Increased Hemoglobin A1c Threshold for Prediabetes Remarkably Improving the Agreement Between A1c and Oral Glucose Tolerance Test Criteria in Obese Population

Jie Li,* Hao Ma,* Lixin Na, Shuo Jiang, Lin Lv, Gang Li, Wei Zhang, Guanqiong Na, Ying Li, and Changhao Sun

National Key Discipline, Department of Nutrition and Food Hygiene, School of Public Health, Harbin Medical University, 150081 Harbin, China

Context: It is unclear why the prevalence of diabetes and prediabetes, especially prediabetes, between diagnosed by oral glucose tolerance test (OGTT) and hemoglobin A1c (HbA1c) criteria, is substantially discordant.

Objective: We aimed to evaluate the effects of obesity on the agreement between HbA1c and OGTT for diagnosing diabetes and prediabetes and identify the optimal HbA1c cutoff values in different body mass index (BMI) classifications.

Design Setting and Participants: In a population-based, cross-sectional study in Harbin, China, 4325 individuals aged 20–74 years without a prior diagnosed diabetes were involved in this study.

Outcome: measure The performance and optimal cutoff points of HbA1c were assessed by receiver-operating characteristic curve. The contribution of BMI to HbA1c was analyzed by structural equational model.

Results: The agreement between HbA1c criteria and OGTT decreased with BMI gain (κ = 0.359, 0.312, and 0.275 in a normal weight, overweight, and obese population, respectively). The structural equational model results showed that BMI was significantly associated with HbA1c in normal glucose tolerance and prediabetes subjects but not in diabetes subjects. At a specificity of 80% for prediabetes and 97.5% for diabetes, the optimal HbA1c cutoff points for prediabetes and diabetes were 5.6% and 6.4% in normal-weight, 5.7% and 6.5% in overweight, and 6.0% and 6.5% in an obese population. When the new HbA1c cutoff values were used, the agreement in obese subjects increased almost to the level in normal-weight subjects.

Conclusions: The poor agreement between HbA1c and OGTT criteria in an obese population can be significantly improved through increasing the HbA1c threshold for prediabetes. (***J Clin Endocrinol Metab*** **100: 1997–2005, 2015)**

In 2010, the American Diabetes Association (ADA) recommended the use of glycated hemoglobin (HbA1c) to diagnose diabetes and prediabetes (1). Although numerous cross-sectional and longitudinal studies indicate that HbA1c is correlated with risk of diabetes and diabetes-related comorbidities (2–4), it is worth noting that not only the of rate of hyperglycemia diagnosed by an oral glucose tolerance test (OGTT) and HbA1c criteria was different but also the overlap between glycemic classification as defined by OGTT and HbA1c criteria was limited

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 18, 2014. Accepted March 3, 2015.
First Published Online March 9, 2015

* J.L. and H.M. contributed equally to this work.

Abbreviations: ADA, American Diabetes Association; AUC, area under the curve; BMI, body mass index; BW, body weight; CI, confidence interval; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; 2h-glucose, 2-hour plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MDA, methane dicarboxylic aldehyde; NGT, normal glucose tolerance; NPV, negative predictive value; OGTT, oral glucose tolerance test; PPV, positive predictive value; SEM, structural equation modeling; T-AOC, total antioxidative capacity; WC, waist circumference; WHO, World Health Organization.

in Chinese (5, 6) and other populations (7–9). However, it is still unclear why the prevalence of diabetes and prediabetes between diagnosed by OGTT and HbA1c criteria is substantially discordant.

HbA1c is the production of the glycation of hemoglobin. The concentration of HbA1c, which reflects the average blood glucose levels over the previous 3 months, depends on both prevailing glucose concentrations and the factors affecting the rate of glycation. It has been reported that oxidative stress is a key determiner of glycation rate and that the elevated oxidative stress is associated with increased HbA1c concentrations in nondiabetic subjects (10, 11). Furthermore, an in vitro study on human erythrocytes demonstrated that lipid peroxides directly affect glycated hemoglobin levels independently of glucose concentration (12). Therefore, oxidative stress may partly explain the discordance between HbA1c and blood glucose diagnosing diabetes and prediabetes.

Obesity is a major public health issue in both developed and developing countries. The recently national survey showed that 31.4% of Chinese adults were overweight, 12.2% were obese, and 27.1% were centrally obese (13). It is reported that obesity per se can induce systemic oxidative stress (14) and is associated with increased glycation of hemoglobin independently of glucose levels (15, 16). Thus, HbA1c concentrations may be disproportionately elevate at a given glycemic level in obese subjects. In other words, HbA1c cannot reflect the real concentration of glucose in obese subjects. Diabetes is a disorder of glucose, not HbA1c, metabolism. It is necessary to clarify whether the diagnostic performance of HbA1c at the given cutoff point depends on the body mass index (BMI) of the target population.

In this study, we aimed to evaluate whether obesity affected the performance of HbA1c in diagnosing diabetes and prediabetes against a standard OGTT, which was partly mediated by oxidative stress, and to identify the optimal HbA1c cutoffs in normal body weight (BW), overweight, and obese population in a large cross-sectional study in Harbin, China.

