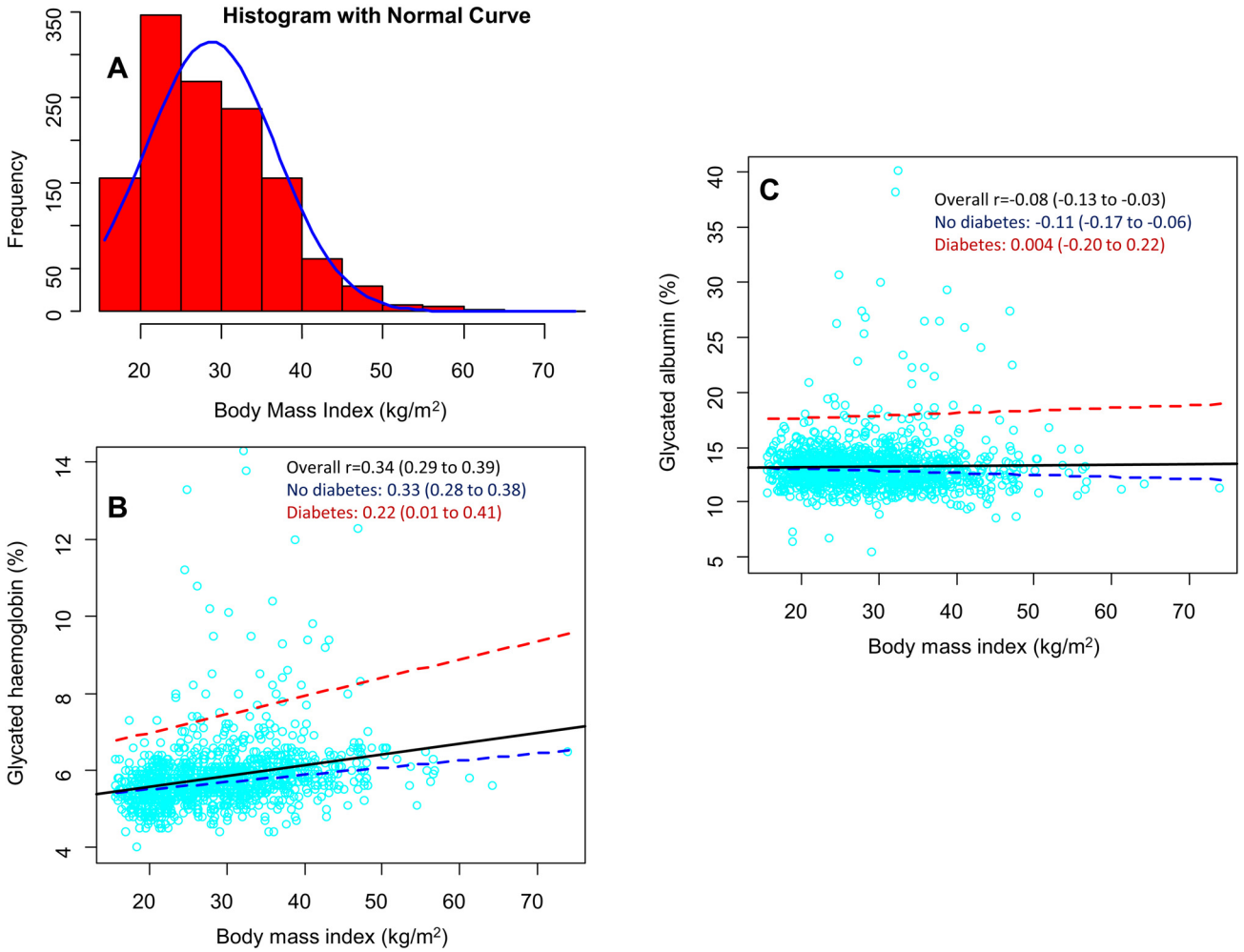

Figure 1. Histogram and superimposed normal curve for the distribution of body mass index (BMI) in the overall sample (Panel A), and Correlation of BMI with HbA_{1c} (Panel B) and glycated albumin (Panel C) in the overall sample and by diabetes status.

