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Glycated haemoglobin (HbA_{1c}) and fasting plasma glucose relationships in sea-level and high-altitude settings

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Abstract

Aim Higher haemoglobin levels and differences in glucose metabolism have been reported among high-altitude residents, which may influence the diagnostic performance of HbA_{1c}. This study explores the relationship between HbA_{1c} and fasting plasma glucose (FPG) in populations living at sea level and at an altitude of > 3000 m.

Methods Data from 3613 Peruvian adults without a known diagnosis of diabetes from sea-level and high-altitude settings were evaluated. Linear, quadratic and cubic regression models were performed adjusting for potential confounders. Receiver operating characteristic (ROC) curves were constructed and concordance between HbA_{1c} and FPG was assessed using a Kappa index.

Results At sea level and high altitude, means were 13.5 and 16.7 g/dl ($P > 0.05$) for haemoglobin level; 41 and 40 mmol/mol (5.9% and 5.8%; $P < 0.01$) for HbA_{1c}; and 5.8 and 5.1 mmol/l (105 and 91.3 mg/dl; $P < 0.001$) for FPG, respectively. The adjusted relationship between HbA_{1c} and FPG was quadratic at sea level and linear at high altitude. Adjusted models showed that, to predict an HbA_{1c} value of 48 mmol/mol (6.5%), the corresponding mean FPG values at sea level and high altitude were 6.6 and 14.8 mmol/l (120 and 266 mg/dl), respectively. An HbA_{1c} cut-off of 48 mmol/mol (6.5%) had a sensitivity for high FPG of 87.3% (95% confidence interval (95% CI) 76.5 to 94.4) at sea level and 40.9% (95% CI 20.7 to 63.6) at high altitude.

Conclusion The relationship between HbA_{1c} and FPG is less clear at high altitude than at sea level. Caution is warranted when using HbA_{1c} to diagnose diabetes mellitus in this setting.

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Introduction

Nowadays, Type 2 diabetes mellitus is a global epidemic, and the prevalence is far from levelling off. The prevalence of diabetes has almost doubled in the last three decades and the chances of achieving the global target of halting the increase in prevalence by 2025 are < 1% [1]. Although originally identified by the presence of glucose in urine, glucose tests for the diagnosis of Type 2 diabetes have been developed over the last century. The oral glucose tolerance test has been used for the diagnosis of Type 2 diabetes over the last three decades. However, this test is laborious for individuals, and thus, has been replaced by fasting plasma glucose (FPG) for use in both clinical settings and epidemiological studies.

HbA_{1c} had been established as the monitoring test of choice to evaluate medium-term diabetic control [2]. Several international societies, including the American Diabetes Association and World Health Organization (WHO) recommend using HbA_{1c} as a diagnostic criterion for Type 2 diabetes in stable haematological circumstances, because it

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What's new?

- Haemoglobin levels and differences in glucose metabolism at high altitude may influence the diagnostic performance of testing for diabetes using HbA_{1c}.
- We found that the relationship between HbA_{1c} and fasting plasma glucose (FPG) differed markedly between high-altitude and sea-level areas.
- The relationship between HbA_{1c} and FPG was quadratic at sea level and linear at high altitude.
- Corresponding FPG values for an HbA_{1c} ≥ 48 mmol/mol (≥ 6.5%) cut-off point, used for the diagnosis of diabetes, were 6.6 and 14.8 mmol/l (120 and 266 mg/dl) at sea level and high altitude, respectively.
- The sensitivity of HbA_{1c} to detect abnormal FPG was 87.3% at sea level and 40.9% at high altitude. This suggests a limitation in the performance of HbA_{1c} to diagnose diabetes at altitude.

has several advantages over glucose tests, such as low intra-individual variation and the convenience of taking the test without fasting. However, this recommendation has been criticized because of the observed discordance between HbA_{1c} and glucose tests, and biological variation in certain ethnic groups [3,4]. As such, alternative population-specific HbA_{1c} cut-off points for the diagnosis of Type 2 diabetes have recently been proposed [5].

Changes in erythrocytes states, for example, due to folic acid deficiency and renal disease, erythrocyte lifespan and levels of haemoglobin can also influence HbA_{1c} levels [6]. One of the mechanisms of adaptation in high-altitude settings is secondary polycythaemia (increase in haemoglobin levels) [7]. A common feature of many Latin American countries, where over the last two decades Type 2 diabetes-related mortality has been the highest worldwide [8], is a significant proportion of people living at high altitude. Over 30 million people currently reside in the Central American highlands, and in Peru, one third of the population live at altitude [9]. Indeed, secondary polycythaemia has been largely reported among Andean natives [10,11]. Additionally, differences in glucose metabolism have been reported among people residing at high altitude [12].

