Postchallenge glucose increment was associated with hemoglobin glycation index in subjects with no history of diabetes

Jun-Sing Wang ,[1,2,3,4] I-Te Lee,[1,2,5] Wen-Jane Lee,[6] Shih-Yi Lin,[1,2] Wen-Lieng Lee,[2,7] Kae-Woei Liang,[2,5,7] Wayne Huey-Herng Sheu[1,2,8]

For numbered affiliations see end of article.

Correspondence to
Dr Jun-Sing Wang, Division of Endocrinology and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung 407204, Taiwan; jswang@vghtc.gov.tw

Accepted 19 January 2021
Published Online First 1 February 2021

ABSTRACT

We investigated the association between postchallenge glucose increment and hemoglobin glycation index (HGI), the difference between observed and predicted glycated hemoglobin (HbA1c), in subjects with no history of diabetes. We enrolled 1381 subjects who attended our outpatient clinic for an oral glucose tolerance test (OGTT) to screen for diabetes. HGI was defined as observed HbA1c minus predicted HbA1c. The predicted HbA1c was calculated by entering fasting plasma glucose (FPG) level into an equation [HbA1c(%)=FPG(mg/dL)*0.029+2.9686] determined from an HbA1c versus FPG regression analysis using data from an independent cohort of 2734 subjects with no history of diabetes. The association between 2-hour glucose increment and HGI was analyzed using linear regression analyses with adjustment of relevant parameters. Overall, the proportions of subjects with normal glucose tolerance, pre-diabetes, and newly diagnosed diabetes were 42.3%, 41.3%, and 16.4%, respectively. Compared with subjects who had an HGI≤0, subjects with an HGI>0 had a lower FPG (95.0±13.3 vs 98.5±15.3 mg/dL, p<0.001) but a higher 2-hour plasma glucose (151.1±52.8 vs 144.6±51.4 mg/dL, p=0.027) and 2-hour glucose increment (56.1±46.1 vs 46.1±45.0 mg/dL, p<0.001). The 2-hour glucose increment after an OGTT was independently associated with HGI (β coefficient 0.003, 95% CI 0.002 to 0.003, p<0.001). Our findings suggested that postchallenge glucose increment was independently associated with HGI in subjects with no history of diabetes.

Significance of this study

What is already known about this subject?

- It is not unusual to find a considerable discordance between glycated hemoglobin (HbA1c) and blood glucose levels.
- The hemoglobin glycation index (HGI, defined as the observed HbA1c minus predicted HbA1c based on glucose levels) has been proposed to quantify those differences (variations).
- The mechanisms that lead to an HGI are not yet clear.

What are the new findings?

- Compared with subjects who had an HGI≤0, subjects with an HGI>0 had a lower fasting plasma glucose but a higher 2-hour plasma glucose and 2-hour glucose increment.
- The findings were observed in those who had abnormal glucose regulation (diabetes or pre-diabetes) determined by an oral glucose tolerance test (OGTT).
- The 2-hour glucose increment was independently associated with HGI.

How might these results change the focus of research or clinical practice?

- For subjects at risk for diabetes who have a high HGI (eg, a higher HbA1c in relation to fasting glucose), an OGTT is recommended as they are likely to have postchallenge hyperglycemia.
- For patients with diabetes with a high HGI (eg, a high HbA1c with a relatively low fasting glucose), monitoring of postprandial glucose is recommended as they are likely to have postprandial hyperglycemia.
- HGI may be useful for individualized glycemic therapy in patients with diabetes.

INTRODUCTION

Glycated hemoglobin (HbA1c) has been widely used for the assessment of glycemic control in people with diabetes and for the diagnosis of abnormal glucose regulation (diabetes or pre-diabetes) in people with no history of diabetes.[1 2] Nevertheless, the correlation between HbA1c and blood glucose levels is only modest.[3 4] Therefore, in assessments of glycemic control[5] and diagnosis of abnormal glucose regulation,[6–8] it is not unusual to find a considerable discordance between HbA1c and blood glucose levels. Such discordances may complicate data interpretation,[9] and it is clinically relevant to understand factors that may alter the association between HbA1c and glucose levels.