Research Design and Methods

Study population

In this study, the participants were from a population-based, cross-sectional diabetes survey that was conducted in Harbin, China, in 2008. A stratified, multistage, random cluster sampling design was used to recruit a representative sample of those in the general population who have lived in Harbin for at least 5 years. The details of the study design had been reported elsewhere (17). Briefly, a total of 8940 individuals aged 20–74 years were invited to participate in the survey, and 8127 responded (90.9%). In this study, the inclusion criteria were as follows: 1) provided written informed consent, 2) without a prior diagnosed diabetes identified based on fasting plasma glucose (FPG) and 2-hour plasma glucose (2h-glucose) criteria or using medications that may affect glucose metabolism, 3) that FPG, OGTT, and HbA1c were measured on the same day, 4) no missing data for BMI and waist circumference (WC), 5) body weights were stable (<3 kg change over the past 3 mo), and 6) without anemia, liver diseases, or chronic kidney disease. A total of 4325 individuals met the inclusion criteria. This study was approved by the Ethics Committee of Harbin Medical University and in accordance with the Declaration of Helsinki.

Procedures

Subjects arrived at the local community clinics at 7:00 AM after a 10-hour overnight fast. The fasting blood samples were collected from the antecubital vein into a vacuum tube containing sodium EDTA2 over the period of 7:00–9:30 AM. The fasting plasma samples were used to detect glucose, HbA1c, insulin, methane dicarboxylic aldehyde (MDA), and total antioxidative capacity (T-AOC). A standard 75-g OGTT was also performed on the same day over the period of 7:00–11:30 AM, and blood samples for glucose determinations were collected the same as the fasting blood samples collection.

The physical examination including height, fasting body weight, and WC were measured according to the standard protocols (17). BMI was calculated as weight in kilograms divided by height in meters squared.

Demographic data including age, gender, cigarette smoking, alcohol use, previous medical history, and family history of diabetes were collected using a standardized questionnaire (17).

Biochemical analyses

Plasma glucose was measured quantitatively by the glucose oxidase method. Plasma insulin was measured by immunofluorescence method (TOSOH automated enzyme immunoassay analyzer AIA-2000ST). The homeostasis model assessment of insulin resistance was calculated with the formula, FPG (millimoles per liter) × fasting insulin (milliinternational units per liter)/22.5, and the homeostasis model of β-cell function was calculated with the formula, 20 × fasting insulin (milliinternational units per liter)/FPG (millimoles per liter) − 3.5 (18). HbA1c levels were measured on the same day of OGTT by HPLC (Bio-Rad VARIANT 2) calibrated against the National Glycosylated Standardization Program. The HbA1c interassay and intraassay coefficients of variation were 1.2% at a

value of 5.8% and 0.7% at a value of 8.0%. Plasma MDA and T-AOC were measured with commercial kits using enzymatic methods (Jiancheng Technology).

Definitions

The 1999 World Health Organization (WHO) OGTT criteria are considered as the gold standard for diagnosing diabetes (19, 20): type 2 diabetes was defined as FPG of 7.0 mmol/L or greater or 2h-glucose of 11.1 mmol/L or greater; prediabetes was defined as an individual showed impaired fasting glucose (IFG; FPG $\geq$ 6.1 and $\leq$ 6.9 mmol/L, and 2h-glucose $<$ 7.8 mmol/L) or impaired glucose tolerance (IGT; FPG $<$ 7.0 mmol/L and 2h-glucose $\geq$ 7.8 and $<$ 11.1 mmol/L) or both. The glycemia status by HbA1c was classified according to ADA criteria (diabetes $\geq$ 6.5% and prediabetes 5.7–6.4%) (1).

Using the Chinese criteria, obesity was defined as a BMI of 28.0 kg/m^2 or greater, and overweight was defined as a BMI of 24.0 kg/m^2 or greater and less than 28.0 kg/m^2 (21).

Statistical methods

The differences between the means of the HbA1c categories were tested using a univariate general linear model with adjustments for age and sex, and categorical data were analyzed by using the χ^2 test. The agreements between the diagnoses resulting from HbA1c and OGTT criteria were estimated by calculating of the Cohen's κ-coefficient. Using OGTT as the gold standard, the diagnostic value for HbA1c was assessed for sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs). The receiver-operating characteristic curve analysis was performed to identify the optimal threshold for HbA1c among different BMI classifications. Diagnostic accuracy was assessed by the area under the curve (AUC) (22).

To assess whether the direct effect of BMI on HbA1c is independent of the indirect effect of BMI on HbA1c mediated by other variables, a statistical analysis was performed using structural equation modeling (SEM) and path diagram analysis by IBM SPSS Amos (23). Values of variables used in SEM were standardized. HbA1c was set as the dependent variable, and BMI was set as the independent variable. MDA, T-AOC, FPG, and 2h-glucose were used as mediator variables. All statistical analyses were performed using SPSS version 20.0 (IBM Corp USA). Two-sided $P < .05$ was considered to be statistically significant.

Results

Characteristics of the participants according to HbA1c categories

The representative sample of this cross-sectional study consisted of 4325 participants (1554 men and 2771 women) aged 51.8 $\pm$ 10.7 (range 20.1–74.0) years. The average BMI and HbA1c level in the entire population were 25.3 $\pm$ 3.5 kg/m^2 and 5.6 $\pm$ 0.6%, respectively. BMI was significantly correlated with HbA1c ($r = 0.133$, $P <$ .001) after adjusting for age, sex, FPG, and 2h-glucose.