In this study, we aim to explore and compare the relationship between HbA_{1c} and FPG in populations living at high altitude and sea level.

Methods

Study settings and participants

We identified eligible individuals from two Peruvian longitudinal population-based studies: the CRONICAS Cohort Study (n = 3601, baseline conducted in 2010–2011), and the rural Ayacucho population of the PERU MIGRANT Study (n = 200, baseline conducted in 2007–2008). The CRONICAS Cohort Study aimed to assess the prevalence and incidence of cardiometabolic and pulmonary conditions at four sites: Lima, highly urban, sea level; Tumbes, semi-urban, sea level; and two high-altitude locations (3825 m above sea level), rural and urban Puno. All participants were aged 35 years or older and full-time residents in the area. The PERU MIGRANT Study was designed to investigate differences in cardiovascular disease risk factors between rural-to-urban migrant and non-migrant groups. This study was performed in participants aged 30 years and over from a rural site in Ayacucho, located at 2900–3100 m above sea level, an urban site in Lima, and rural-to-urban migrants from Ayacucho currently residing in Lima. In both studies, participants were sex- and age-stratified, a single-stage random sampling was used, and only one participant per household was enrolled. The studies are described in detail elsewhere [13,14].

The original pooled dataset had 3801 cases. We excluded 187 individuals with self-reported diagnosis of diabetes or use of anti-diabetic medications. In addition, one person was excluded during regression analysis because that individual was an influential point. The final number of people included in this analysis was 3613.

Participants were classified into two geography-based categories: (1) sea-level population (those from Lima and Tumbes), and (2) high-altitude population (those from Ayacucho and Puno).

Study variables

We evaluated clinical variables, including BMI, hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or current use of antihypertensive medications), and current smoking status (self-report of having smoked at least one cigarette in the last 30 days). We also explored sociodemographic variables, such as wealth index based on asset possessions [15] and educational level (primary or less, secondary and higher).

Besides FPG (mmol/l) and HbA_{1c} (mmol/mol; %), additional laboratory variables included lipid measurements (total cholesterol, triglycerides, HDL-C and Friedewald-estimated LDL-C, in mg/dl), and haematological parameters, such as total haemoglobin (g/dl), mean corpuscular volume (fl/red blood cell), mean corpuscular haemoglobin (pg/cell) and mean corpuscular haemoglobin concentration (g/dl). HbA_{1c} was measured using high-performance liquid chromatography (HPLC, D10-BIORAD, Germany), which is traceable to the Diabetes Control and Complications Trials reference study as certified by the National Glycohemoglobin Standardization Program.

Statistical analysis

For descriptive purposes, study variables were compared between sea-level and high-altitude settings using analysis of variance, chi-squared or Fisher's exact tests. Linear, quadratic and cubic regression models were performed to assess the relationship between HbA_{1c} and FPG, crude and adjusted by age, sex, education, wealth, BMI and total haemoglobin. Models were performed separately for each sea-level and high-altitude subgroup.

Beta coefficients and 95% confidence intervals (95% CI) were calculated for FPG, squared-FPG and/or cubic-FPG. Maximum likelihood optimization (Newton–Raphson) and robust variance estimations [16] were used in these models to compensate for heteroscedasticity and non-normality. Information from Wald's test and Bayesian information criteria helped select the best models.

We evaluated diagnostic performance for diabetes and prediabetes in the sea-level and high-altitude subgroups using FPG as the gold standard. We used the cut-off points recommended by the American Diabetes Association for HbA_{1c} (normal < 39 mmol/mol, < 5.7%; prediabetes 39 to < 48 mmol/mol, 5.7 to < 6.5%; diabetes ≥ 48 mmol/mol, ≥ 6.5%) and FPG (normal < 5.6 mmol/l; prediabetes 5.6–6.9 mmol/l; diabetes ≥ 7.0 mmol/l). We evaluated sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios of HbA_{1c}, and receiver operating characteristic (ROC) curves.

Finally, we compared concordance between diagnosis by HbA_{1c} and FPG using a Kappa index. All analyses were conducted using Stata/IC v. 12 (Stata Corp, College Station, TX, USA). Ethical approval was obtained for the original studies.

