

Glycated hemoglobin for the diagnosis of diabetes and prediabetes: Diagnostic impact on obese and lean subjects, and phenotypic characterization

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ABSTRACT

Aims/Introduction: Measurement of glycated hemoglobin (HbA1c) has been recommended for the diagnosis of diabetes and prediabetes. However, epidemiological studies have shown significant discordance between HbA1c and glucose-based tests. Of the factors that could influence agreement between HbA1c and the oral glucose tolerance test (OGTT), bodyweight has not been fully evaluated. The aims of the present study were to evaluate the impact of HbA1c criteria to diagnose diabetes and prediabetes compared with OGTT, and to examine HbA1c in relation to body mass index.

Materials and Methods: Two cohorts were studied, one from an obesity clinic ($n = 592$) and one from subjects undergoing screening for diabetes ($n = 462$). All underwent OGTT and HbA1c measurement.

Results: In the obese cohort, HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol) showed a sensitivity of 69.3% for diabetes, whereas HbA1c 5.7–6.4% (39–46 mmol/mol) did not identify prediabetes well (sensitivity 39.1%). In the diabetes screening cohort, HbA1c had low sensitivities for both diabetes (39.2%) and prediabetes (53.3%). When participants were stratified according to body mass index class I–III, HbA1c agreement with the OGTT for diabetes was much higher (80%, $P < 0.005$) in class I obesity compared with class II–III obesity; whereas for prediabetes, HbA1c had a low sensitivity in all obesity classes.

Conclusions: The agreement between HbA1c, fasting plasma glucose and 2-h glucose post-OGTT for the diagnosis of prediabetes was poor in our Italian population; whereas HbA1c $\geq 6.5\%$ showed a relatively good agreement with OGTT for the diagnosis of diabetes. For the first time, we have shown that obesity class influences the diagnostic performance of HbA1c.

INTRODUCTION

Glycated hemoglobin (HbA1c) is the gold standard for monitoring glycemic control in patients with diabetes mellitus. The HbA1c assay provides an accurate, precise measure of chronic glycemic levels, and correlates with the risk of diabetes

complications. The use of this test has been extended to diagnose and screen for diabetes mellitus with the endorsement of several international diabetes societies and the World Health Organization. In 2010, the International Expert Committee and the American Diabetes Association proposed diagnostic criteria for diabetes and prediabetes based on HbA1c levels. These are HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol) to diagnose diabetes mellitus and between 5.7–6.4% (39–46 mmol/mol) for prediabetes¹.

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Epidemiological studies have shown significant discordance between HbA1c and glucose-based tests for defining diabetes and prediabetes. For the diagnosis of diabetes, HbA1c showed 24% sensitivity and 99% specificity in the Dutch population². These levels of sensitivity and specificity were replicated in several other studies^{3–7}, all suggesting poor agreement between HbA1c, fasting plasma glucose (FPG) and 2-h plasma glucose (2 hPG).

Furthermore, the degree of diagnostic agreement of HbA1c criteria with the fasting and 2 h glucose-based criteria for prediabetes was also questioned^{8,9}, and might be different across ethnic groups and populations¹⁰, thus suggesting that the diagnostic performance of HbA1c will depend also on the target population. In a study by Mann *et al.*⁸, for example, prediabetes by the HbA1c criterion showed 27% sensitivity and 93% specificity, with 61% positive predictive value, a result confirmed by Heinaza *et al.*⁹, where a threshold of HbA1c 5.7% again showed low sensitivity (24%) with high specificity (91%), whereas HbA1c of 5.5% gave the highest combination of specificity (76%) and sensitivity (46%).

Obesity is one of the major risk factors for diabetes and impaired glucose regulation¹¹, and has reached epidemic proportions. It might be postulated that in obese subjects, at increased risk for glucose abnormalities, the efficacy of HbA1c could be higher than in normal weight people, and therefore of increased clinical utility. One recent study has shown a modest increased risk of prediabetes associated with obesity¹². However, to our knowledge, no studies have explored the impact of different grades of obesity (class I–III) on the efficacy of HbA1c to diagnose diabetes and prediabetes.