In Table 1, we stratified the population according to 2010 ADA HbA1c criteria: 62.2% were in the normal

Table 1. Characteristics of Participants According to HbA1c Categories

	All (n = 4325)	HbA1c < 5.7 (n = 2692)	HbA1c ≥ 5.7 to ≤ 6.4(n = 1390)	HbA1c > 6.4 (n = 243)
Demographic				
Age, y	51.82 ± 10.66	49.92 ± 10.52	54.75 ± 10.12[a]	56.12 ± 10.08[a]
Sex (M/F)	1554/2771	907/1785	550/840	97/146
BMI, kg/m^2	25.28 ± 3.49	24.69 ± 3.48	26.27 ± 3.28[a]	26.21 ± 3.37[a]
WC, cm	86.16 ± 10.74	84.47 ± 10.72	88.72 ± 10.28[a]	90.21 ± 9.57[a]
Smokers, %	17.1	16.3	18.1	21.0
Drinkers, %	34.9	35.9	33.4	33.3
Glucose metabolism				
FPG, mmol/L	4.71 ± 1.18	4.38 ± 0.67	5.01 ± 0.98[a]	7.03 ± 2.74[a]
2h-glucose, mmol/L	6.39 ± 2.87	5.54 ± 1.63	7.00 ± 2.59[a]	12.31 ± 5.84[a]
Fasting insulin, μU/L	8.75 ± 7.89	8.14 ± 7.60	9.69 ± 8.48[a]	10.13 ± 6.95[a]
HOMA-IR	1.90 ± 2.04	1.63 ± 1.78	2.20 ± 2.22[a]	3.18 ± 2.80[a]
HOMA-B	155.07 ± 148.82	166.89 ± 155.51	148.15 ± 143.12[a]	91.72 ± 90.94[a]
HbA1c, %	5.64 ± 0.59	5.32 ± 0.24	5.97 ± 0.23[a]	7.32 ± 0.86[a]
Oxidative stress				
MDA, nmol/mL	2.86 ± 1.43	2.60 ± 1.38	3.15 ± 1.88[a]	4.24 ± 3.52[a]
T-AOC, U/mL	4.15 ± 2.10	4.51 ± 1.97	3.59 ± 1.90[a]	2.18 ± 1.76[a]

Abbreviations: HOMA-B, homeostasis model of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance. Data are means ± SD or percentage.

[a] $P < .001$ for the difference between the indexed and the HbA1c less than 5.7% category using a univariate general linear model adjusted for age and sex.

Table 2. Glycemic Classification by WHO OGTT and ADA HbA1c Criteria in Different BMI Categories

	ADA HbA1c			
WHO OGTT	< 5.7	≥ 5.7 to ≤ 6.4	> 6.4	All
Normal				
NGT	1181	230	21	1432
Prediabetes	84	118	12	214
Diabetes	8	29	30	67
Total	1273	377	63	1713
Overweight				
NGT	860	382	20	1262
Prediabetes	107	199	23	329
Diabetes	9	59	60	128
Total	976	640	103	1719
Obesity				
NGT	380	245	9	634
Prediabetes	57	106	20	183
Diabetes	6	22	48	76
Total	443	373	77	893

Data are n. κ-Coefficients with 95% CI were 0.359 (0.312–0.406), 0.312 (0.273–0.351), and 0.275 (0.222–0.328) in normal-BW, overweight, and obese subjects, respectively.

glucose tolerance (NGT) category, 32.1% were in the prediabetes category, and 5.6% were in the diabetes category. Across the HbA1c categories, subjects tended to be older and heavier in the prediabetes and diabetes groups ($P < .001$). Subjects in these latter two categories also had higher FPG, 2h-glucose, fasting insulin, and insulin resistance ($P < .001$), and lower β-cell function ($P < .001$) than subjects in the NGT category. Compared with the subjects in the NGT category, the subjects in both prediabetes and diabetes categories had higher plasma MDA levels and lower plasma T-AOC levels ($P < .001$).

Agreement between HbA1c categories and OGTT status

Among the 4325 participants, the OGTT showed that 16.8% had prediabetes (IFG, IGT, and IFG/IGT) and 6.3% had diabetes. By the ADA HbA1c criteria, 32.1% had prediabetes and 5.6% had diabetes. Although the majority of the subjects who were NGT by OGTT criteria (72.7%) were classified as NGT by HbA1c criteria, 25.8% were misclassified with prediabetes and 1.5% were misclassified with diabetes. Of the subjects with prediabetes by OGTT criteria, 34.2% were NGT and 7.6% were diabetes by HbA1c criteria. Of the subjects with diabetes by OGTT criteria, there were 8.5% classified as NGT and 40.6% classified as prediabetes by HbA1c criteria. The agreement between OGTT and HbA1c criteria decreased with the BMI gain (κ-coefficients were 0.359, 0.312, and 0.275 in normal BW, overweight, and obese subjects, respectively) (Table 2).

Performance of HbA1c on the diagnosis of diabetes and prediabetes

In the whole population, HbA1c had 54.3% sensitivity, 79.5% specificity, 35.1% PPV, and 89.5% NPV for diagnosis of prediabetes compared with OGTT as well as 50.8% sensitivity, 97.4% specificity, 56.1% PPV, and 96.6% NPV for diagnosis of diabetes compared with OGTT (Table 3).

The specificity of HbA1c for diagnosis of prediabetes, but not diabetes, decreased from 89.4% in normal BW subjects to 60.8% in obese subjects and the NPV of HbA1c for diagnosis of prediabetes, but not diabetes, decreased from 92.0% in normal BW subjects to 87.0% in obese subjects (Table 3).

Diagnostic accuracy of A1c

The AUCs shown in Figure 1 represented the diagnostic accuracy of the HbA1c, compared with OGTT, for prediabetes and diabetes in different BMI classifications. In the whole population, the AUCs for detecting prediabetes and newly diagnosed diabetes were 0.74 [95% confidence interval (CI) 0.72–0.75] and 0.89 (95% CI 0.88–0.90), respectively.