Results

In total, 3613 individuals were included in the analysis: Lima, n = 1036; Tumbes, n = 963; Ayacucho, n = 200; and Puno, n = 1414. Haemoglobin levels were significantly lower in individuals at sea level (13.5 ± 1.4 g/dl) than those at high altitude (16.7 ±1.9 g/dl) (P < 0.001). Mean HbA_{1c} was 41 mmol/mol (5.9 ± 0.88%) at sea level, and 40 mmol/mol (5.8 ± 0.48%) at high altitude. Individuals at sea level had higher mean FPG (5.3 ± 1.4 mmol/l) compared with those from high altitude (4.9 ± 0.9 mmol/l) (P < 0.001). The cardiovascular risk factor profile, in terms of adiposity, lipid markers, hypertension and smoking status, was poorer among those living at sea level. Diabetes, defined both by HbA_{1c} and FPG, was more prevalent at sea level than high altitude. In both high-altitude and sea-level settings, the estimates of HbA_{1c}-defined diabetes were three times higher than those based on FPG. We had haematological parameters from one site at high altitude (Ayacucho, n = 167). Levels of mean haematocrit were 48.5 ± 4.1%; mean corpuscular volume was 94.9 ± 4.9 fl/red blood cell; mean corpuscular haemoglobin was 31.2 ± 1.7 pg/cell; and mean corpuscular haemoglobin concentration was 32.8 ± 1.2 g/dl (Table 1).

In both crude and adjusted models, we found differences between predictions of HbA_{1c} by FPG at sea level and high altitude (Figs 1 and 2). Whereas HbA_{1c} and FPG showed a non-linear, quadratic relationship at sea level, we found a linear association at high altitude (Table S1). Differences in relationship patterns and intercept values (3.9 for high altitude, 4.6 for sea level) display notable differences in the shape of each curve (Fig. 1). This effect has a repercussion on values for diagnosis: to predict an HbA_{1c} value of 48 mmol/mol (6.5%), mean FPG values of 6.6 and 14.8 mmol/l were needed at sea level and high altitude, respectively.

Among those with diabetes, at sea level, the number of individuals diagnosed by HbA_{1c} was 13.5 times greater than diagnosed by FPG only (108 vs. 8), and this relationship was 4.6 times greater at high altitude (60 vs. 13). Individuals diagnosed by HbA_{1c} only were older, but metabolically healthier at high altitude than at sea level. Similar results were found among individuals diagnosed by FPG, and by the combination of HbA_{1c} and FPG (Table 2).

Using HbA_{1c} instead of FPG to diagnose diabetes increased the number of cases by 159% at sea level and 215% at high altitude. Finally, when evaluating the agreement between diagnosis of diabetes and prediabetes by HbA_{1c} and FPG, we found poor agreement at sea level (Kappa = 0.19) and at high altitude (Kappa = 0.04) (Table 3).

The sensitivity of an HbA_{1c} cut-off value 48 mmol/mol (6.5%) for diabetes diagnoses, using FPG as a gold standard, was much higher in the sea-level groups (87.3%) than in the high-altitude groups (40.9%), with specificities of 94.2% and 95.0%, respectively. Positive likelihood ratios were 15.1 and 8.1, respectively. Sensitivities for diagnosis of prediabetes were similar, 74.7% and 71.4% in the sea-level and high-altitude groups, respectively (Table 4). ROC areas for sea level (0.95) and high altitude (0.74) were significantly different ($chi^2(1) = 9$, P < 0.01) using HbA_{1c} standard cut-points and FPG as the gold standard of diabetes (Fig. S1). ROC areas for sea level (0.68) and high altitude (0.57) were also significantly different ($chi^2(1) = 12$, P < 0.001) using HbA_{1c} standard cut-points and FPG as the gold standard of prediabetes (Fig. S2).

The prevalence of diabetes is higher when HbA_{1c} is used (≥ 48 mmol/mol; ≥ 6.5%) rather than FPG (≥ 7.0 mmol/l) for sea-level populations (diabetes prevalence of 12.3% with HbA_{1c} and 6.5% with FPG) and high-altitude populations (diabetes prevalence of 7.9% with HbA_{1c} and 3.8% with FPG) (Table S2).

Discussion

In this study, we found that the relationship between HbA_{1c} and FPG differed markedly between populations living at high altitude and sea level. Using current recommended HbA_{1c} cut-off points for the diagnosis of diabetes

Table 1 Characteristics of study participants at sea-level and high-altitude settings

