Supplementary Materials

Clinical utility of glycated albumin and 1,5-anhydroglucitol in the screening and prediction of diabetes: A multi-center study

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Supplemental Notes

Study participants

Obese Chinese Cohort (OCC)

OCC consisted of participants, aged over 18 years old, who were screened for eligibility for bariatric surgery at the First Affiliated Hospital of Jinan University (Huaqiao Hospital) from January 2019 to March 2022. Details of this cohort have been described previously [1,2]. In brief, 621 morbidly obese patients were consecutively recruited in this cohort. The participants' clinical and demographic data were obtained, and their blood samples were collected within one week before the bariatric surgery. Patients on diabetic medications (n =88) were excluded from the current study. Among the 533 eligible participants, 462 subjects with plasma samples and complete biomarker data available for the analysis were included in the current study to examine the diagnostic performance of the biomarkers (Supplemental Figure 1). To assess whether GA and 1,5-AG can serve as sensitive biomarkers for glycemic monitoring, blood samples of patients with diabetes collected before the bariatric surgery (n =24), and at one (n =13) and three months (n =14) after the surgery were analyzed. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg or with the use of anti-hypertensive drugs. For assessment of the agreement in classifications between different biomarkers, prediabetes and diabetes were also defined according to the optimal values defined by the Youden J index.

Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS)

CRISPS is a community-based prospective study in Hong Kong [3,4]. In brief, a total of 2895 unrelated participants of Chinese ancestry were invited randomly by their telephone numbers for a detailed assessment of the cardiovascular risk factors in 1995-1996 (CRISPS-1) [3,4]. The participants were invited for follow-up assessments in CRISPS-2 (2000-2004), CRISPS-3 (2005-2008), CRISPS-4 (2010-2012) and CRISPS-5 (2016-2018). During each assessment, the participants' medical, drug and family histories were recorded. The anthropometric and biochemical parameters were obtained as previously described [4]. A

75g-OGTT was performed in those who were not taking antidiabetic medications. Hypertension was defined as blood pressure ≥140/90mmHg or on antihypertensive drugs. The third part of the current study was conducted based on a sub-group of CRISPS, which included 322 subjects with impaired glucose tolerance (IGT) and 322 subjects with normal glucose tolerance (NGT) at the CRISPS-1 time-point [5]. The glycemic status of these 644 subjects was reassessed by OGTT at 2-year in 1997-1998. GA and 1,5-AG were measured in the plasma samples collected at 2-year and this time point was set as the baseline of the current study. A total of 538 participants who did not have diabetes at 2-year (baseline) had returned for at least one follow-up assessment. These subjects were reassessed at ~5-year intervals, similar to the rest of the CRISPS cohort [4]. The nested case-control study was conducted on 158 incident diabetes cases who had developed diabetes by CRISPS-5 and 158 age and sex-matched controls who remained free from diabetes (Supplemental Figure 2).

Quantitative measurements of biomarkers

FPG and HbA1c were measured as a part of standard clinical examination by the accredited clinical laboratories of the First Affiliated Hospital of Jinan University in Guangdong and the Queen Mary Hospital in Hong Kong. The commercially available enzymatic assay kits (Cat. No.; GA: 51970 and 1,5-AG: 51990; Immunodiagnostics Co., Ltd, Hong Kong) were used to measure the circulating levels of GA and 1,5-AG according to the manufacturer's instructions. For the quantification of GA percentage (%), 2 distinct assays were performed, including the quantification of GA using the ketoamine oxidase enzymatic method and the quantification of total albumin using the bromocresol green method. The quantification of 1,5-AG was accomplished using the pyranose oxidase enzymatic method. The assay range of GA, 1,5-AG and total albumin enzymatic assay kits were 0-1.78 g/dL, 0-150 μmol/L and 0-3.71 g/dL, respectively. The lower detection limits for the 3 assay kits were 0.0493 g/dL, 6.033 μmol/L and 0.0734 g/dL, respectively. The intra-assay and inter-assay coefficient of variations were as follows: GA: 1.1-2.7%; 1,5-AG: 0.8-1.4%; total albumin: 2.8-8.5%. Five different sample controls were applied to the testing of each cohort to ensure the

consistency and stability of reagents. The average recovery of sample testing results for the 3 assay kits was 4.6% for GA, 4.2% for 1,5-AG and 6.9% for total albumin, respectively. The spiking recovery rates and linearity rates of the 3 assay kits were as follows: GA: 96.3-104.8% and 88.3-114.7%; 1,5-AG: 101.2-103.4% and 87.4-108.5%; total albumin: 97.5-102.4% and 91.3-112.4%.