For the correlation plots (B and C), the correlation coefficients and accompanying 95% confidence intervals (95%CI) are shown overall and separately for participants with diabetes and those without. All correlations are statistically significant except for BMI vs. glycated albumin in people with diabetes, where the 95%CI include the absolute zero.

Variable[1]	Overall	Normal weight	Overweight	Obese	p-value[2]
N (%)	1274 (100)	492 (38.6)	273 (21.4)	509 (39.9)	
Female, n (%)	946 (74.2)	281 (57.1)	203 (74.4)	462 (90.8)	<0.001
Age, years	47.8 (15.7)	44.1 (16.1)	48.0 (15.1)	51.3 (14.3)	<0.001
BMI, kg/m^2	28.7 (8.0)	21.1 (2.3)	27.4 (1.5)	36.7 (5.9)	<0.001
Waist circumference, cm	90 (17)	75 (9)	89 (8)	105 (12)	<0.001
Hip circumference, cm	103 (16)	89 (9)	101 (7)	117 (13)	<0.001
SBP, mmHg	127 (24)	123 (26)	127 (23)	131 (23)	<0.001
DBP, mmHg	81 (14)	78 (16)	80 (13)	84 (13)	<0.001
Total cholesterol, mmol/L	5.1 (1.2)	4.8 (1.1)	5.4 (1.2)	5.3 (1.1)	<0.001
Measured LDL, mmol/L	3.2 (1.0)	2.8 (0.9)	3.4 (1.1)	3.4 (1.0)	<0.001
HDL Cholesterol, mmol/L	1.34 (0.38)	1.45 (0.45)	1.31 (0.32)	1.25 (0.30)	<0.001
Median Triglycerides, mmol/L (Q1-Q3)	1.16 (0.84−1.65)	0.94 (0.71−1.33)	1.24 (0.91−1.80)	1.35 (1.01−1.88)	<0.001
HbA_{1c},% (SD)	5.8 (0.8)	5.6 (0.6)	5.8 (0.7)	6.0 (1.0)	<0.001
Fasting glucose, mmol/L (SD)	5.1 (1.4)	4.7 (0.9)	5.1 (1.1)	5.5 (1.8)	<0.001
2 h glucose	6.7 (3.2)	5.7 (2.6)	6.6 (2.8)	7.6 (3.5)	<0.001
Glucose tolerance status					<0.001
Normal	963 (75.6)	422 (85.8)	213 (78.0)	328 (64.4)	
Prediabetes	221 (17.3)	53 (10.8)	43 (15.7)	125 (24.6)	
Diabetes	90 (7.1)	17 (3.4)	17 (6.2)	56 (11.0)	
Glycated albumin,% (SD)	13.2 (2.4)	13.1 (1.8)	13.2 (2.2)	13.3 (3.0)	0.351
Albumin, g/L (SD)	42.4 (2.8)	42.4 (3.2)	42.9 (2.8)	42.1 (2.4)	0.030
HOMA-IR	1.4 (0.8−2.3)	0.8 (0.5−1.3)	1.4 (1.0−2.0)	2.2 (1.4−3.5)	<0.001

***Table 1*: General characteristics of the sample by body mass index (BMI) status.**
(1) Data presented as mean and standard deviation (SD), median and 25th-75th percentiles (Q1-Q3), or count and percentages.
(2) Analyses by body mass index categories (normal weight, overweight, obese).
Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; SD, standard deviation.

Estimates of the discrimination after adjustment for covariates are shown in Supplemental Table 2. Adjustment for BMI enhanced the discrimination power of GA for dysglycaemia, but attenuated the discriminatory ability of other markers; while adjustment for age and sex, with and without BMI systematically attenuated the discriminatory power of all biomarkers. The effect of covariates on the discrimination was less important for 2 h glucose than the other three biomarkers.