Variable	Total (n = 3613)	Sea level (n = 1999)	High altitude (n = 1614)	P*
Sociodemographic				
Age (mean ± SD)	3611	55.2 ± 12.7	55.0 ± 13.0	0.77
Male, n (%)	3610	986 (49.3)	770 (47.8)	0.36
Wealth index (mean ± SD)	3613	251.9 ± 153.8	167.9 ± 161.8	< 0.001
Education				
Primary or less, n (%)	1709	971 (48.6)	738 (45.7)	< 0.001
Secondary, n (%)	1137	707 (35.4)	430 (26.7)	
Higher, n (%)	764	319 (16.0)	445 (27.6)	
Cardiovascular risk factors				
BMI (kg/m^2, mean ± SD)	3248	28.3 ± 4.6	25.9 ± 4.2	< 0.001
Waist circumference, cm (mean ± SD)	3243	93.2 ± 10.4	86.6 ± 12.1	< 0.001
Total cholesterol, mg/dL (mean ± SD)	2947	202.4 ± 38.7	194.7 ± 40.8	< 0.001
Triglycerides, mg/dL [median (IQR)]	3147	139 (97)	125 (83)	< 0.001
HDL-C, mg/dL (mean ± SD)	2947	40.9 ± 11.5	43.0 ± 11.3	< 0.001
LDL-C, mg/dL (mean ± SD)†	200	–	85.7 ± 27.1	
Hypertension, n (%)	3047	291 (15.0)	106 (9.6)	< 0.001
Current smoker, n (%)	3610	268 (13.4)	130 (8.1)	< 0.001
Haematological variables				
Haemoglobin, g/dL (mean ± SD)	3146	13.5 ± 1.4	16.7 ± 1.9	< 0.001
Mean corpuscular volume, fl/red blood cell†	167	–	94.9 ± 4.9	
Mean corpuscular haemoglobin, pg/cell†	167	–	31.2 ± 1.7	
Mean corpuscular haemoglobin concentration, g/dl†	167	–	32.8 ± 1.2	
Diabetes-related markers				
HbA_{1c} (mean mmol/mol)	3146	41	40	0.10
HbA_{1c} (mean % ± SD)		5.9 ± 0.88	5.8 ± 0.48	
Fasting plasma glucose (mean mmol/l ± SD)	3146	5.3 ± 1.4	4.9 ± 0.9	< 0.001
Diabetes diagnosed by HbA_{1c}‡				
Normal, n (%)	1227	789 (40.9)	438 (36.0)	< 0.001
Prediabetes, n (%)	1687	978 (50.6)	709 (58.3)	
Diabetes, n (%)	232	163 (8.5)	69 (5.7)	
Diabetes diagnosed by FPG§				
Normal, n (%)*	2493	1433 (74.3)	1060 (87.2)	< 0.001
Prediabetes, n (%)*	568	434 (22.5)	134 (11.0)	
Diabetes, n (%)*	85	63 (3.3)	22 (1.8)	

*ANOVA one-way for mean differences; Kruskal–Wallis or median differences; chi square for distribution differences.
†Only available for Ayacucho.
‡Prediabetes and diabetes were diagnosed using the American Diabetes Association recommended HbA_{1c} cut-off point: diabetes, HbA_{1c} ≥ 48 mmol/mol (≥ 6.5%); prediabetes, ≥ 48 mmol/mol (6.5%) > HbA_{1c} ≥ 39 mmol/mol (≥ 5.7%); normal, HbA_{1c} < 39 mmol/mol (< 5.7%).
§Prediabetes and diabetes were diagnosed using the American Diabetes Association recommended FPG cut-off point: diabetes, FPG ≥ 7.0 mmol/l; prediabetes, 7.0 mmol/l > FPG ≥ 5.6 mmol/l; normal: FPG < 5.6 mmol/l.
FPG, fasting plasma glucose.

(≥ 48 mmol/mol, ≥ 6.5%), our models showed a discrepancy of up to 8.2 mmol/l units of FPG. In other words, corresponding FPG values for such HbA_{1c} cut-off point were 6.6 and 14.8 mmol/l at sea level and high altitude, respectively. This translated into major discrepancies in diagnostic performance, as shown by differences in the sensitivity of HbA_{1c} at sea level (89%) compared with at high altitude (41%). In terms of new cases of diabetes, greater discordance was observed in high-altitude settings, which was confirmed by the poor agreement found. Taken together, our findings show that high altitude is another setting in which HbA_{1c} might not be appropriate when used as a diagnostic tool for Type 2 diabetes.

Discordance between FPG and HbA_{1c} has been reported in American, European and Asian populations, as well as in older and female individuals. However, this is the first study reporting discordance in Andean populations. Differences in the glycation process, of genetic or adaptive origin, have been shown to play a significant role in inter-individual variance by causing abnormally high or low levels of HbA_{1c} for a given plasma glucose level [18]. However, other physiological or environmental pathways may contribute to discordance observed between FPG and HbA_{1c} in our study settings.