Furthermore, as several studies have shown a large proportion of patients that are discordantly categorized by HbA1c or OGTT, their phenotypic characterization needs to be assessed, in order to identify those parameters that could be of help in the choice of the most appropriate diagnostic tests.

Finally, only one study¹³ so far has analyzed the relationship between HbA1c and plasma glucose values for the diagnosis of prediabetes in the Italian population, showing again poor agreement between HbA1c and FPG.

Hence, the aims of the present study were to evaluate the impact of HbA1c criteria to diagnose diabetes and prediabetes in two large cohorts of participants undergoing OGTT, one recruited from an obesity clinic and one from a cohort undergoing screening for diabetes. Then, we aimed to investigate whether differences exist between obesity classes I–III with respect to the relationship of HbA1c and blood glucose. Finally, we examined the phenotypic characteristics of those participants who had a diagnosis of prediabetes with the OGTT, but had a normal HbA1c, comparing them with those that were concordant with both tests, aiming to identify specific clinical variables that might help to direct the choice of the most appropriate diagnostic test.

MATERIALS AND METHODS

Participants and Measurements

A total of 1,054 Caucasian Italian participants (mean age 48.8 ± 14.4 years) were selected. Of these, 592 were recruited from the Obesity Outpatient Clinic of the Endocrinology Unit, Department of Clinical Sciences, Sapienza University of Rome, Rome, Italy. These participants were referred for a complete evaluation of their excess weight, and undertook a medical and laboratory evaluation. The other 462 participants were recruited from patients undergoing a screening for diabetes at the Endocrinology and Diabetes Unit, Department of Medical Sciences, University of Cagliari, Cagliari, Italy. These participants had the indication from their GPs to screen for diabetes owing to the presence of risk factors, such as obesity, hypertension, dyslipidemia and diabetes in first-degree relatives. All 1,054 participants underwent a standard oral glucose tolerance test (OGTT).

For all patients, whole blood and serum samples were collected for measurement of HbA1c and serum glucose concentrations, respectively. Each method was carried out according to the 2013 American Diabetes Association recommendations¹. Serum glucose concentrations were measured by an automated enzymatic ultraviolet method, glucose-6-phosphate dehydrogenase/hexokinase, using a Miura 200 Chemistry analyzer (I.S.E. SRL, Rome, Italy). HbA1c was measured using a G8 analyzer (THOSO Diagnostics, Tokyo, Japan) by high-performance liquid chromatography, aligned with International Federation of Clinical Chemistry standardization, according to Diabetes Control and Complications Trial/Uniter Kingdom Prospective Diabetes guidelines.

Glucose tolerance status was assessed by the 75-g OGTT. In all participants, diagnosis of type 2 diabetes was based on either fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL) or plasma glucose ≥ 11.1 mmol/L (200 mg/dL) 2 h after the 75-g glucose load. Diagnosis of prediabetes was based on a fasting plasma glucose ≥ 5.6 mmol/L (100 mg/dL impaired fasting glucose [IFG]) or plasma glucose between 7.8–11.05 mmol/L (140–199 mg/dL impaired glucose tolerance [IGT]) 2 h after the OGTT.

For the entire cohort, after the OGTT, 0 min and 120 min insulin levels, body mass index (BMI) and blood pressure were recorded. For the subset of 592 patients from the obesity clinic, we also collected data regarding lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides), aspartate aminotransferase and alanine aminotransferase by standard laboratory methods. For the majority of patients, it was also possible to assess the presence or absence of lipid-lowering or antihypertensive therapy. Participants were considered dyslipidemic if presenting high lipid levels according to National Cholesterol Education Program – Adult Treatment Panel III¹⁴ and/or were treated with lipid-lowering agents, and the diagnosis of hypertension was based on the presence of elevated systolic (>140 mmHg) and/or diastolic (>90 mmHg) blood pressure, and/or the current use of antihypertensive medications.

The present study was reviewed and approved by the Ethics Committee of Policlinico Umberto I, Sapienza University of Rome and carried out in conformance with the Helsinki Declaration. Written consent was obtained from all patients before the study.

Statistical Analysis

Differences between groups were analyzed by Student's *t*-test for normally-distributed variables, by Mann–Whitney non-parametric independent sample test and by χ^2 -test for categorical variables, as appropriate.