For prediabetes, the AUC was significantly lower in obese subjects than in normal BW and overweight subjects (obesity vs normal, 0.66 vs 0.77, $P < .001$; and obesity vs

Table 3. Sensitivity, Specificity, PPVs, and NPVs of HbA1c for Diagnosis of Prediabetes and Diabetes Stratified by BMI (OGTT Was Considered as the Gold Standard)

	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Prediabetes				
All (n = 4325)	54.3 (50.4–58.1)	79.5 (78.0–80.8)	35.1 (32.2–38.1)	89.5 (88.3–90.5)
Normal (n = 1713)	46.0 (39.0–53.2)	89.4 (87.7–91.0)	38.4 (32.3–44.9)	92.0 (90.5–93.4)
Overweight (n = 1719)	53.9 (48.2–59.6)	77.5 (75.1–79.8)	37.2 (32.7–41.8)	87.2 (85.1–89.1)
Obesity (n = 893)	65.0 (57.2–72.3)	60.8 (56.8–64.6)	30.2 (25.4–35.3)	87.0 (83.4–90.0)
Diabetes				
All (n = 4325)	49.5 (43.3–55.6)	97.4 (96.9–97.9)	56.1 (49.5–62.5)	96.6 (96.0–97.2)
Normal (n = 1713)	44.8 (32.6–57.4)	98.0 (97.2–98.6)	47.6 (34.8–60.7)	97.8 (96.9–98.4)
Overweight (n = 1719)	46.1 (37.2–55.1)	97.3 (96.4–98.0)	57.8 (47.6–67.6)	95.7 (94.6–96.7)
Obesity (n = 893)	59.2 (47.3–70.4)	96.5 (94.9–97.6)	60.8 (48.7–72.0)	96.2 (94.7–97.4)

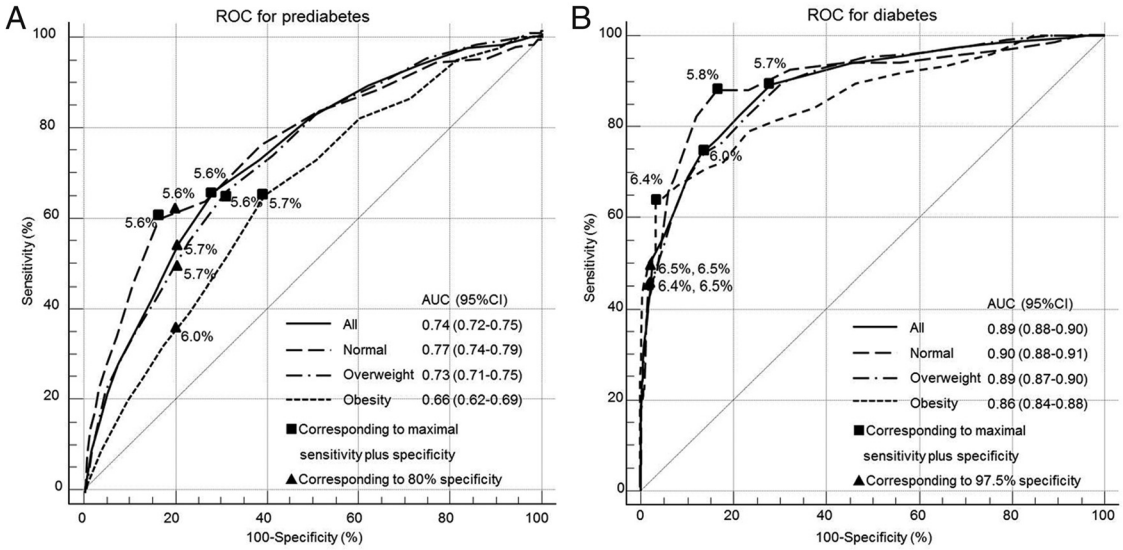

Figure 1. Receiver operating characteristics (ROC) curve of HbA1c for detecting prediabetes (A) and newly diagnosed diabetes (B) in all, normal body weight, overweight, and obese subjects

overweight, 0.66 vs 0.73, $P = .004$). The optimal threshold for maximal sensitivity and specificity of HbA1c for prediabetes in normal BW, overweight, and obese subjects were 5.6% (with a sensitivity of 60.4% and specificity of 83.7%), 5.6% (with a sensitivity of 65.0% and specificity of 69.2%), and 5.7% (with a sensitivity of 65.6% and specificity of 60.8%), respectively. However, these cutoff values yielded inconsistent specificity. At a 80% specificity, the cutoff values for prediabetes were 5.6%, 5.7%, and 6.0% in the normal BW, overweight, and obese population, respectively (Table 4 and Figure 1A).

In the diabetes category, the diagnostic accuracy of HbA1c was not significantly different among normal BW, overweight, and obese subjects. The optimal threshold for maximal sensitivity and specificity of HbA1c for diabetes in normal BW, overweight, and obese subjects were 5.7% (with a sensitivity of 88.1% and specificity 83.3%), 6.0% (with a sensitivity of 74.2% and specificity 86.3%), and 6.4% (with a sensitivity of 63.2% and specificity 96.5%), respectively. At a 97.5% specificity, the cutoff values for diabetes were 6.4%, 6.5%, and 6.5% in the normal BW, overweight, and obese population, respectively (Table 4 and Figure 1B).