Performance of GA and HbA_{1c} at their optimal threshold, and in linear combinations

By the Youden's index the optimal threshold for dysglycaemia diagnosis by HbA_{1c} was 5.95% (5.75 to 6.15) and for GA was 13.44% (12.72 to 14.71). Optimal thresholds for other outcomes coding are shown in Supplemental Table 3. The sensitivity at optimal threshold to diagnose dysglycaemia in the overall sample was 63.0 (57.4 −68.4) for HbA_{1c}-alone, 53.0 (47.3−58.7) for GA-alone and 76.2 (71.1−80.8) for the combination of HbA_{1c} and GA, Figure 3. Equivalent figures were: non-obese 50.8 (41.9−59.6), 54.6 (45.6−63.4) and 68.5 (59.7−76.3); and obese 71.8 (64.7−78.2), 51.9 (44.4−59.4) and 81.8 (75.4−87.1); Figure 3, Supplemental Table 3 and Supplemental Figure 2. The characteristics of participants with dysglycaemia and those without across different combinations of HbA_{1c} and GA ate their optimal thresholds, are shown in Supplemental Table 4, with suggestions of differential distribution of age and blood pressure levels.

The performance of HbA_{1c} and GA at recommended/published threshold as well as while using the 75th percentile of their distribution as threshold to diagnose dysglycaemia is described in Supplemental Table 3. Patterns of performance measures were broadly similar those observed at the optimal thresholds.

The C-statistics for the joint prediction of diabetes and dysglycaemia from logistic regressions are available in Supplemental Table 5. Estimates of C-statistics for model containing HbA_{1c} and GA were always higher than those for each markers, although differences with HbA_{1c} alone were not always significant. Covariates only model (age, BMI, sex) was always inferior to HbA_{1c}+GA, while combining both markers and covariates further enhanced the discrimination power.

Discussion

Analyses in this large sample of mixed-ancestry South Africans confirmed the positive correlation of BMI and HbA_{1c} and the negative correlation of BMI and GA.