HbA1c values are influenced by both glycemic and non-glycemic factors.[10] There are considerable between-individual differences (variations) in the relationship of HbA1c to glucose

To cite: Wang J-S, Lee I-T, Lee W-J, *et al*. *J Investig Med* 2021;**69**:1044–1049.

levels.[4 11] The hemoglobin glycation index (HGI) has been proposed to quantify those differences (variations).[12–14] HGI is defined as the observed HbA1c minus predicted HbA1c based on glucose levels. The predicted HbA1c can be derived by using a linear regression between HbA1c and blood glucose levels.[11–13] Hence, subjects with a high HGI have an HbA1c level higher than that estimated from their blood glucose levels. As HGI has been associated with cardiovascular diseases[15] and mortality,[16] it is important to clarify factors that may be associated with HGI.

Although the mechanisms that lead to between-individual variations in the relationship of HbA1c to glucose levels are not yet clear,[15 17] postprandial hyperglycemia has been suggested to be associated with increased glycation gap/HGI.[17] However, there are few data showing an association between postprandial hyperglycemia and glycation gap/HGI.[18] We hypothesized that HGI was associated with postprandial glucose increment (postprandial hyperglycemia). In this study, we investigated the association between postchallenge glucose increment and HGI in subjects with no history of diabetes who underwent an oral glucose tolerance test (OGTT) by using fasting glucose levels to determine the predicted HbA1c levels and HGI.[15 19]

MATERIALS AND METHODS

We enrolled subjects with no history of diabetes who attended our outpatient clinic for an OGTT to screen for diabetes between 2011 and 2019. We excluded subjects from the analysis if they had blood transfusion within 3 months or anemia, defined as a hemoglobin level less than 100 g/L.

All subjects underwent a 75 g OGTT.[20] Briefly, subjects fasted overnight and attended our outpatient clinic in the morning. A trained nurse interviewed the subjects and measured their height, weight, waist circumference, and blood pressure. For the measurements of plasma glucose, insulin, HbA1c, and lipids profiles, we collected a fasting blood sample before the OGTT. We then conducted a 75 g OGTT[20] for study subjects, and collected a blood sample after 2 hours to determine the postchallenge plasma glucose levels.

We measured HbA1c by using boronate-affinity high-performance liquid chromatography with an interassay and intra-assay coefficient of variation of <2.9% and <0.9%, respectively. We determined plasma glucose by using the glucose oxidase-peroxidase method. The interassay and intra-assay coefficients of variation were both <1.5%. We measured plasma insulin by using an electrochemiluminescence immunoassay with an interassay and intra-assay coefficient of variation of 2.5% and 1.8%, respectively.

We divided the study population into 2 groups according to their HGI (the observed HbA1c minus predicted HbA1c). We developed an equation to estimate HbA1c by fasting plasma glucose (FPG) in the study population.[15 19] The equation was derived using data from an independent cohort (developmental database) of 2734 subjects with no history of diabetes who had visited our hospital for a health check-up (mean age 47.4±11.2 years, male 66.4%, mean body mass index 24.4±3.6 kg/m^2, mean FPG 91.7±17.7 mg/dL, mean HbA1c 5.6%±0.6%). We excluded subjects from the developmental database if they had blood transfusion within 3 months or anemia, defined as a hemoglobin level less than 100 g/L. An equation [HbA1c(%)=FPG(mg/dL)*0.029+2.9686] was determined based on the correlation between HbA1c and FPG in the developmental database (figure 1).