When the HbA1c cutoff values corresponding to 80% specificity for prediabetes and 97.5% specificity for diabetes were used to diagnose prediabetes and diabetes in this study population, the aforementioned agreement between HbA1c criteria and OGTT decreasing with BMI grain disappeared ($\kappa = 0.35$, 0.34, and 0.32 in normal BW, overweight, and obese population, respectively, Supplemental Table 1).

Decomposition of direct and indirect effect of BMI on HbA1c

Figure 2 showed the SEM for HbA1c in each glucose tolerance group. The degree of the direct effect of BMI on

Table 4. HbA1c Cutoff Values for Detecting Newly Diagnosed Diabetes and Prediabetes Corresponding to Maximal Youden Index or the Default Specificities

	HbA1c Cutoff, %[a]	Sensitivity, %[a]	Specificity, %[a]	HbA1c Cutoff, %[b]	Sensitivity, %[b]
Prediabetes					
All	5.6	65.6 (61.8–69.2)	71.7 (70.1–73.2)	5.7	53.1 (48.6–57.5)
Normal	5.6	60.4 (53.3–67.2)	83.7 (81.7–85.6)	5.6	61.9 (55.5–68.6)
Overweight	5.6	65.0 (59.4–70.4)	69.2 (66.6–71.8)	5.7	49.7 (42.9–56.2)
Obesity	5.7	65.0 (57.2–72.3)	60.8 (56.8–64.6)	6.0	35.5 (27.7–44.3)
Diabetes					
All	5.8	82.7 (77.6–87.0)	78.6 (77.3–79.9)	6.5	48.6 (41.7–55.8)
Normal	5.7	88.1 (77.8–94.7)	83.3 (81.4–85.1)	6.4	45.7 (33.1–57.5)
Overweight	6.0	74.2 (65.7–81.5)	86.3 (84.5–88.0)	6.5	45.2 (36.6–53.5)
Obesity	6.4	63.2 (51.3–73.9)	96.5 (94.9–97.6)	6.5	50.9 (38.7–64.5)

[a] HbA1c cutoff values with sensitivity and specificity for detecting newly diagnosed diabetes and prediabetes corresponding to maximal Youden index.

[b] HbA1c cutoff values with sensitivity corresponding to 80% (for prediabetes) and 97.5% (for diabetes) specificity.

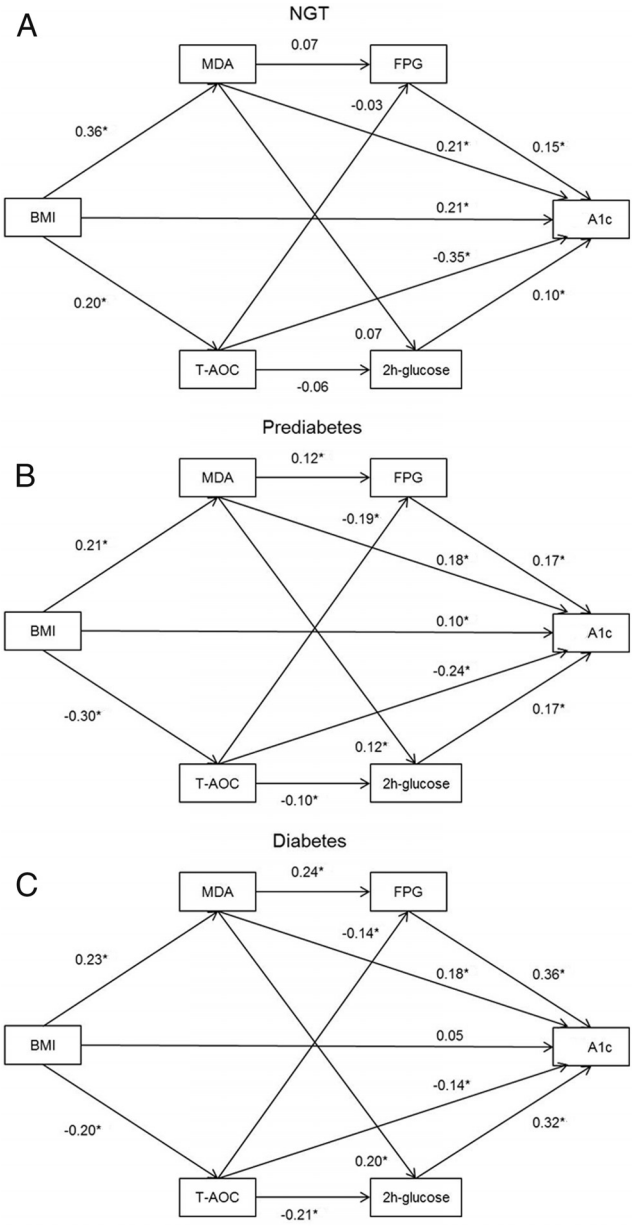

Figure 2. Structural equational models for the HbA1c levels in NGT (A), prediabetes (B), and diabetes groups (C). *, $P < .001$.

HbA1c was the most prominent in the NGT group (0.21, $P < .001$), followed by the prediabetes (0.10, $P < .001$) and diabetes (0.05, $P = .089$) groups, in that order.

In each group, there were significant effects of BMI on MDA and T-AOC; moreover, the indirect effects of BMI on HbA1c, which is mediated by MDA and T-AOC, were significant (MDA→HbA1c: 0.21, T-AOC→HbA1c: −0.35 in the NGT group; MDA→HbA1c: 0.18, T-AOC→HbA1c: −0.24 in the prediabetes group; MDA→HbA1c: 0.18, T-AOC→HbA1c: −0.14 in the diabetes group). In the NGT group, the effects of MDA and T-AOC on FPG and 2h-glucose were not observed. In contrast, in the prediabetes and diabetes groups, the indirect effects of BMI on HbA1c, which were mediated by MDA→FPG or 2h-glucose and T-AOC→FPG or 2h-glucose, were significant.