Variables/outcomes	C-statistic (95% CI)	C-statistics comparison (p-values)			Optimal threshold
		Vs. 2-h	Vs. HbA_{1c}	Vs. GA.	
Screen-detected diabetes (*N* = 1274)					
Overall sample (*n* = 1274)					
FBG (mmol/l)	0.940 (0.910−0.967)	0.381	0.081	0.0013	5.65 (5.45−5.85)
2-h glucose (mmol/l)	0.960 (0.929−0.990)		0.018	<0.0001	10.55 (10.25−11.10)
HbA_{1c} (%)	0.899 (0.855−0.944)			0.050	6.15 (6.05−6.45)
Glycated albumin (%)	0.842 (0.787−0.896)				14.90 (13.68−15.28)
Normal weight (*n* = 492)					
FBG (mmol/l)	0.884 (0.804−0.964)	0.093	0.156	0.607	5.45 (4.65−5.85)
2-h glucose (mmol/l)	0.971 (0.922−1.000)		0.020	0.074	11.25 (8.25−11.55)
HbA_{1c} (%)	0.763 (0.598−0.929)			0.188	5.85 (5.75−6.55)
Glycated albumin (%)	0.852 (0.734−0.969)				14.58 (13.10−15.24)
Overweight (*n* = 273)					
FBG (mmol/l)	0.915 (0.823−1.000)	0.071	0.239	0.893	5.85 (5.85−6.50)
2-h glucose (mmol/l)	1.000		0.033	0.093	11.35 (11.10−11.95)
HbA_{1c} (%)	0.972 (0.946−0.998)			0.218	6.35 (5.95−6.55)
Glycated albumin (%)	0.906 (0.796−1.000)				13.94 (13.88−16.34)
Normal + Overweight (*n* = 765)					
FBG (mmol/l)	0.897 (0.837−0.958)	0.015	0.498	0.699	5.45 (5.25−5.85)*
2-h glucose (mmol/l)	0.983 (0.955−1.000)		0.014	0.018	11.35 (8.25−11.55)*
HbA_{1c} (%)	0.863 (0.771−0.956)			0.726	6.15 (5.85−6.35)*
Glycated albumin (%)	0.880 (0.800−0.959)				13.92 (13.68−15.24)*
Obese (*n* = 509)					
FBG (mmol/l)	0.968 (0.948−0.987)	0.273	0.016	<0.0001	5.75 (5.55−6.80)
2-h glucose (mmol/l)	0.933 (0.877−0.989)		0.402	0.001	10.55 (10.02−11.1)
HbA_{1c} (%)	0.908 (0.859−0.956)			0.024	6.45 (6.05−6.65)
Glycated albumin (%)	0.828 (0.758−0.897)				14.90 (13.30−15.62)
Normal waist (*n* = 658)					
FBG (mmol/l)	0.872 (0.788−0.957)	0.059	0.323	0.516	5.25 (4.95−5.65)
2-h glucose (mmol.l)	0.973 (0.925−1.000)		0.023	0.033	11.35 (8.25−11.65)
HbA_{1c} (%)	0.792 (0.646−0.939)			0.679	6.15 (5.75−6.35)
Glycated albumin (%)	0.824 (0.700−0.948)				13.81 (13.67−15.24)
High waist (*n* = 616)					
FBG (mmol/l)	0.958 (0.932−0.984)	0.733	0.071	0.0008	5.85 (5.75−6.25)
2-h glucose (mmol.l)	0.948 (0.905−0.992)		0.234	0.0003	10.55 (10.25−11.10)
HbA_{1c} (%)	0.919 (0.879−0.960)			0.023	6.35 (6.15−6.45)
Glycated albumin (%)	0.853 (0.794−0.912)				14.12 (13.38−15.16)
Dysglycemia (*n* = 1274)					
Overall (*n* = 1274)					
FBG (mmol/l)	0.830 (0.801−0.859)	<0.0001	0.0006	<0.0001	5.15 (5.05−5.35)
2-h glucose (mmol/l)	0.968 (0.953−0.983)		<0.0001	<0.0001	7.75 (7.75−7.75)
HbA_{1c} (%)	0.765 (0.731−0.799)			<0.0001	5.95 (5.75−6.15)
Glycated albumin (%)	0.673 (0.637−0.710)				13.44 (12.72−14.71)
Normal weight (*n* = 492)					
FBG (mmol/l)	0.826 (0.768−0.884)	0.0004	0.0004	0.0003	4.95 (4.75−5.15)
2-h glucose (mmol/l)	0.961 (0.923−0.998)		<0.0001	<0.0001	7.75 (7.75−7.75)
HbA_{1c} (%)	0.663 (0.584−0.742)			0.879	5.85 (5.65−6.05)
Glycated albumin (%)	0.657 (0.580−0.734)				13.67 (12.84−14.74)
Overweight (*n* = 273)					
FBG (mmol/l)	0.742 (0.659−0.825)	<0.0001	0.975	0.431	5.15 (5.05−3.45)*
2-h glucose (mmol/l)	0.994 (0.986−1.000)		<0.0001	<0.0001	7.45 (7.15−7.75)*
HbA_{1c} (%)	0.744 (0.664−0.823)			0.393	5.95 (5.75−5.95)*
Glycated albumin	0.704 (0.628−0.780)				13.06 (12.72−13.68)*