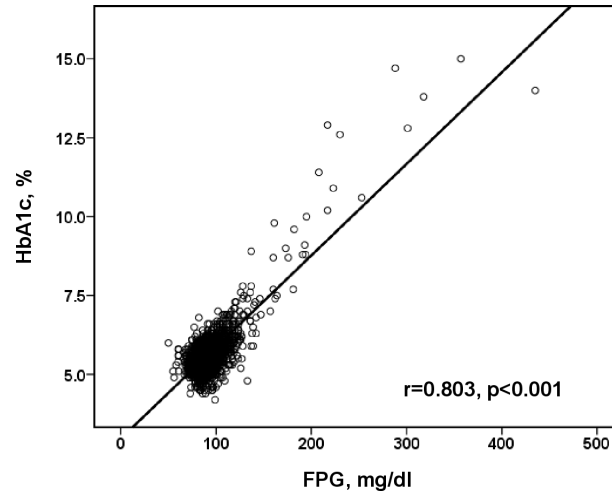

Figure 1 The association of FPG and HbA1c in 2734 subjects with no history of diabetes (developmental database). FPG, fasting plasma glucose; HbA1c, glycated hemoglobin.

We determined glucose regulation status of our study subjects according to OGTT, as recommended by the American Diabetes Association.[21] We assessed insulin resistance and β-cell function using the homeostasis model assessment (HOMA-IR and HOMA-β, respectively).[22] To investigate the association between postchallenge hyperglycemia and HGI, 2-hour glucose increment (2-hour plasma glucose—FPG) was calculated using OGTT results.

The Statistical Package for the Social Sciences (IBM SPSS V.22.0; International Business Machines, New York, USA) was used to perform the statistical analyses. Categorical and continuous data are expressed as numbers (percentages) and mean±SD, respectively. Kolmogorov-Smirnov test was applied to determine whether a variable was normally distributed. To examine statistical differences in continuous variables between groups, the Student's t-test was used. For categorical variables, the χ^2 test was used. To examine the association between 2-hour glucose increment and HGI, linear regression analyses were used with adjustment of relevant parameters. Bonferroni correction was applied for multiple comparisons. Data that were not normally distributed were logarithmically transformed before analysis. Statistical significance was considered for a two-sided p value <0.05.

RESULTS

A total of 1381 outpatients (mean age 60.5±11.6 years, male 82.3%, mean body mass index 26.1±3.6 kg/m^2) underwent an OGTT. Using the equation [HbA1c(%)=FPG(mg/dL)*0.029+2.9686] derived from the developmental database (figure 1) to predict HbA1c and calculate HGI, we divided the study population into 2 groups (HGI≤0 vs HGI>0, table 1). Subjects with an HGI>0 had a lower FPG (95.0±13.3 vs 98.5±15.3 mg/dL, p<0.001) and a lower predicted HbA1c (5.7%±0.4% vs 5.8%±0.4%, p<0.001),

Table 1 Characteristics of study subjects according to HGI

Variables	HGI≤0	HGI>0	P value
n	495	886	
Fasting plasma glucose (mg/dL)	98.5±15.3	95.0±13.3	<0.001
Predicted HbA1c (%)	5.8±0.4	5.7±0.4	<0.001
Observed HbA1c (%)	5.5±0.5	6.1±0.5	<0.001
HGI (%)	−0.3±0.3	0.4±0.4	<0.001
Age (y)	59.8±11.7	60.9±11.5	0.108
Male, n (%)	413 (83.4)	723 (81.6)	0.393
Body mass index (kg/m^2)	25.8±3.5	26.3±3.6	0.013
Waist circumference (cm)	90.2±9.2	91.1±8.7	0.056
Current smoker, n (%)	72 (14.5)	161 (18.2)	0.084
Systolic blood pressure (mm Hg)	126±17	128±18	0.107
Diastolic blood pressure (mm Hg)	74±10	74±11	0.733
Hemoglobin (g/L)	139±15	139±15	0.695
Total cholesterol (mg/dL)	180±39	186±40	0.011
LDL cholesterol (mg/dL)	117±61	118±53	0.638
HDL cholesterol (mg/dL)	46.9±12.7	46.0±12.3	0.247
Triglycerides (mg/dL)	142±97	156±114	0.035
OGTT results, n (%)			<0.001
Normal glucose tolerance	211 (42.6)	373 (42.1)	
Isolated IFG	59 (11.9)	39 (4.4)	
Combined IFG and IGT	45 (9.1)	94 (10.6)	
Isolated IGT	105 (21.2)	228 (25.7)	
Newly diagnosed diabetes	75 (15.2)	152 (17.2)	