We observed that FPG was, on average, 0.4 mmol/l higher at sea-level sites than at high-altitude sites, yet mean HbA_{1c} was similar in both study groups. Glucose metabolism has been shown to differ at altitude; for instance, an association between polycythaemia and glucose intolerance has previously been described in an Andean population [12]. A study in rats showed that exposure to hypobaric hypoxia is associated with reduced insulin release due to inhibition of corticotrophin-releasing hormone [19]. This has also been replicated in clinical research; a recent

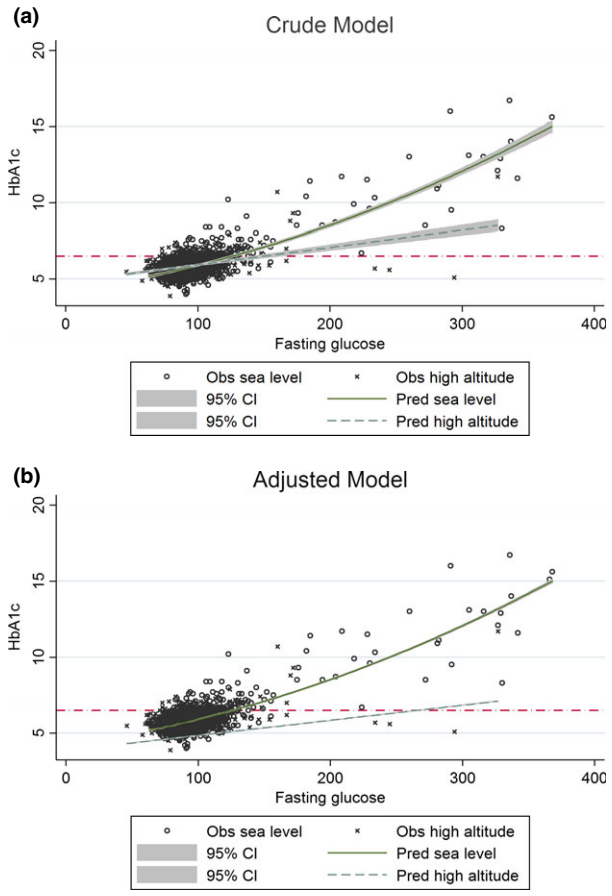

FIGURE 1 Graphical representation of the quadratic model (sea level) and linear model (high altitude) for HbA$_{1c}$ (dependent variable) and fasting plasma glucose (independent variable), crude and adjusted by age, sex, education, wealth, BMI and total haemoglobin levels. After comparison of linear, quadratic and cubic models of the relationship between HbA$_{1c}$ and fasting plasma glucose, a quadratic adjusted model was selected as the best for people at sea level, and a linear adjusted model was selected as the best for people at high altitude (Table S1). The red line was established at an HbA$_{1c}$ value of 48 mmol/mol (6.5%) to represent the current recommended diagnostic cut-point for diabetes [17].

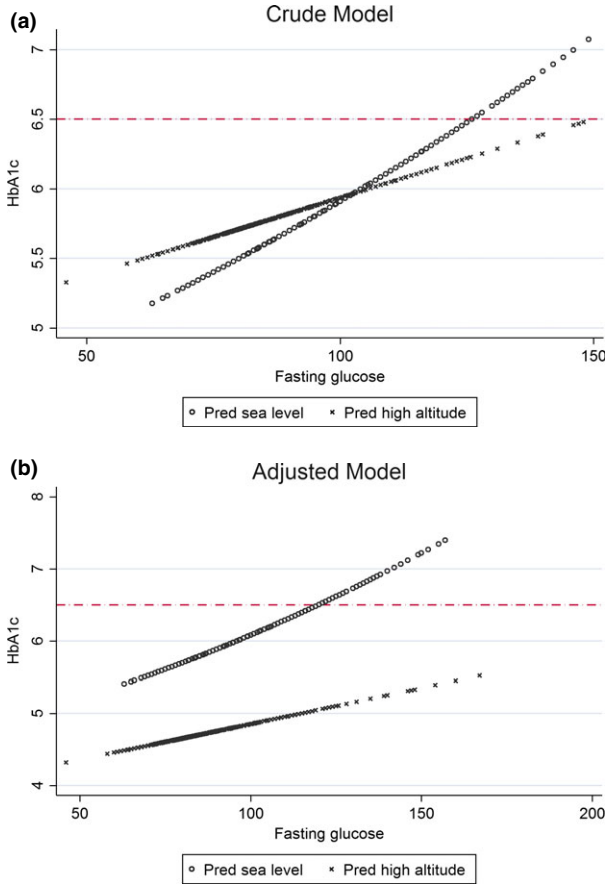

FIGURE 2 Amplification of the zone near to an HbA1c value of 48 mmol/mol (6.5%) in the graphical representation of the quadratic model (sea level) and linear model (high altitude) for HbA$_{1c}$ (dependent variable) and fasting plasma glucose (independent variable) both crude and adjusted by age, sex, education, wealth, BMI and total haemoglobin. The red line was established at a HbA$_{1c}$ value of 48 mmol/mol (6.5%) to indicate the standard diagnostic cut-point for diabetes [17].