Table 2 *(Continued)*

Variables/outcomes	C-statistic (95% CI)	C-statistics comparison (p-values)			Optimal threshold
		Vs. 2-h	Vs. HbA_{1c}	Vs. GA.	
Normal + overweight (*n* = 765)					
FBG (mmol/l)	0.795 (0.747–0.842)	<0.0001	0.006	0.0004	5.05 (4.95–5.15)*
2-h glucose (mmol/l)	0.975 (0.953–0.996)		<0.0001	<0.0001	7.75 (7.15–7.75)*
HbA_{1c} (%)	0.705 (0.649–0.761)			0.359	5.75 (5.65–5.95)*
Glycated albumin	0.677 (0.622–0.731)				13.17 (12.82–13.68)*
Obese (*n* = 509)					
FBG (mmol/l)	0.840 (0.801–0.878)	<0.0001	0.027	<0.0001	5.25 (5.15–5.35)*
2-h glucose (mmol/l)	0.954 (0.930–0.979)		<0.0001	<0.0001	7.65 (7.65–7.75)*
HbA_{1c} (%)	0.787 (0.743–0.830)			0.0009	5.95 (5.95–6.05)*
Glycated albumin	0.692 (0.643–0.741)				13.02 (12.72–13.3)*
Normal waist (*n* = 658)					
FBG (mmol/l)	0.804 (0.751–0.857)	<0.0001	0.0007	0.0006	4.95 (4.85–5.15)
2-h glucose (mmol.l)	0.969 (0.938–0.999)		<0.0001	<0.0001	7.75 (7.75–7.75)
HbA_{1c} (%)	0.671 (0.603–0.738)			0.820	5.95 (5.65–6.15)
Glycated albumin (%)	0.663 (0.598–0.727)				13.52 (12.74–14.78)
High WC (*n* = 616)					
FBG (mmol/l)	0.821 (0.783–0.858)	<0.0001	0.064	<0.0001	5.35 (5.25–5.75)
2-h glucose (mmol.l)	0.961 (0.942–0.981)		<0.0001	<0.0001	7.75 (7.75–7.75)
HbA_{1c} (%)	0.779 (0.738–0.819)			0.001	6.05 (5.95–6.35)
Glycated albumin (%)	0.697 (0.652–0.741)				12.94 (12.72–14.23)

***Table 2*: Discrimination of indices of glucose homeostasis for dysglycaemia diagnosis.**
95% CI, 95% confidence intervals; FBG, fasting blood glucose; GA, glycated albumin; 2-h, 2 h glucose; WC, waist circumference.

Furthermore, the correlation of both markers with fasting and 2 h glucose was dependent on adiposity with point estimates consistently increasing across increasing BMI categories. The discriminatory ability of the two markers for prevalent dysglycaemia was better in obese than in non-obese participants. At their respective data-specific optimal threshold for dysglycaemia diagnosis, performance measures were mostly better for HbA_{1c} than GA, whereas combining the two markers improved sensitivity, particularly in non-obese participants. Nonetheless, we continue to recommend the simultaneous measurement of HbA_{1c} and GA independent of BMI because in any given clinical setting BMI may not have been properly calculated or a factor which compromises HbA_{1c} interpretation such as anemia may be present and undiagnosed. Similarly, hypoalbuminemia is not routinely assessed and may be present compromising GA interpretation.

The correlations of HbA_{1c} and GA with fasting and 2-h glucose have been previously investigated including in African populations.[37–39] In the latter however, the effects of adiposity on the correlations have been seldom investigated.[40] The correlation of HbA_{1c} and GA with each other and with glucose-based markers in our sample was low-to-modest, with estimates being better for HbA_{1c} than GA against glucose-based tests. In analyses stratified by BMI status estimates significantly improved in obese participants, with substantial attenuation of the HbA_{1c} vs. GA differences. This confirms recent findings in Black South Africans and supports the suggestion that these biomarkers could become more relevant for glucose tolerance status assessment with the increasing obesity in Africa.[40] However, the rather modest correlation of the two biomarkers argues against their interchangeability for this purpose.

The diverging association of HbA_{1c} and GA with BMI, which was apparent in our population, has been described in previous studies,[15,20,41,42] with the negative association of GA with BMI attributed to increased albumin catabolism from obesity-related chronic inflammation,[43] and defective insulin secretion and subsequent post-prandial hyperglycemia.[44,45] In short, if obesity-induced inflammation affects GA levels for reasons other than degree of glycemia, GA cannot be used to assess glycemia in the obese.