Values are mean±SD or n (%).
HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HGI, hemoglobin glycation index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test.

but a higher observed HbA1c (6.1%±0.5% vs 5.5%±0.5%, p<0.001), compared with those who had an HGI≤0. Body mass index (26.3±3.6 vs 25.8±3.5 kg/m^2, p=0.013), total cholesterol (186±40 vs 180±39 mg/dL, p=0.011), and triglycerides (156±114 vs 142±97 mg/dL, p=0.035) were higher in subjects with an HGI>0 (table 1). No significant difference was observed with regard to the other parameters between the 2 groups.

Overall, the proportions of subjects with normal glucose tolerance, pre-diabetes, and newly diagnosed diabetes were 42.3%, 41.3%, and 16.4%, respectively. The corresponding values in subjects with an HGI≤0 were 42.6%, 42.2%, and 15.2%. In subjects with an HGI>0, these values were 42.1%, 40.7%, and 17.2%. Nevertheless, we observed a lower rate of isolated impaired fasting glucose (4.4% vs 11.9%) and a higher rate of isolated impaired glucose tolerance (25.7% vs 21.2%) in subjects with an HGI>0 (table 1).

Table 2 shows fasting and 2-hour plasma glucose, 2-hour glucose increment, HOMA-IR, and HOMA-β according to HGI. Compared with subjects who had an HGI≤0, subjects with an HGI>0 had a lower FPG but a higher 2-hour plasma glucose and 2-hour glucose increment. The aforementioned findings were observed in those who had abnormal glucose regulation (diabetes or pre-diabetes) by OGTT, but not in those who had normal glucose tolerance. We did not observe significant differences in insulin resistance (HOMA-IR) and β-cell function (HOMA-β) between the 2 groups.

Table 3 shows the association between 2-hour glucose increment and HGI. Using linear regression analysis, we demonstrated that 2-hour glucose increment after an OGTT was positively associated with HGI (β coefficient 0.002, 95% CI 0.002 to 0.003, p<0.001). The association remained significant (β coefficient 0.003, 95% CI 0.002 to 0.003, p<0.001) after adjustment for some parameters, such as age and sex (table 3).

DISCUSSION

In this study, we demonstrated that subjects with no history of diabetes who had an HGI>0 had a higher 2-hour

Table 2 Results of OGTT, HOMA-IR, and HOMA-β according to HGI

Variables	HGI≤0	HGI>0	P value
All (n=1381)			
Fasting plasma glucose (mg/dL)	98.5±15.3	95.0±13.3	<0.001
2 h plasma glucose (mg/dL)	144.6±51.4	151.1±52.8	0.027
2 h glucose increment (mg/dL)	46.1±45.0	56.1±46.1	<0.001
HOMA-IR	2.7±2.5	2.5±2.0	0.127
HOMA-β	117±82	126±87	0.057
Normal glucose tolerance by OGTT (n=584)			
Fasting plasma glucose (mg/dL)	90.6±5.7	87.8±6.0	<0.001
2 h plasma glucose (mg/dL)	107.7±20.0	108.0±21.5	0.891
2 h glucose increment (mg/dL)	17.1±20.7	20.2±21.6	0.096
HOMA-IR	2.1±1.4	1.9±1.3	0.205
HOMA-β	125±83	134±88	0.212
Diabetes or pre-diabetes by OGTT (n=797)			
Fasting plasma glucose (mg/dL)	104.3±17.4	100.2±14.7	<0.001
2 h plasma glucose (mg/dL)	172.0±50.4	182.4±46.3	0.003
2 h glucose increment (mg/dL)	67.7±46.1	82.2±41.3	<0.001
HOMA-IR	3.2±2.9	2.9±2.3	0.181
HOMA-β	111±81	120±86	0.140

Values are mean±SD.
HGI, hemoglobin glycation index; HOMA, homeostasis model assessment; IR, insulin resistance; OGTT, oral glucose tolerance test.