publication by our group found that a 5% decrease in oxyhaemoglobin saturation was strongly associated with a HbA$_{1c}$ value ≥ 48 mmol/mol (≥ 6.5%) (JC Bazo-Alvarez, R Quispe, TD Pillay, A Bernabé-Ortiz, L Smeeth, W Checkley, RH Gilman, G Málaga, JJ Miranda, personal communication). It is possible that these processes of relative intolerance lead to a serum glucose level that is not represented by a FPG test obtained in a fasting state, but is identified by HbA$_{1c}$.

We hypothesize that the discrepancy observed in our study might be partially explained by an increase in haemoglobin production. An erythropoietin-driven increase in haemoglobin production is the most important mechanism of adaptation and acclimatization seen at altitude, especially in the Andes [20,21]. This observation was further confirmed in our study, because we found significantly higher mean haemoglobin levels at high altitude than at sea level. Increased erythropoiesis due to other causes, such as intravenous iron or erythropoietin-stimulating agents, has also been shown to influence HbA$_{1c}$ levels [22–24]. In high-altitude native populations, the utilization of iron appears to be 25% greater than in people from sea-level settings [25], and higher HbA$_{1c}$ values have been reported in individuals with iron deficiency [26,27]. Haemoglobin levels may affect the extent of glycation, however, the effect of altitude on lifespan remains unclear [28]. Our haematological data were limited and thorough evaluation of haematological parameters in relationship to glucose and other metabolic markers will add to this understanding. Another potential mechanism to explain our observations might be related to haemoglobin glycation itself at altitude, although the literature is limited in this field. Indeed, many of the mechanisms presented deserve to be fully studied in high-altitude settings.

Table 2 Clinical characteristics of individuals with diagnosis of diabetes by different criteria*

Variable	Overall			Sea-level population			High-altitude population		
	HbA_{1c} only	HbA_{1c} and FPG	FPG only	HbA_{1c} only	HbA_{1c} and FPG	FPG only	HbA_{1c} only	HbA_{1c} and FPG	FPG only
$n^{\dagger}$	168	64	21	108	55	8	60	9	13
Age (mean ± SD)	60.4 ± 12.8	57.2 ± 10.5	58.4 ± 12.3	59.1 ± 12.9	57.0 ± 10.3	53.8 ± 14.1	62.7 ± 12.2	58.7 ± 12.0	61.3 ± 10.6
Male, *n* (%)	59 (35.1)	26 (40.6)	12 (57.1)	**42 (38.9)**	**23 (41.8)**	**5 (62.5)**	**17 (28.3)**	**3 (33.3)**	**7 (53.9)**
BMI (kg/m^2)	29.6 ± 6.0	30.6 ± 5.1	28.5 ± 6.1	**30.7 ± 5.8**	30.9 ± 5.0	**33.0 ± 7.8**	**27.6 ± 5.9**	28.9 ± 5.3	**25.7 ± 2.4**
Waist circumference, cm (mean ± SD)	95.1 ± 13.4	99.0 ± 9.2	94.4 ± 15.0	**98.3 ± 11.0**	99.7 ± 11.3	**104.9 ± 17.5**	**89.3 ± 15.4**	95.0 ± 13.5	**87.9 ± 8.9**
Total cholesterol, mg/dL (mean ± SD)	208.8 ± 42.9	218.0 ± 39.4	220.0 ± 42.3	211.8 ± 39.4	217.7 ± 40.8	236.6 ± 43.2	201.9 ± 49.7	219.5 ± 29.9	209.6 ± 39.9
Triglycerides, mg/dL [median (IQR)]	149 (101)	184 (100)	112 (81)	148 (97)	183 (108)	138 (66)	149 (105)	185 (96)	111 (84)
HDL-C, mg/dL (mean ± SD)	39.0 ± 10.3	38.6 ± 10.6	50.1 ± 15.3	38.7 ± 9.8	38.5 ± 11.1	48.1 ± 17.8	39.8 ± 11.6	39.4 ± 7.8	51.3 ± 14.2
Hypertension, *n* (%)	34 (22.1)	20 (31.8)	2 (9.5)	28 (25.9)	20 (36.4)	2 (25.0)	6 (13.0)	0 (0)	0 (0)
Current smoker, *n* (%)	23 (13.7)	11 (17.2)	4 (19.1)	16 (14.8)	10 (18.2)	2 (25.0)	7 (11.7)	1 (11.1)	2 (15.4)
Haemoglobin, g/dL (mean ± SD)	14.5 ± 2.6	14.3 ± 2.2	16.0 ± 2.0	**13.2 ± 1.7**	**13.6 ± 1.1**	**14.3 ± 0.98**	**16.9 ± 2.3**	**18.5 ± 2.5**	**17.0 ± 1.8**