Our cohort was characterized by the availability of the full range of the BMI distribution from 15 to 74 kg/m^2 (Figure 1, Panel A), allowing a comprehensive assessment of the relationships of BMI with HbA_{1c} and GA. Variable strengths of those relationships have been reported in existing studies. One such study has for instance reported a one kg/m^2 higher BMI to be associated with a 0.13% decrease in GA,[20] which is compatible with the significant positive correlation found in our sample.

HbA_{1c} has been promoted as biomarkers for glucose tolerance status classification for over a decade.[9,11,12] However, studies on the performance of HbA_{1c} in

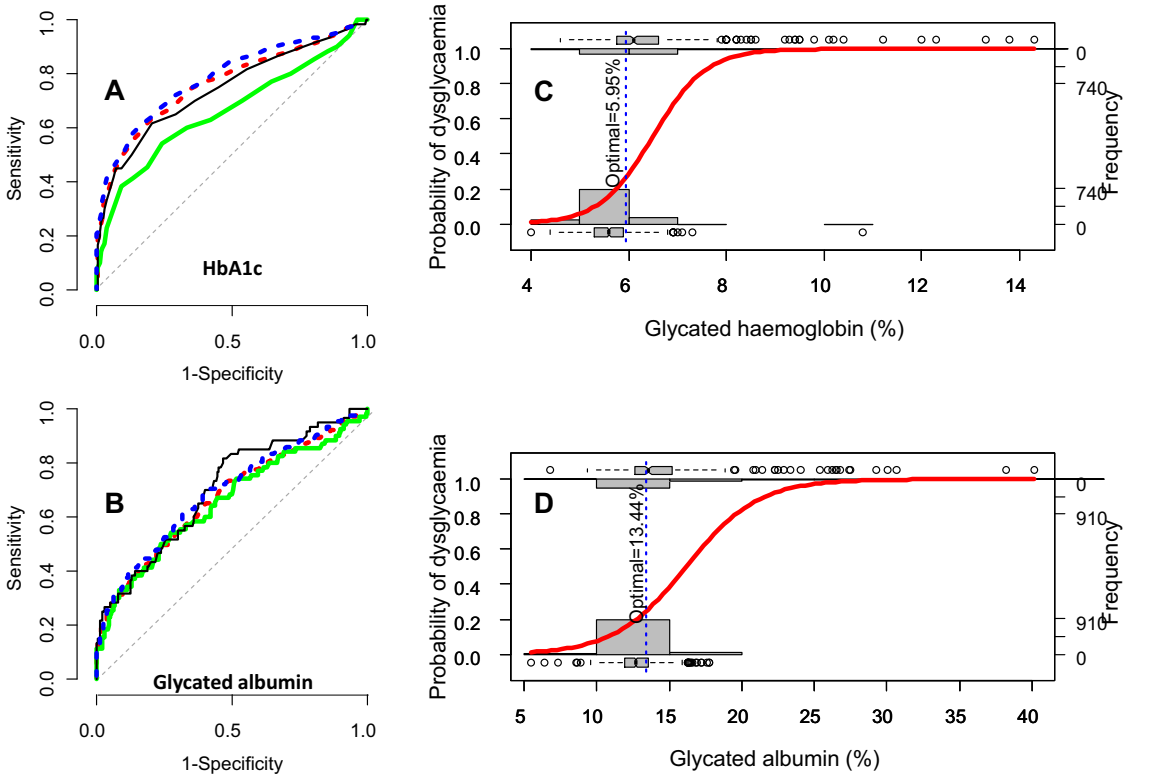

Figure 2. Receiver operating characteristic (ROC) curves (panels A and B) and logistic curves (panels C and D) for the ability of HbA_{1c} and glycated albumin (GA) to diagnose dysglycaemia.

For the ROC curves: the dotted diagonal line at 45° is the line of no discrimination (c-statistics=0.50). The broken red lines are for the total sample with the accompanying c-statistics (95% confidence interval) being 0.76 (0.73−0.80) for HbA_{1c} (panel A) and 0.67 (0.64−0.71) for GA (panel B). The solid green lines are for normal weight [corresponding c-statistics 0.66 (0.58−0.74) and 0.66 (0.58−0.73)]. The solid black lines are for the overweight [corresponding c-statistics 0.74 (0.66−0.82) and 0.70 (0.63−0.78)]; and the broken blue lines are for the obese [corresponding c-statistics 0.79 (0.74−0.83) and 0.69 (0.64−0.74)].