Discussion

This population-based study showed that the agreement between HbA1c and OGTT criteria in classifying subjects' glycemia decreased with the increased BMI. We observed that the specificity of HbA1c with OGTT as the reference for screening prediabetes, but not newly diagnosed diabetes, was significantly lowered in the obese subjects compared with the normal-BW subjects. Corresponding to the 80% specificity, the HbA1c cutoff values for prediabetes increased from 5.6% in normal BW to 6.0% in obese subjects; by contrast, the HbA1c cutoff values for diabetes were relatively stable among subjects with different BMIs. This phenomenon was partly explained by the results of SEM analysis, which showed that BMI was significantly associated with HbA1c levels in the NGT and prediabetes population but not in the diabetes population, defined by OGTT criteria. Furthermore, the association between BMI and HbA1c was partly mediated by MDA and T-AOC.

The concerns about the utility of HbA1c for diagnosing diabetes and prediabetes have been recently raised. Many studies showed the poor agreement between HbA1c and OGTT for the diagnosis of prediabetes in different populations (9, 24, 25) and the better concordance between HbA1c and OGTT to diagnose diabetes (26, 27), which were in agreement with our study. However, we designed this study not to simply verify the performance of HbA1c in predicting diabetes and prediabetes but mainly to investigate the effects of obesity on the performance of HbA1c for the diagnosis of diabetes and prediabetes and to identify the optimal HbA1c cutoff points in the normal BW, overweight, and obese population. Unlike glucose, HbA1c does not directly reflect glycemia, but rather measures the proportion of hemoglobin proteins that have been bound by glucose. Thus, the HbA1c level is affected by a multitude of factors in addition to prevailing glucose concentrations (28). BMI is reported to be associated with HbA1c independent of glucose concentration (15, 16). However, the effect of obesity on the diagnostic performance of HbA1c has not yet been well studied in adults. Our results demonstrated that HbA1c represented a poorer diagnostic tool for prediabetes, but not diabetes, in obese adults compared with that in normal BW subjects.

Although different HbA1c cutoff points have been reported to diagnose diabetes and prediabetes in previous population-based studies (5, 29, 30), BMI of the target population was not considered seriously in these studies,

which may induce lowered efficiency of HbA1c in the obese population. In this study, we determined the cutoff values using the maximal value of the Youden index for prediabetes (5.7% with a sensitivity of 54.3% and specificity of 79.5%) and diabetes (6.5% with a sensitivity of 49.5% and specificity of 97.4%) in the whole population at first, which were consistent with the ADA criteria (1). It is worth noting that the specificity of the cutoff of 5.7% for diagnosing prediabetes significantly decreased from 89.4% in the normal BW population to 60.8% in the obese population. The ADA International Expert Committee agreed to emphasize specificity rather than sensitivity after balancing the stigma and costs of mistakenly identifying individuals as diabetic against the minimal clinical consequences of delaying the diagnosis (3). Therefore, to make sure the specificity of HbA1c diagnosing prediabetes and diabetes among every BMI classification was at the unified high level, we calculated the cutoff values again at the default specificities of 80% for prediabetes and 97.5% for diabetes in the normal BW, overweight, and obese population, respectively. The specificities of 80% and 97.5% were selected based on our results showing that the specificities of HbA1c of 5.7% and 6.5% diagnosing prediabetes and diabetes in our whole population were 79.5% and 97.4%, respectively. We first found that the HbA1c cutoff values for diagnosing prediabetes increased with BMI (5.6% in normal BW, 5.7% in overweight, and 6.0% in obese subjects) corresponding to 80% specificity. Although the HbA1c cutoff values for diagnosing diabetes remained relatively stable in every BMI classification corresponding to 97.5% specificity.

When the new HbA1c cutoff values corresponding to the default specificities were used, the agreement between HbA1c and OGTT criteria in the obese population increased to the level in the normal-BW population, which suggests that the part of the discordance induced by obesity is improved. However, the improved agreement is still low ($\kappa < 0.4$). This can be interpreted as that the contribution of obesity on the discordance between HbA1c and OGTT for glycemia classification is limited. Further research is needed to identify much more important factors that significantly influence the agreement between HbA1c and OGTT. Nevertheless, given that obesity has become one of the most serious worldwide public health problems (31), it is necessary to consider subjects' BMI when HbA1c is used for glycemia classification; otherwise, a large amount of obese subjects may be misdiagnosed.

The glycation of hemoglobin is determined not only by ambient glucose concentrations but also by many factors unrelated to glucose metabolism (28), which influence the HbA1c level in NGT more than in diabetes (32). To illustrate why BMI influenced the HbA1c cutoff points for diagnosing prediabetes, but not diabetes, we used SEM to decompose the association between BMI and HbA1c in different glucose tolerance groups. Our data show that the associations between BMI and HbA1c are reduced when glucose values are close to abnormal because the relative contribution of glucose concentration becomes more important, which is consistent with the previous studies (6, 16). The variations in HbA1c in diabetes subjects are not less than that in NGT and prediabetes subjects in our study. So it is not possible that the lack of variation in HbA1c in overt diabetes impedes the ability to detect a linear relationship between BMI and HbA1c. Therefore, we speculate that BMI is associated with HbA1c level in NGT and prediabetes, but not in diabetes, in this study because hyperglycemia may mask the contribution of BMI on HbA1c in diabetes. We also can conclude that in NGT and prediabetes categories, HbA1c cannot reflect the real glucose level in obese subjects, which results in the discordance between HbA1c and OGTT.