Sensitivity, specificity, positive predictive value and negative predictive value for HbA1c were calculated. Cohen's kappa coefficient was used to measure the level of agreement between HbA1c and the diagnosis of diabetes or prediabetes by OGTT.

Data are expressed as means \pm standard deviation or SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of the Study Cohort

A total of 1,054 participants were studied, 592 from the obesity clinic cohort and 462 from the diabetes screening cohort.

The clinical characteristics of the two cohorts are shown in Table 1. As expected, significant differences in all metabolic and clinical parameters were observed between participants recruited from the obesity clinic compared with those coming from the diabetes screening program. To be noted, participants from the obesity clinic had significantly lower age, higher HbA1c, basal insulin and homeostatic model assessment of insulin resistance (all $P < 0.0001$). In Table 1, values for lipid, blood pressure and liver enzymes, available only for the obese participants, are also shown. As the two cohorts differed significantly in all parameters, we carried out the analyses in the two groups separately.

In the obesity clinic cohort, after the OGTT (Table 2), 166 (28.0%) of the 592 participants were diagnosed with diabetes, 173 (29.3%) with prediabetes (IFG and/or IGT) and 253 (42.7%) had normal glucose tolerance (NGT). In this cohort, 173 (29.3%) of the 592 participants were diagnosed with prediabetes; 78 (13.2%) were IFG, 51 (8.6%) were IGT and 44 (7.4%) had both conditions. Using HbA1c for diagnosis, 140 (23.6%) of the participants had a value $\geq 6.5\%$ (≥ 48 mmol/mol), 157 (26.57%) were within the prediabetes range (HbA1c 5.7–6.4%, 39–46 mmol/mol) and 295 (49.8%) had values $< 5.7\%$ (< 39 mmol/mol). Mean HbA1c levels were $7.7 \pm 1.8\%$, 5.8 ± 0.7 and $5.4 \pm 0.5\%$ in individuals diagnosed by OGTT as diabetes mellitus (166), IFG/IGT (173), and NGT (253), respectively.

In the diabetic screening cohort, after the OGTT (Table 2), 51 (11.0%) of the 462 participants were diagnosed with diabetes, 300 (65.0%) with prediabetes (IFG and/or IGT) and 111 (24.0%) had normal glucose tolerance (NGT). Within the 300 (65.0%) of the 462 participants diagnosed with prediabetes, 171 (37.0%) were IFG, 29 (6.3%) were IGT and 100 (21.6%) had

Table 1 | Phenotypic characteristics of study population

	Obesity clinic <i>n</i> = 592	Diabetes screening <i>n</i> = 462	<i>P</i> -value
AGE (years)	45.8 \pm 13.1	52.8 \pm 15.1	<0.0001
SEX (male/female)	180/412	165/297	0.070
BMI (kg/m ²)	41.9 \pm 7.6	26.2 \pm 4.3	<0.0001
SBP (mmHg)	129.0 \pm 15.7	–	–
DBP (mmHg)	82.9 \pm 10.5	–	–
FPG (mg/dL)	113.5 \pm 47.9	107 \pm 20.7	0.003
2hPG (mg/dL)	136.1 \pm 48.7	132.5 \pm 46.2	0.023
HbA1c (%)	6.1 \pm 1.4	5.7 \pm 0.8	<0.0001
Insulin basal (μ U/mol)	33 \pm 10.2	10.2 \pm 6.4	<0.0001
Insulin 120 (μ U/mol)	103.5 \pm 63.5	70.5 \pm 54.6	<0.0001
HOMA-IR (U)	9.2 \pm 10.1	2.7 \pm 1.7	<0.0001
AST (U/L)	24.6 \pm 15.4	–	–
ALT (U/L)	36.1 \pm 30.4	–	–
TC (mg/dL)	203.7 \pm 42.6	–	–
HDL-C (mg/dL)	47.9 \pm 12.6	–	–
LDL-C (mg/dL)	126.5 \pm 36.1	–	–
TG (mg/dL)	151.2 \pm 130.3	–	–
Hypertensive	77.4%	37.5%	<0.0001
Smokers*	69%	–	–
Physical activity	15.5%	–	–

2hPG, 2-h plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, total triglycerides. ²Data are expressed as mean \pm standard deviation. Differences between groups were analyzed by Student's *t*-test for normally-distributed variables and by Mann–Whitney *U*-test for non-parametric independent samples. Categorical variables were analyzed by the χ^2 -test. *Smokers include current and former smokers. *P*-values < 0.05 are considered significant.