For the logistic curve panels: The red curve spanning figure is the logistic curve. The histogram on the top is for the distribution of participants with dysglycaemia and the histogram at the bottom of the figure panel is for the distribution of participants without dysglycaemia across the continuum of HbA_{1c} (panel C) and GA (panel D). the dotted vertical blue line is for the optimal threshold from ROC curves analyses. The horizontal boxplots are for the distribution of HbA_{1c} and GA in participants with dysglycaemia (upper boxplots) and those without (bottom boxplots).

African populations have been inconsistent.[46] This has been ascribed at least in part to the high prevalence in the African setting of interfering factors/conditions that can affect the diagnostic utility of HbA_{1c}.[41] These factors include among others ethnic differences in HbA_{1c} levels,[47] the high burden of infectious diseases such as HIV and tuberculosis, anemia of various etiologies, and hemoglobin variants. One recent systematic review[46] has concluded that the commonly advocated threshold for diabetes of >=6.5%, HbA_{1c} will substantially misclassified the status of many African people for dysglycaemia, while HbA_{1c}>6.0% was associated with the highest sensitivity for OGTT-diagnosed diabetes mellitus.

GA responds faster than HbA_{1c} to increases in glucose levels, and therefore could be a potentially sensitive biomarker of early stages dysglycaemia.[48] Few studies on the performance of GA to diagnose dysglycaemia in African populations have been consistent in showing a modest performance of GA,[38,40] with comparative studies suggesting that GA is less sensitive but more specific than HbA_{1c}.[38] Beside adiposity, one study has suggested age, gender and ethnicity to be potential determinants of GA levels in African populations.[49] But the potential impact of these factors on the performance of GA to diagnose dysglycaemia in African populations has yet to be explored. A systematic review and meta-analysis of worldwide studies on the diagnostic performance of GA for diabetes concluded based on 16 eligible studies that the optimal threshold was 14.0%.[19] This threshold was associated with a sensitivity of 76.6% and a specificity of 68.7%. There was however substantial heterogeneity across included studies, reflecting the diversity of the populations across studies, but also the spectrum of