Table 3 Linear regression analysis with HGI as the dependent variable

Independent variable	β coefficient	95% CI	P value
2 h glucose increment (mg/dL)			
Model 1	0.002	0.002 to 0.003	<0.001
Model 2	0.002	0.002 to 0.003	<0.001
Model 3	0.002	0.001 to 0.003	<0.001
Model 4	0.003	0.002 to 0.003	<0.001

Model 1, unadjusted. Model 2, adjusted for age and sex. Model 3, adjusted for variables in model 2 plus body mass index and waist circumference. Model 4, adjusted for variables in model 3 plus smoking, total cholesterol, HOMA-IR, and HOMA-β.
HGI, hemoglobin glycation index; HOMA, homeostasis model assessment; IR, insulin resistance.

plasma glucose and 2-hour glucose increment after OGTT, compared with those who had an HGI≤0 (table 2). The findings were mainly observed in subjects with diabetes or pre-diabetes defined by an OGTT (table 2). Moreover, 2-hour glucose increment was independently associated with HGI (table 3). Our findings suggest that postchallenge glucose increment may contribute, at least partly, to a higher than predicted HbA1c (HGI>0) in subjects with no history of diabetes.

The mechanisms involved in the 'glycation gap' (HGI) are not yet clear. It has been reported that there were ethnic differences in the relationship between glucose concentrations and HbA1c levels.[23–25] For a given blood glucose level in patients with type 2 diabetes,[23] non-Caucasians had a higher HbA1c level (ie, a high HGI) than Caucasians. Similar findings related to racial differences in the glycation gap were reported in patients with type 1 diabetes[24] and impaired glucose tolerance.[25] However, race only partially explains the observed glycation gaps.[24] In our study, we investigated the association between 2-hour glucose increment and HGI in a Chinese population with no history of diabetes. Our findings suggest that 2-hour glucose increment after OGTT was independently associated with HGI. In a previous study conducted in Europeans with no diabetes,[26] there was no significant difference in 2-hour glucose levels among HGI tertiles. Similar finding was noted in Americans at risk for diabetes.[27] In contrast, there was a higher 2-hour glucose level and a greater 2-hour glucose increment in the highest HGI tertile in an Asian population with pre-diabetes or diabetes.[18] Hence, ethnicity may have some effects on HGI which may help explain our results and previous findings.[18 26 27]

It is reasonable to speculate that postchallenge hyperglycemia may be associated with a higher than predicted HbA1c. Treatment guidelines suggest treating postprandial glucose excursions in patients with diabetes with an HbA1c above target despite having FPG at target.[28] Moreover, treatment targeting fasting or postprandial glucose may have different effects on HGI. In a randomized trial[29] which compared the effects of treating prandial versus fasting glycemia on cardiovascular outcomes in patients with type 2 diabetes, patients treated with basal insulin had a lower fasting glucose than those treated with prandial insulin (7.0±0.2 vs 8.1±0.2 mmol/L, p<0.001). However, there was no significant difference in HbA1c between the 2 groups (7.8%±0.1% vs 7.7%±0.1%, p=0.4).[29] Thus, the patients would have a lower estimated HbA1c and a higher HGI after treatment with basal insulin, compared with treatment with prandial insulin. Similar findings could be observed in the Treating to Target in Type 2 Diabetes trial.[30] In a recent study[31] using data from flash glucose monitoring in a Chinese population of patients with type 2 diabetes, the authors reported differences in glycation gap (estimated HbA1c vs measured HbA1c) in subjects with different mean glucose levels. Their findings suggest that different glucose levels may have some effects on glycation gap. It might be possible that glucose regulation status may influence HGI. People with normal glucose regulation may have much smaller difference in glycation gap, or a low glucose peak might be less likely to trigger glycation in vivo. Our results and the aforementioned observations suggest an association between postchallenge glucose increment and HGI.