Table 2 | Prevalence of participants diagnosed by the oral glucose tolerance test and glycated hemoglobin in the two studied cohorts

	HbA1c			Total
	<5.7%	5.7–6.4%	$\geq 6.5\%$	
<i>Diabetes screening cohort</i>				
OGTT				
DM	10 (19.6%)	21 (41.2%)	20 (39.2%)	51 (11.0%)
IFG/IGT	126 (42.0%)	144 (48.0%)	30 (10.0%)	300 (65.0%)
NGT	77 (69.4%)	33 (29.7%)	1 (0.9%)	111 (24.0%)
Total	213 (46.1%)	198 (42.9%)	51 (11.0%)	462
<i>Obesity clinic cohort</i>				
OGTT				
DM	17 (10.2%)	34 (20.5%)	115 (69.3%)	166 (28.0%)
IFG/IGT	89 (51.4%)	66 (38.2%)	18 (10.4%)	173 (29.3%)
NGT	189 (74.7%)	57 (22.5%)	7 (2.8%)	253 (42.7%)
Total	295 (49.8%)	157 (26.5%)	140 (23.6%)	592

DM, diabetes mellitus; HbA1c, glycated hemoglobin; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; NGT, normal glucose tolerance.

both conditions. Using HbA1c for diagnosis, 51 (11.0%) of the participants had a value $\geq 6.5\%$ (≥ 48 mmol/mol), 198 (42.9%) were within the prediabetes range (HbA1c 5.7–6.4%, 39–46 mmol/mol) and 213 (46.1%) had values $< 5.7\%$ (< 39 mmol/mol). Mean HbA1c levels were $6.6 \pm 1.6\%$, 5.8 ± 0.6 and $5.4 \pm 0.4\%$ in individuals diagnosed by OGTT as diabetes mellitus (51), IFG/IGT (300) and NGT (111), respectively.

Agreement Between HbA1c and OGTT Results

In the obesity clinic cohort, HbA1c performed better for the diagnosis of diabetes mellitus: 69.3% of the participants diagnosed as diabetic by OGTT had a HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol). However, merely 38.2% of the prediabetic participants by OGTT from the obesity clinic cohort had HbA1c between 5.7 and 6.4% (39–46 mmol/mol) (Table 2). It should be pointed out that the obesity clinic cohort had a low prevalence of IFG/IGT (29.3%); a possible explanation could be that these participants were relatively young, conceivably requiring more time to develop glucose abnormalities. The normoglycemic participants were well identified by HbA1c, with 74.7% of NGT participants by OGTT having HbA1c $< 5.7\%$ (< 39 mmol/mol). Agreement between HbA1c and diabetes was good (Cohen's $\kappa = 0.666$), but was poor between HbA1c and prediabetes ($\kappa = 0.202$).

In the diabetic screening cohort, just 39.2% of the participants diagnosed as diabetic by OGTT had HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol), and just 48% of the prediabetic participants by OGTT had HbA1c between 5.7 and 6.4% (39–46 mmol/mol; Table 2). In contrast, the normoglycemic participants were well identified by HbA1c, with 69.4% of NGT participants by OGTT having HbA1c $< 5.7\%$ (< 39 mmol/mol). Agreement between HbA1c and diabetes was fair ($\kappa = 0.317$), but was poor between HbA1c and prediabetes ($\kappa = 0.187$).

As HbA1c might reflect more post-glucose load values in the prediabetic stage¹⁵, we evaluated the agreement between HbA1c and each glycemic component of prediabetes. In the whole population (obese and diabetes cohorts), the concordance was very low with IGT (30%), intermediate with IFG (44.9%) and higher with IFG + IGT (51.4%). The results were similar when the two cohorts were individually analyzed (data not shown).