Furthermore, the results of the SEM analysis showed that the association between BMI and HbA1c was mediated by oxidative stress as determined by plasma MDA and T-AOC levels. Although BMI does not distinguish fat mass and lean mass, it is the most frequently used index of obesity. Obesity has been reported to be a strong independent predictor of systemic oxidative stress (14, 33). Oxidative stress affects the HbA1c level through two ways. First, the glycation of hemoglobin is a two-step Maillard reaction, which involves the initial formation of a labile Schiff base and a subsequent Amadori rearrangement (28). Oxidative stress facilitates the autoxidation of glucose to dicarbonyl intermediates in an early step of the Maillard reaction and then enhances the glycation of proteins (34). Second, oxidative stress results in insulin resistance within adipose and skeletal muscle tissues and subsequent development of hyperglycemia (35), which further increases oxidative stress (36). Elevated blood glucose increases the amount of glucose entering erythrocytes and then the HbA1c level (28).

Our study has several strengths. It was performed in a large representative population without a prior diagnosed diabetes and confounding comorbidities (eg, hepatic and renal disease, anemia, or pregnancy). Furthermore, HbA1c was measured on the same day of the OGTT by an internationally accepted method. To illustrate the mechanism underlying the phenomenon that the performance of HbA1c diagnosing prediabetes, but not diabetes, was poorer in obese population, SEM analysis was used to demonstrate the contribution of BMI to HbA1c in every glucose tolerance group. We also measured plasma MDA and T-AOC concentrations, which were used to explain the association between BMI and HbA1c.

There are three potential limitations to this study. First, the cross-sectional design of this study limited the ability to investigate the performance of HbA1c as a screening tool. Second, in addition to BMI, there are multiple non-glucose factors such as aging (37) and iron deficiency anemia (38) that affect the HbA1c measurement. To address this limitation, we excluded the subjects with anemia or were receiving medications for anemia and controlled age in statistical analyses. Third, it has been reported that, for a given BMI, the diabetes risks are different among different ethnic populations (39), and race and ethnicity affect HbA1c level independent of blood glucose (16, 40). Therefore, the racial and ethnic disparity should be considered when extrapolating our results to other populations.

In summary, when the ADA HbA1c criteria are used for glycemia classification, HbA1c between 5.7% and 6.4% should be interpreted considering BMI, but not in the case of HbA1c of 6.5% or greater. Under the background of the incidence of obesity quickly increasing all over the world, a fixed HbA1c cutoff value is not suitable for screening prediabetes in the population with a large BMI variation. This study raises the possibility that personalized cutoff values considering BMI and other confounding factors for glycemia classification may be more appropriate.

Acknowledgments

Author contributions included the following: J.L. and H.M. analyzed the data and wrote the manuscript. L.N., S.J., L.L., G.L., W.Z., and G.N. contributed to the discussion and reviewed the manuscript. C.S. and Y.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Address all correspondence and requests for reprints to: Changhao Sun, PhD, and Ying Li, Department of Nutrition and Food Hygiene, School of Public Health, Harbin Medical University, Harbin 150081, China. E-mail: sun2002changhao@126.com; or liying_helen@163.com.

This work was supported by National Natural Science Foundation of China Grants 81130049 and 81302417 and China Postdoctoral Science Foundation Grant 2014M551279.

Disclosure Summary: The authors having nothing to disclose.