In addition to ethnicity and postchallenge glucose excursions, several factors were shown to be related to HGI.[15 17 18 32] For example, Dunmore *et al*[33] demonstrated that variations in the level of intracellular deglycating enzyme fructosamine-3-kinase were associated with the glycation gap in human subjects. Liu *et al*[34] reported an independent association between inflammatory markers and HGI in a population with no history of diabetes. As postprandial hyperglycemia has been associated with peripheral inflammation gene expression,[35] this may help explain our finding that 2-hour glucose increment was associated with HGI. Genetic factors[32 36] and differences in mean red cell age[36 37] had also been suggested to account for the glycation gap. These non-glycemic factors related to HGI may help explain the only modest (though significant) correlation between 2-hour glucose increment and HGI in our subjects (table 3). Furthermore, HGI was found to be associated with chronic diabetes complications,[38] cardiovascular disease,[15 39] and mortality.[16 40] Although the mechanisms underlying the relationships between the aforementioned factors and glycation gap/HGI have yet to be fully elucidated, our findings may have important implications for clinical practice with respect to screening for diabetes and treatment for patients with diabetes. For subjects at risk for diabetes who have a high HGI (eg, a higher HbA1c in relation to fasting glucose), an OGTT is recommended as they are likely to have postchallenge hyperglycemia. For patients with diabetes with a high HGI (eg, a high HbA1c with a relatively low fasting glucose), monitoring of postprandial glucose is recommended as they are likely to have postprandial hyperglycemia. We suggest HGI may be useful for individualized glycemic therapy in patients with diabetes.[19 41]

There are several limitations in this study. First, this study was conducted in a Chinese population. The association between postchallenge glucose increment and HGI needs to be investigated in other ethnicities. Second, this study investigated subjects at risk for diabetes and thus our findings were not confounded by glucose-lowering therapies. Nevertheless, whether our findings could be generalized to subjects with known diabetes or the general population merits further study. Third, postchallenge hyperglycemia was assessed using an OGTT in this study. Thus, the poor reproducibility of OGTT might confound our results.[42] Last, non-glycemic factors which may impact hemoglobin glycation[43] were not addressed in our study. With these

limitations in mind, the novel association between postchallenge glucose increment and HGI in this study may help identify subjects with postchallenge glucose excursions in daily practice.

In conclusion, we demonstrated that postchallenge glucose increment was independently associated with HGI in subjects with no history of diabetes. Our findings need to be confirmed in other populations.

Author affiliations
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan
[2]Department of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan
[3]Rong Hsing Research Center for Translational Medicine, Institute of Biomedical Science, College of Life Science, National Chung Hsing University, Taichung, Taiwan
[4]Ph.D. Program in Translational Medicine, National Chung Hsing University, Taichung, Taiwan
[5]Department of Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[6]Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan
[7]Cardiovascular Center, Taichung Veterans General Hospital, Taichung, Taiwan
[8]Institute of Medical Technology, College of Life Science, National Chung Hsing University, Taichung, Taiwan

Contributors JSW, WJL, and WHHS designed the research. JSW, ITL, SYL, WLL, and KWL conducted the research and collected the data. JSW and WLL analyzed the data. JSW, ITL, and WHHS wrote the first draft of the manuscript. WJL, SYL, WLL and KWL revised the manuscript critically for important intellectual content. All authors approved the final draft of the manuscript.

Funding This study was supported by Taichung Veterans General Hospital, Taichung, Taiwan (TCVGH-YM1070102 and TCVGH-1073505C, 2018; TCVGH-1083505C, 2019).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study protocol was reviewed and approved by the Institutional Review Boards of Taichung Veterans General Hospital, Taichung, Taiwan (approval number C08215B). This study was conducted in accordance with the Declaration of Helsinki, and written informed consent was provided by all subjects prior to the procedures.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data cannot be made publicly available due to the 'Personal Information Protection Act'.