*Diagnosed by HbA_{1c} only: HbA1c ≥ 48 mmol/mol (≥ 6.5%) and FPG < 7.0 mmol/l; diagnosed by FPG only: HbA_{1c} < 48 mmol/mol (< 6.5%) and FPG ≥ 7.0 mmol/l; diagnosed by both: HbA_{1c} ≥ 48 mmol/mol (≥ 6.5%) and FPG ≥ 7.0 mmol/l.

†Of a total 3613 people in the study, we excluded those without diabetes (*n* = 2892) and those without complete data to evaluate diabetes status (HbA_{1c} and FPG, *n* = 468), therefore data from *n* = 253 people is included.

Entries in bold represent $P < 0.05$ in comparisons between sea-level and high-altitude populations. For categorical variables (%) we used Fisher's exact test (2 × 3 cross table). For continuous variables (means) we used one-way ANOVA, comparing each criterion by altitude (separately). For non-normal variables (medians) we used Kruskal–Wallis test, comparing each criterion by altitude (separately).

FPG, fasting plasma glucose.

Table 3 Concordance of diabetes and prediabetes diagnostics at sea level and high altitude settings considering HbA1c or FPG standard cut-points

		Sea-level population				High-altitude population			
		HbA_{1c}				HbA_{1c}			
Test		Normal	Prediabetes	Diabetes	Total	Normal	Prediabetes	Diabetes	Total
Fasting plasma glucose	Normal	692	691	50	1433	402	619	38	1059
	Prediabetes	95	281	58	434	32	80	22	134
	Diabetes	2	6	55	63	4	9	9	22
	Total*	789	978	163	1930	438	708	69	1215

*Of a total of 3613 people in the study, we excluded those without complete data to evaluate diabetes status (HbA_{1c} and FPG, n = 468), therefore, data from n = 3145 people are included.
Diagnostic criteria for HbA_{1c}: diabetes, $HbA_{1c} \geq 48$ mmol/mol (≥ 6.5%); prediabetes, ≥ 48 mmol/mol (6.5%) > $HbA_{1c} \geq 39$ mmol/mol (≥ 5.7%); normal: $HbA_{1c} < 39$ mmol/mol (< 5.7%).
Diagnostic criteria for FPG: diabetes, FPG ≥ 7.0 mmol/l; prediabetes, 7.0 > FPG ≥ 5.6 mmol/l; normal, FPG < 5.6 mmol/l.
Concordance at sea-level settings: kappa = 0.19, expected agreement = 42.0%; agreement = 53.3%.
Concordance at high-altitude settings: kappa = 0.04, expected agreement = 38.0%; agreement = 40.4%.
FPG, fasting plasma glucose.

Table 4 Diagnostic test characteristics for HbA_{1c} standard cut-points using FPG as the gold standard

		Sea-level population (n = 1930)*		High-altitude population (n = 1215)*	
	Test	%	95% CI	%	95% CI
Diabetes, $HbA_{1c} \geq 48$ mmol/mol (≥ 6.5%)	Sensitivity	87.3	(76.5–94.4)	40.9	(20.7–63.6)
	Specificity	94.2	(93.1–95.2)	95.0	(93.6–96.1)
	PPV	51.2	(46.1–56.3)†	25.3	(16.2–37.2)†
	NPV	99.1	(98.2–99.5)†	97.5	(96.5–98.2)†
	LR+	15.1	(12.3–18.5)	8.1	(4.7–14.2)
	LR−	0.14	(0.07–0.26)	0.62	(0.44–0.88)
Prediabetes, $HbA_{1c} \geq 39$ mmol/mol (≥ 5.7%) and < 48 mmol/mol (< 6.5%)	Sensitivity	74.7	(70.0–79.0)	71.4	(62.1–79.6)
	Specificity	50	(47.4–52.7)	39.4	(36.4–42.4)
	PPV	32.1	(30.4–33.8)†	13.8	(12.4–15.4)†
	NPV	86.2	(83.9–88.3)†	91.0	(88.2–93.2)†
	LR+	1.5	(1.4–1.6)	1.2	(1.1–1.3)
	LR−	0.51	(0.42–0.61)	0.73	(0.54–0.98)