References

1. **American Diabetes Association.** Standards of medical care in diabetes—2010. *Diabetes Care*. 2010;33(suppl 1):S11–S61.
2. **Selvin E, Steffes MW, Zhu H, et al.** Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N Engl J Med*. 2010; 362(9):800–811.
3. **International Expert Committee.** International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care*. 2009;32(7):1327–1334.
4. **Pradhan AD, Rifai N, Buring JE, Ridker PM.** Hemoglobin A1c predicts diabetes but not cardiovascular disease in nondiabetic women. *Am J Med*. 2007;120(8):720–727.
5. **Bao Y, Ma X, Li H, et al.** Glycated haemoglobin A1c for diagnosing diabetes in Chinese population: cross-sectional epidemiological survey. *BMJ*. 2010;340(7757):c2249.
6. **Zhou X, Pang Z, Gao W, et al.** Performance of an A1C and fasting capillary blood glucose test for screening newly diagnosed diabetes and pre-diabetes defined by an oral glucose tolerance test in Qingdao, China. *Diabetes Care*. 2010;33(3):545–550.
7. **Picón MJ, Murri M, Muñoz A, Fernández-García JC, Gomez-Huelgas R, Tinahones FJ.** Hemoglobin A1c versus oral glucose tolerance test in postpartum diabetes screening. *Diabetes Care*. 2012;35(8): 1648–1653.
8. **Pinelli NR, Jantz AS, Martin ET, Jaber LA.** Sensitivity and specificity of glycated hemoglobin as a diagnostic test for diabetes and prediabetes in Arabs. *J Clin Endocrinol Metab*. 2011;96(10):E1680–E1683.
9. **Saukkonen T, Cederberg H, Jokelainen J, et al.** Limited overlap between intermediate hyperglycemia as defined by A1C 5.7–6.4%, impaired fasting glucose, and impaired glucose tolerance. *Diabetes Care*. 2011;34(10):2314–2316.
10. **Mohan Kumar KM, Bobby Z, Selvaraj N, et al.** Possible link between glycated hemoglobin and lipid peroxidation in hyperthyroidism. *Clin Chim Acta*. 2004;342(1–2):187–192.
11. **Sathiyapriya V, Bobby Z, Vinod Kumar S, Selvaraj N, Parthibane V, Gupta S.** Evidence for the role of lipid peroxides on glycation of hemoglobin and plasma proteins in non-diabetic asthma patients. *Clin Chim Acta*. 2006;366(1–2):299–303.
12. **Selvaraj N, Bobby Z, Sathiyapriya V.** Effect of lipid peroxides and antioxidants on glycation of hemoglobin: an in vitro study on human erythrocytes. *Clin Chim Acta*. 2006;366(1–2):190–195.
13. **Hou X, Lu J, Weng J, et al.** Impact of waist circumference and body mass index on risk of cardiometabolic disorder and cardiovascular disease in Chinese adults: a national diabetes and metabolic disorders survey. *PLoS One*. 2013;8(3):e57319.
14. **Furukawa S, Fujita T, Shimabukuro M, et al.** Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004;114(12):1752–1761.
15. **Sathiyapriya V, Selvaraj N, Nandeesha H, et al.** Increased glycation of hemoglobin and plasma proteins in normotensive, non-diabetic obese Indian subjects: putative role of lipid peroxides. *Clin Chem Lab Med*. 2007;45(8):996–999.
16. **Ziemer DC, Kolm P, Weintraub WS, et al.** Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. *Ann Intern Med*. 2010;152(12):770–777.
17. **Huang L, Xue J, He Y, et al.** Dietary calcium but not elemental calcium from supplements is associated with body composition and obesity in Chinese women. *PLoS One*. 2011;6(12):e27703.
18. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412–419.
19. **World Health Organization.** Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva, Switzerland: World Health Organization; 1999.
20. **Chinese Diabetes Society.** China guideline for type 2 diabetes. Beijing, China: Chinese Diabetes Society; 2013.
21. **Li LM, Rao KQ, Kong LZ, et al.** A description on the Chinese national nutrition and health survey in 2002. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2005;26(7):478–484.
22. **Akobeng AK.** Understanding diagnostic tests 3: receiver operating characteristic curves. *Acta Paediatr*. 2007;96(5):644–647.
23. **Stein CM, Morris NJ, Nock NL.** Structural equation modeling. *Methods Mol Biol*. 2012;850:495–512.

24. **Heianza Y, Hara S, Arase Y, et al.** HbA1c 5.7–6.4% and impaired fasting plasma glucose for diagnosis of prediabetes and risk of progression to diabetes in Japan (TOPICS 3): a longitudinal cohort study. *Lancet*. 2011;378(9786):147–155.
25. **Mann DM, Carson AP, Shimbo D, Fonseca V, Fox CS, Muntner P.** Impact of A1C screening criterion on the diagnosis of pre-diabetes among US adults. *Diabetes Care*. 2010;33(10):2190–2195.
26. **Kramer CK, Araneta MR, Barrett-Connor E.** A1C and diabetes diagnosis: The Rancho Bernardo Study. *Diabetes Care*. 2010;33(1):101–103.
27. **Cowie CC, Rust KF, Byrd-Holt DD, et al.** Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988–2006. *Diabetes Care*. 2010;33(3):562–568.
28. **Hare MJ, Shaw JE, Zimmet PZ.** Current controversies in the use of haemoglobin A1c. *J Intern Med*. 2012;271(3):227–236.
29. **Buell C, Kermah D, Davidson MB.** Utility of A1C for diabetes screening in the 1999–2004 NHANES population. *Diabetes Care*. 2007;30(9):2233–2235.
30. **Colagiuri S, Hussain Z, Zimmet P, Cameron A, Shaw J, AusDiab.** Screening for type 2 diabetes and impaired glucose metabolism: the Australian experience. *Diabetes Care*. 2004;27(2):367–371.
31. **Barness LA, Opitz JM, Gilbert-Barness E.** Obesity: genetic, molecular, and environmental aspects. *Am J Med Genet A*. 2007;143A(24):3016–3034.
32. **Modan M, Meytes D, Rozeman P, et al.** Significance of high HbA1 levels in normal glucose tolerance. *Diabetes Care*. 1988;11(5):422–428.
33. **Keaney JF Jr, Larson MG, Vasan RS, et al.** Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol*. 2003;23(3):434–439.
34. **Jain SK, Palmer M.** The effect of oxygen radicals metabolites and vitamin E on glycosylation of proteins. *Free Radic Biol Med*. 1997;22(4):593–596.
35. **Henriksen EJ, Diamond-Stanic MK, Marchionne EM.** Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radic Biol Med*. 2011;51(5):993–999.
36. **Brownlee M.** Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414(6865):813–820.
37. **Tay TL, Foo JP, Tan E, et al.** HbA1c may not be a sensitive determinant of diabetic status in the elderly. *Diabetes Res Clin Pract*. 2011;92(2):e31–e33.
38. **Hardikar PS, Joshi SM, Bhat DS, et al.** Spuriously high prevalence of prediabetes diagnosed by HbA1c in young Indians partly explained by hematological factors and iron deficiency anemia. *Diabetes Care*. 2012;35(4):797–802.
39. **McNeely MJ, Boyko EJ.** Type 2 diabetes prevalence in Asian Americans: results of a national health survey. *Diabetes Care*. 2004;27(1):66–69.
40. **Oza-Frank R, Ali MK, Vaccarino V, Narayan KM.** Asian Americans: diabetes prevalence across US and World Health Organization weight classifications. *Diabetes Care*. 2009;32(9):1644–1646.