ORCID iD
Jun-Sing Wang http://orcid.org/0000-0002-0887-6432

REFERENCES

1 International Expert Committee. International expert Committee report on the role of the A1c assay in the diagnosis of diabetes. *Diabetes Care* 2009;32:1327–34.
2 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010;33 Suppl 1:S62–9.
3 van 't Riet E, Alssema M, Rijkelijkhuizen JM, *et al*. Relationship between A1C and glucose levels in the general Dutch population: the new Hoorn study. *Diabetes Care* 2010;33:61–6.
4 Diabetes Research in Children Network (DirecNet) Study Group. Relationship of A1c to glucose concentrations in children with type 1 diabetes: assessments by high-frequency glucose determinations by sensors. *Diabetes Care* 2008;31:381–5.
5 Shu H, Lu J, Zhang P, *et al*. Clinical characteristics of type 2 diabetes patients with discordance between HbA_{1c} and fasting plasma glucose in the real world: An analysis of the ORBIT study. *Diabetes Metab Res Rev* 2018;34:e2977.
6 Xu Y, Wang L, He J, *et al*. Prevalence and control of diabetes in Chinese adults. *JAMA* 2013;310:948–59.
7 Wang J-S, Lee I-T, Lee W-J, *et al*. Comparing HbA1c, fasting and 2-h plasma glucose for screening for abnormal glucose regulation in patients undergoing coronary angiography. *Clin Chem Lab Med* 2015;53:1441–9.
8 Evron JM, Herman WH, McEwen LN. Changes in Screening Practices for Prediabetes and Diabetes Since the Recommendation for Hemoglobin A_{1c} Testing. *Diabetes Care* 2019;42:576–84.
9 Cohen RM, Lindsell CJ. When the blood glucose and the HbA(1c) don't match: turning uncertainty into opportunity. *Diabetes Care* 2012;35:2421–3.
10 Dagogo-Jack S. Pitfalls in the use of HbA_1(c) as a diagnostic test: the ethnic conundrum. *Nat Rev Endocrinol* 2010;6:589–93.
11 Wilson DM, Xing D, Cheng J, *et al*. Persistence of individual variations in glycated hemoglobin: analysis of data from the juvenile diabetes research Foundation continuous glucose monitoring randomized trial. *Diabetes Care* 2011;34:1315–7.
12 Hempe JM, Gomez R, McCarter RJ, *et al*. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. *J Diabetes Complications* 2002;16:313–20.
13 Soros AA, Chalew SA, McCarter RJ, *et al*. Hemoglobin glycation index: a robust measure of hemoglobin A1c bias in pediatric type 1 diabetes patients. *Pediatr Diabetes* 2010;11:455–61.
14 Chalew SA, McCarter RJ, Hempe JM. Biological variation and hemoglobin A1c: relevance to diabetes management and complications. *Pediatr Diabetes* 2013;14:391–8.
15 Hempe JM, Liu S, Myers L, *et al*. The hemoglobin glycation index identifies subpopulations with harms or benefits from intensive treatment in the Accord trial. *Diabetes Care* 2015;38:1067–74.
16 Basu S, Raghavan S, Wexler DJ, *et al*. Characteristics associated with decreased or increased mortality risk from glycemic therapy among patients with type 2 diabetes and high cardiovascular risk: machine learning analysis of the Accord trial. *Diabetes Care* 2018;41:604–12.
17 Riddle MC, Gerstein HC. Comment on Hempe, et al. The hemoglobin glycation index identifies subpopulations with harms or benefits from intensive treatment in the Accord trial. *Diabetes Care 2015; 38: 1067-1074. Diabetes Care* 2015;38:e170–1.
18 Ahn CH, Min SH, Lee D-H, *et al*. Hemoglobin glycation index is associated with cardiovascular diseases in people with impaired glucose metabolism. *J Clin Endocrinol Metab* 2017;102:2905–13.
19 Wang J-S, Hung Y-J, Lu Y-C, *et al*. Difference between observed and predicted glycated hemoglobin at baseline and treatment response to vildagliptin-based dual oral therapy in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2018;138:119–27.
20 World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Part I: diagnosis and classification of diabetes mellitus. Report of a who consultation, 1999. Available: https://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf [Accessed 04 Oct 2019].
21 American Diabetes Association. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes-2018*. *Diabetes Care* 2018;41:S13–27.
22 Matthews DR, Hosker JP, Rudenski AS, *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.
23 Wolffenbuttel BHR, Herman WH, Gross JL, *et al*. Ethnic differences in glycemic markers in patients with type 2 diabetes. *Diabetes Care* 2013;36:2931–6.
24 Bergenstal RM, Gal RL, Connor CG, *et al*. Racial Differences in the Relationship of Glucose Concentrations and Hemoglobin A_{1c} Levels. *Ann Intern Med* 2017;167:95–102.
25 Herman WH, Ma Y, Uwaifo G, *et al*. Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. *Diabetes Care* 2007;30:2453–7.
26 Marini MA, Fiorentino TV, Succurro E, *et al*. Association between hemoglobin glycation index with insulin resistance and carotid atherosclerosis in non-diabetic individuals. *PLoS One* 2017;12:e0175547.
27 Hsia DS, Rasouli N, Pittas AG, *et al*. Implications of the hemoglobin glycation index on the diagnosis of prediabetes and diabetes. *J Clin Endocrinol Metab* 2020;105:e130–8.
28 American Diabetes Association. 9. Pharmacologic Approaches to Glycemic Treatment: *Standards of Medical Care in Diabetes-2019*. *Diabetes Care* 2019;42:S90–102.
29 Raz I, Wilson PWF, Strojek K, *et al*. Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: the HEART2D trial. *Diabetes Care* 2009;32:381–6.
30 Holman RR, Thorne KI, Farmer AJ, *et al*. Addition of biphasic, prandial, or basal insulin to oral therapy in type 2 diabetes. *N Engl J Med* 2007;357:1716–30.
31 Hu Y, Shen Y, Yan R, *et al*. Relationship between estimated glycosylated hemoglobin using flash glucose monitoring and actual measured glycosylated hemoglobin in a Chinese population. *Diabetes Ther* 2020;11:2019–27.