Sensitivity and Specificity of HbA1c for Diabetes and Prediabetes

In the obesity clinic cohort, HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol) for the diagnosis of diabetes, when compared with OGTT, showed a sensitivity of 69.3% (95% confidence interval [CI] 61.7–76.2%), with high specificity of 94.1% (95% CI 91.5–96.2%), and good positive and negative predictive values (82.1 and 88.7%).

On the contrary, HbA1c between 5.7% (39 mmol/mol) and 6.4% (46 mmol/mol) did not identify prediabetic subjects well. Sensitivity was just 39.1% (95% CI 31.1–47.5%), with a specificity of 76.8% (95% CI 71.1–81.9%), and positive and negative predictive values of 50.0 and 67.9%, respectively.

In the diabetes screening cohort, HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol) for the diagnosis of diabetes showed very poor sensitivity of 39.2% (95% CI 25.8–53.9%), with high specificity of 92.5% (95% CI 89.5–94.8%), and positive and negative predictive values of 39.2 and 92.5%, respectively.

Also, HbA1c between 5.7% (39 mmol/mol) and 6.4% (46 mmol/mol) did not identify prediabetic subjects in this cohort well. Sensitivity was just 53.3% (95% CI 47.2–59.4%), with a specificity of 70.0% (95% CI 60.5–78.4%), and positive and negative predictive values of 81.4 and 37.9%, respectively.

Effect of Obesity on the Performance of HbA1c on the Diagnosis of Diabetes and Prediabetes

As the obesity clinic cohort was selected by BMI criteria, we aimed to assess the possible influence of bodyweight on glycemic variables. Individuals were divided according to obesity class I–III. In this cohort, 111 participants (18.8%) were classified as class I obese (BMI 30–34.9 kg/m²), 150 (25.3%) as class II obese (BMI 35–39.9 kg/m²) and 331 (55.9%) as class III obese (BMI ≥ 40 kg/m²). The mean ages were: class I 54 ± 13 years, class II 46 ± 14 years and class III 44 ± 12 years.

In participants with class I obesity ($n = 111$), HbA1c performed very well for the diagnosis of diabetes mellitus: 80.0% of the participants diagnosed as diabetic by OGTT had HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol). As observed in the whole obesity cohort, just 35.5% of the prediabetic participants with class I obesity by OGTT had HbA1c between 5.7 and 6.4% (39–46 mmol/mol). The normoglycemic participants were well identified by HbA1c, with 75.6% of NGT patients by OGTT having HbA1c $< 5.7\%$ (< 39 mmol/mol).

In participants belonging to class II and class III obesity, the agreement between HbA1c and OGTT for the diagnosis of diabetes was significantly lower ($P < 0.005$) than class I obese participants; it was 58.3% for class II and 69.5% for class III obesity. Again, the diagnosis for prediabetes by HbA1c was inadequate, being just 44.7% for class II and 35.8% for class III obesity, and this could be related to the decreasing age in each BMI class I–III.

Phenotypic Characterization According to HbA1c Results

In our populations, we observed a high level of discordance between results from HbA1c and OGTT, particularly when analyzing data in the prediabetic range. In the whole population of 1,054 participants, just 210 (44.4%) of the prediabetic participants by OGTT had a concordant HbA1c of 5.7–6.4% (39–46 mmol/mol). In contrast, 215 (45.5%) participants had a positive OGTT for prediabetes, but had HbA1c within the normal range. Finally, 266 (73.1%) of the participants had both HbA1c and OGTT in the normal range. When the same analyses were carried out in the two cohorts separately, the results were very similar (data not shown).

We therefore examined the clinical and biochemical characteristics of three groups (NGT concordant with both OGTT and HbA1c, concordant for prediabetes with both OGTT and

HbA1c, and discordant for prediabetes), aiming to identify possible factors that might help to select those at higher risk for prediabetes that should be preferentially analyzed by OGTT.