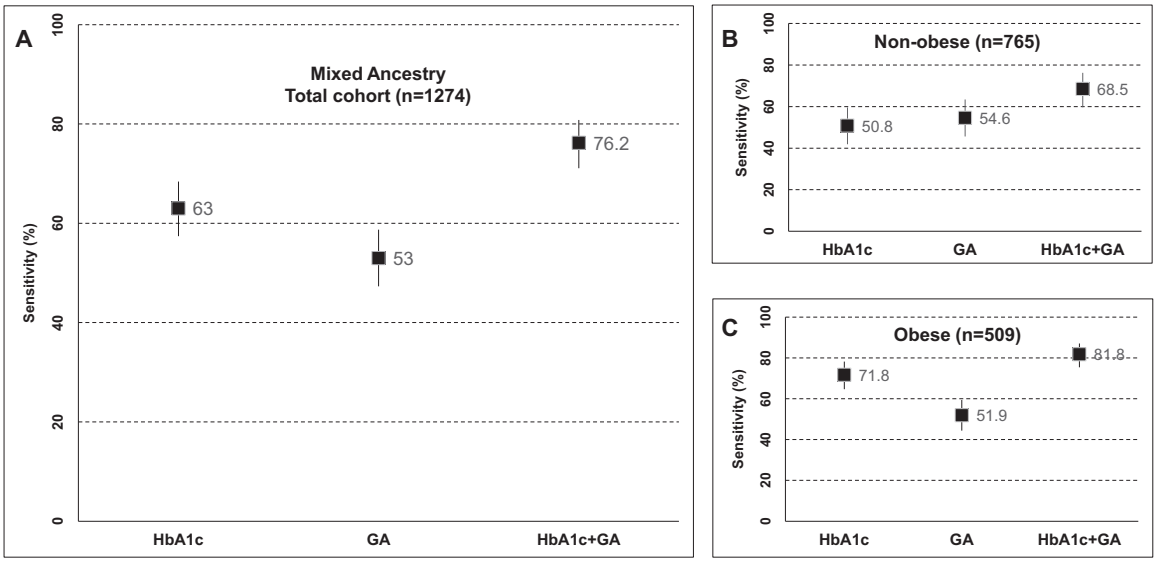

Figure 3. Sensitivities of HbA_{1c} and glycated albumin (GA) singly and in combination for the diagnosis of dysglycaemia in the overall sample (panel A), and separately in non-obese (panel B) and obese participants (panel C).

The black boxes are for the effect estimates (sensitivity), with the absolute value displayed next to each box. The vertical bars about the black boxes are for the 95% confidence intervals.

assays used to measure GA across studies.[19] Indeed, unlike HbA_{1c},[50] there are currently no international standards for GA measurement.

Our teams have in two previous studies in African-born Blacks living in America, shown that combining HbA_{1c} and GA resulted in improved sensitivity to diagnose dysglycaemia than using HbA_{1c} alone.[15,21] In one of the studies, we have further demonstrated that this increase in sensitivity was primarily driven by improved performance in nonobese participants.[15] While this pattern was also apparent in the current study, due to the largely overweight and obese profile of our sample (mean BMI 28.7 kg/m^2), the advantage of combining HbA_{1c} and GA to diagnose dysglycaemia would apply across the entire population. Internationally, attention has focused primarily on the combination of HbA_{1c} with fasting glucose for abnormal glucose tolerance diagnosis.[11,51] Accordingly, data is currently lacking on the combined performance of HbA_{1c} and GA in diverse settings.

Our study has major strengths. It is the first detailed study on the effect of adiposity on the performance of HbA_{1c} and GA in Africa, and it is the largest study to investigate those issues in any populations of African descent. Some limitations should also be accounted for while interpreting the findings from our study. The sample was restricted to mixed-ancestry adults residing in an urban environment, which may therefore not be representative of the diverse African population. We lacked data on the presence of haemoglobin variants and other factors than can interfere with HbA_{1c} and/or GA measurement, and were therefore unable to account for their possible effects on the observed findings. However, haemoglobinopathies are much less common in southern African countries than West, Central and East Africa.[52] The low representation of men in our sample precluded sex-specific analyse and therefore, our findings are largely driven by the performance of the HbA_{1c} and GA in women.

In conclusion, the population of people with diabetes and other forms of dysglycaemia is rapidly increasing in Africa, against a background of very low detection rates, inviting more effort into the development for better screening tests for diabetes in these populations. The many challenges associated with implementing OGTT preclude its widespread application. Our study supports previous suggestions that whereas HbA_{1c} and GA are not optimal for dysglycaemia screening when only one is used, combining these two markers could become increasing important for dysglycaemia screening in African populations.[15,21] Both HbA_{1c} and GA are non-fasting biomarkers and this enhances their ease of administration. Population-based screening for common chronic infectious disease in Africa such as HIV is already taking place across many settings on the continent using minimally invasive blood sample collection. Adding HbA_{1c} and GA provides an opportunity to co-screen people for dysglycaemia.

Contributors

Funding acquisition (APK, TEM, RTE), Study conception (APK, TEM, RTE, AES), operationalization and supervision of data collection (TEM), data analysis and

interpretation and drafting the manuscript (APK, AES), critical revision of manuscript (TEM, RTE, DBS, AEZ), approval of the final version (all co-authors)

APK and TEM had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis; both are guarantors.

Data sharing statement

The data can be accessed from the corresponding author upon a reasonable request, and subject to approval by the Ethics Communities. The original approval for this data collection did not make provision for data sharing.

Declaration of interests

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2022.101443.

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