32 Nayak AU, Singh BM, Dunmore SJ. Potential clinical error arising from use of HbA1c in diabetes: effects of the glycation gap. *Endocr Rev* 2019;40:988–99.
33 Dunmore SJ, Al-Derawi AS, Nayak AU, *et al*. Evidence that differences in fructosamine-3-kinase activity may be associated with the glycation gap in human diabetes. *Diabetes* 2018;67:131–6.
34 Liu S, Hempe JM, McCarter RJ, *et al*. Association between inflammation and biological variation in hemoglobin A1c in U.S. nondiabetic adults. *J Clin Endocrinol Metab* 2015;100:2364–71.
35 Honma K, Jin F, Tonaka R, *et al*. Changes in peripheral inflammation-related gene expression by postprandial glycemic response in healthy Japanese men. *Nutrition* 2020;2:111026.
36 Cohen RM, Snieder H, Lindsell CJ, *et al*. Evidence for independent heritability of the glycation gap (glycosylation GAP) fraction of HbA1c in nondiabetic twins. *Diabetes Care* 2006;29:1739–43.
37 Cohen RM, Franco RS, Smith EP, *et al*. When HbA1c and blood glucose do not match: how much is determined by race, by genetics, by differences in mean red blood cell age? *J Clin Endocrinol Metab* 2019;104:707–10.
38 McCarter RJ, Hempe JM, Gomez R, *et al*. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. *Diabetes Care* 2004;27:1259–64.
39 Kim MK, Jeong JS, Yun J-S, *et al*. Hemoglobin glycation index predicts cardiovascular disease in people with type 2 diabetes mellitus: a 10-year longitudinal cohort study. *J Diabetes Complications* 2018;32:906–10.
40 van Steen SCJ, Schrieks IC, Hoekstra JBL, *et al*. The haemoglobin glycation index as predictor of diabetes-related complications in the AleCardio trial. *Eur J Prev Cardiol* 2017;24:858–66.
41 Chen Y-W, Wang J-S, Sheu WH-H, *et al*. Hemoglobin glycation index as a useful predictor of therapeutic responses to dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes. *PLoS One* 2017;12:e0171753.
42 Sacks DB. A1C versus glucose testing: a comparison. *Diabetes Care* 2011;34:518–23.
43 Welsh KJ, Kirkman MS, Sacks DB. Role of glycated proteins in the diagnosis and management of diabetes: research gaps and future directions. *Diabetes Care* 2016;39:1299–306.