As shown in Table 3, age increased significantly (all $P < 0.001$) from participants with both OGTT and HbA1c in the normal range, who were a mean age of 38 ± 12 years, to participants with a prediabetic OGTT, but a normal HbA1c (mean age 49 ± 15 years), to the participants with both tests in the prediabetic range (mean age 55 ± 12 years). Also, male sex was more associated with having both tests in the prediabetic diagnostic range. The homeostatic model assessment of insulin resistance was, as expected, lower in the NGT group with both tests compared with the others. Surprisingly, BMI was significantly higher in the group that was NGT with both tests. This could be explained by the fact that participants from the obesity clinic cohort were significantly younger than the participants recruited from the diabetes screening program. As, in our whole cohort, altered glucose levels were associated with increasing age, it follows that the younger obese participants tended to cluster in the NGT group. In agreement with this point, the obese participants in the Diabetes clinic cohort had a 65% prevalence of prediabetes, and had a mean age of 54 ± 12 years. Multivariate linear regression confirmed the independent association of increasing age, male sex and BMI (data not shown). All other variables, including insulin, number of hypertensive patients and number of smokers, were not significantly different between the groups.

Finally, a worst lipid profile was significantly ($P < 0.01$) associated with increasing age in the obesity clinic cohort, where this data was available (data not shown).

DISCUSSION

The present study shows the poor agreement between HbA1c, FPG and 2-h glucose post-OGTT for the diagnosis of prediabetes in this Italian population. We observed that HbA1c

was inadequate to diagnose prediabetes in two different cohorts, one characterized by the presence of only obese participants and one representative of the general population undergoing a screening for diabetes. Also, HbA1c performed quite badly in the diagnosis of prediabetes compared with OGTT independently of BMI. In particular, BMI obesity classes did not determine a change in performance of HbA1c, where only in the 35–40% of all cases HbA1c agreed with the diagnosis of prediabetes made through the OGTT. The poor agreement between HbA1c and OGTT for the diagnosis of prediabetes has been reported in other studies in different populations^{8,9,13}. The different pathophysiological mechanisms underlying abnormal glucose homeostasis could explain the differences between HbA1c and OGTT that were observed for the diagnosis of prediabetes. Hepatic insulin resistance and defective early-phase insulin secretion characterize IFG, resulting in the loss of control of fasting hepatic glucose production. Instead, muscle insulin resistance combined with defective late-phase insulin secretion, with almost normal hepatic insulin sensitivity, characterizes IGT, thus determining post-challenge hyperglycaemia¹⁶. Both IFG and IGT show fast glucose changes; HbA1c, in contrast, represents the chronic exposure to both basal and postprandial hyperglycemia over the previous 2–3 months. HbA1c, therefore, could reflect a combination of the pathophysiological defects underlying IFG and IGT over time. In fact, we observed the highest concordance with HbA1c when the two conditions of IFG + IGT were present together. These different pathophysiological mechanisms might explain the discordant diagnoses of prediabetes based on FPG, 2 hPG and HbA1c. However, when overt diabetes develops, all the aforementioned underlying mechanisms are operating, and this might explain the better concordance that we observed between OGTT and HbA1c to diagnose diabetes^{17–19}. Accordingly, in the obesity clinic cohort, the agreement between HbA1c and OGTT resulted much better for diabetes, a condition that, in this population, HbA1c

Table 3 | Clinical and biochemical characteristics of participants stratified according to glycated hemoglobin and oral glucose tolerance test results

OGTT HbA1c	Group 1 NGT <5.7% (n = 266)	Group 2 IFG/IGT <5.7% (n = 214)	P_1 1 vs 2	Group 3 IFG/IGT 5.7–6.4% (n = 210)	P_2 2 vs 3	P_3 1 vs 3
Age (years)	38 ± 12	49 ± 15	<0.001	55 ± 12	<0.001	<0.001
Sex (male/female)	65/201	67/147	0.094	101/109	<0.001	<0.001
BMI (kg/m ²)	36.2 ± 9.1	31.9 ± 9.2	<0.001	31.6 ± 9.1	0.782	<0.001
Insulin basal (μU/mol)	24.8 ± 16.9	25.4 ± 22.7	0.787	28.8 ± 24	0.280	0.730
Insulin 120 (μU/mol)	93.5 ± 61.9	107.8 ± 64.5	0.041	100.1 ± 63.7	0.404	0.367
HOMA-IR (U)	5.2 ± 3.7	6.4 ± 5.7	0.335	7.4 ± 6.0	0.210	0.008
Hypertensive	54%	52%	0.628	55%	0.493	0.800
Smokers*	65%	77%	0.131	68%	0.368	0.708

BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance. Data are expressed as mean \pm standard deviation. Differences between groups were analyzed by Student's *t*-test for normally-distributed variables, by Mann–Whitney non-parametric independent sample-test and by χ^2 -test for categorical variables, as appropriate. *P*-values <0.05 are considered significant. *Smokers include current and former smokers. Group 1: participants with both oral glucose tolerance test (OGTT) and glycated hemoglobin (HbA1c) in the normal glucose tolerance (NGT) range. Group 2: participants with OGTT in the prediabetes range and normal HbA1c. Group 3: participants with both OGTT and HbA1c in the prediabetes range.

identifies with a sensitivity of 69.3%. Furthermore, 80% of the participants with class I obesity that were diagnosed with diabetes by the OGTT were identified by HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol), suggesting that, in this category of subjects, HbA1c is a very good marker of diabetes. HbA1c identified diabetic participants with class II and III obesity with less sensitivity. As class II and class III participants were significantly younger than class I obese participants, it could be speculated that they might not have had the time to develop the chronic hyperglycemia that is necessary to affect HbA1c levels. Also, the pathophysiological mechanisms that underlie severe obesity could differ from those present in class I obesity²⁰.

Finally, participants from the diabetes screening cohort, representative of the general population undergoing screening for diabetes mellitus, were not well identified as diabetic by HbA1c, with just 39% of the diabetic participants diagnosed by OGTT that were identified by HbA1c.

As pointed out by the American Diabetes Association¹, the characterization of subjects discordantly categorized by HbA1c or OGTT is warranted, in order to identify variables that could help to indicate the best possible test to be prescribed. To this aim, we evaluated the phenotype of our participants that were diagnosed as prediabetic by the OGTT, but were not detected by HbA1c. The most significant variable that differed between participants discordantly categorized by HbA1c and OGTT compared with participants concordant for NGT or for prediabetes was their age (Table 3). Above the age of 55 years, participants were most likely to have both tests above the diagnostic thresholds, whereas participants aged < 50 years were highly discordant, with just 32.6% of them having HbA1c in the prediabetes range. Furthermore, male sex was also significantly associated with having both HbA1c and OGTT tests concordant for prediabetes. Thus, it might be hypothesized that in male subjects above the age of 55 years, HbA1c could be the test of choice for the diagnosis of prediabetes; whereas in younger subjects, the use of the OGTT might be preferable. Obviously, if possible, the combination of more tests (for example HbA1c and FPG) might be the best option, although less cost-effective, as previously shown by other studies⁹.

The present study had some limitations. First, the cross-sectional design of the study did not allow measuring long-term outcomes. However, the present results are in line with other studies that have found similar data in longitudinal observations^{9,21}. Second, our results are derived from single blood measurements, reflecting standard clinical practice. Thus, individual and daily changes in FPG and 2h post-OGTT glucose cannot be evaluated, and this is a common limitation of most epidemiological studies. Finally we did not look simultaneously for blood disorders, and this might have carried over some cases of low hemoglobin levels due to other causes. However, the present results are highly consistent with other studies, suggesting that the prevalence of hemoglobinopathies and of other blood disorders, if any present, was not high enough to affect the final results.

In summary, we have shown that the agreement between HbA1c, FPG and 2-h glucose post-OGTT for the diagnosis of prediabetes was very poor in our two Italian cohorts. We have also observed a relatively good agreement between HbA1c $\geq 6.5\%$ (48 mmol/mol) and the diagnosis of diabetes.

For the first time, to our knowledge, we have shown that a specific class of obesity positively influences the diagnostic performance of HbA1c for the diagnosis of diabetes, which is much better in class I obese subjects. However, when searching for prediabetes, OGTT and HbA1c were persistently in poor agreement. Finally, in class I obese subjects, we identified specific characteristics, such as male sex and age > 55 years, that might help to ascertain subjects that are more likely to be recognized as prediabetic with both tests, and in which the use of HbA1c might be preferred over OGTT for economic and practical reasons.

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