*Of a total 3613 people in the study, we excluded those without complete data to evaluate diabetes status (HbA_{1c} and FPG, n = 468), therefore data from n = 3145 people are included.
†Values and confidence intervals are based on likelihood ratios, using prevalence estimated in this study (using explained FPG cut-off points).
Diabetes sea level = 6.5%; diabetes high altitude = 4%; prediabetes sea level = 24%; prediabetes high altitude = 12%.
The gold standard for diabetes is defined as FPG ≥ 7.0 mmol/l and for prediabetes 7.0 mmol/l > FPG ≥ 5.6 mmol/l.
95% CI, 95% confidence interval; FPG, fasting plasma glucose; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio.

Mounting evidence from observational and controlled clinical trials has demonstrated a strong association between HbA_{1c} levels and retinopathy and other microvascular complications of diabetes. Moreover, HbA_{1c} is also associated with increased risk of cardiovascular disease, even in individuals without diabetes [29]. As such, the role of HbA_{1c} as a key biomarker is undeniable. Yet, the discordance between FPG and HbA_{1c} at altitude observed in our study, particularly in high-altitude settings, merits further and deeper scrutiny because the number of people classified as having diabetes would treble if HbA_{1c} was used as a diagnosis tool. This discrepancy between HbA_{1c} and FPG has recently been highlighted in a global data-pooling study signalling difficulties for monitoring of diabetes targets at a policy level [30]. Consequently, many individuals residing at high altitude, who were shown to have a more favourable cardiometabolic risk profile than those residing at sea level, would initiate glucose-lowering medications and be exposed to the unnecessary harm associated with such treatments. Given the large populations living at high altitude and the rising prevalence of diabetes worldwide, especially in the southern hemisphere, inappropriate prescription of anti-diabetic medications might lead to inefficient public health policies in countries with limited economic resources.

This study has benefited from leveraging data from well-defined population-based studies and relatively large sample sizes. Peru has a particular geographical distribution characterized by a large variety of climates and altitudes. The CRONICAS Cohort Study and the PERU MIGRANT Study cohorts are unique in that they have a relatively large

proportion of individuals living at high altitude, > 3000 m above sea level, where increases in haemoglobin levels are mostly observed. Despite this, our study did not have data on oral glucose tolerance test, the gold standard test used for diabetes research, multiple FPG readings over time to more accurately represent glucose levels in people with diabetes, or a detailed evaluation of all haematological markers in all sites. The cross-sectional approach of this study precludes the ascertainment of causal relationships; therefore, longitudinal studies are better placed to explore the long-term consequences of the discordant patterns reported, particularly in terms of progression of diabetes-related complications. Prospective evaluations are also required to evaluate clinical and economic consequences that may result from modification of current diagnostic criteria.

Conclusions

These findings provide unique evidence that the relationship between HbA_{1c} and FPG differs considerably between sea-level and high-altitude settings. Our models show that an HbA_{1c} of 48 mmol/mol (6.5%) would correspond to different FPG levels in each setting, as shown by a discrepancy of up to 7.8 mmol/l. Such a substantial difference hampers potential strategies for expanding diabetes diagnosis and public health planning in high-altitude settings, and therefore FPG and the oral glucose tolerance test should be used as diagnostic criteria under these circumstances.

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Competing interests

None declared.

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The PERU MIGRANT Study data are publicly available at: https://figshare.com/articles/PERU_MIGRANT_Study_Baseline_dataset/3125005. The CRONICAS Cohort Study data will be available at NHLBI's open repository (https://biolincc.nhlbi.nih.gov/home/).

Author contributions

JJM and GM conceived the original idea. JCBA led the statistical analysis. RQ, TDP and JCBA wrote the first draft of the manuscript. ABO, WC and JJM aided with conceptualizing the study and edited/reviewed the manuscript. GM supervised analytical work, provided clinical feedback and edited/reviewed the manuscript. LS and RHG also provided critical inputs to earlier versions of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Linear, quadratic and cubic regression models for HbA_{1c} using glucose-like predictor (crude and adjusted models).
Table S2. Distribution of diabetes and prediabetes at sea level and high altitude considering HbA_{1c} or FPG standard cut-off points and including cases of diabetes diagnosed by physician and pharmacological treatment.
Figure S1. Comparison between ROC curves at sea level (blue) and high altitude (red), for HbA_{1c} standard cut-off points using FPG as the gold standard of diabetes.
Figure S2. Comparison between ROC curves at sea level (blue) and high altitude (red), for HbA_{1c} standard cut-off points using FPG as the gold standard of prediabetes.