Statistical analyses

All statistical analyses were conducted using SPSS Statistics 27 (SPSS, Chicago, IL), GraphPad Prism (version 9) and R (version 4.3.2). Data are presented as mean ± SD or median (interquartile range), where appropriate. Non-normally distributed data were natural-logarithmically transformed to near normality before analysis. Continuous variables were compared using One-way ANOVA. Categorical variables were compared using Pearson $\chi 2$ tests. The multiple conditional logistic model with adjustments for the most important clinical parameters was used to evaluate the independent associations of GA and 1,5-AG with incident diabetes. A two-tailed p-value <0.05 was considered statistically significant.

References

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Supplementary Table 1. Baseline clinical characteristics of subjects in the Obese Chinese Cohort.

	Normoglycemia (n=164)	Prediabetes (n=171)	Diabetes (n=127)	*P* value
Age (Years)	28.89 ± 8.62	30.97 ± 8.26	36.63 ± 11.55	< 0.001
Sex (Male %)	28.21	36.75	40.48	0.080
BMI (kg/m^2)	36.73 ± 6.46	39.32 ± 8.16	39.65 ± 8.84	0.002
Waist circumference (cm)	114.55 ± 13.87	120.26 ± 17.96	123.52 ± 19.18	< 0.001
FPG (mmol/L)	4.90 ± 0.44	5.54 ± 0.64	8.92 ± 3.23	< 0.001
HbA1c (%)	5.29 ± 0.25	5.83 ± 0.31	7.92 ± 1.81	< 0.001
GA† (%)	11.52(10.07-13.19)	14.44(13.29-15.37)	19.77(16.94-23.94)	< 0.001
1,5-AG[a] (μmol/L)	134.10(93.80-170.00)	100.05(72.46-139.49)	35.55(16.11-79.21)	< 0.001

Data are presented as mean ± SD or median (Interquartile range). BMI, Body mass index; FPG, fasting glucose; HbA1c, glycated hemoglobin; GA, glycated albumin; 1,5-AG, 1,5-anhydroglucitol. †Natural log-transformed before analysis.

Supplementary Table 2. Diagnostic performances of GA and 1,5-AG on prediabetes and diabetes.

	Prediabetes		Diabetes	
	GA (%)	1,5-AG (μmol/L)	GA (%)	1,5-AG (μmol/L)
AUC(95%CI)	0.838(0.795-0.880)	0.635(0.576-0.695)	0.919(0.884-0.955)	0.829 (0.782-0.876)
Optimal value	13.06	130.39	16.39	68.87
Sensitivity (%)	68.37	61.48	81.60	73.60
Specificity (%)	58.53	60.00	95.68	83.09
PPV (%)	42.68	58.87	87.18	61.74
NPV (%)	80.38	62.59	93.52	89.64

HbA1c, glycated hemoglobin; 1,5-AG, 1,5-anhydroglucitol; PPV, positive predictive value; NPV, negative predictive value.

Supplementary Table 3. Agreement in diagnostic classification for diabetes between different biomarkers in 2x2 table.

a. Concordance in diagnostics classification (HbA1c vs. FPG)

		Classification based on FPG		
		Non-diabetes	Diabetes	Total
Classification based on HbA1c	Non-diabetes	323	24	347
	Diabetes	27	88	115
	Total	350	112	462

b. Concordance in diagnostics classification (GA vs. FPG)

		Classification based on FPG		
		Non-diabetes	Diabetes	Total
Classification based on GA	Non-diabetes	315	30	345
	Diabetes	35	82	117
	Total	350	112	462

c. Concordance in diagnostics classification (GA vs. HbA1c)

		Classification based on HbA1c		
		Non-diabetes	Diabetes	Total
Classification based on GA	Non-diabetes	323	22	345
	Diabetes	24	93	117
	Total	347	115	462

d. Concordance in diagnostics classification (HbA1c/GA vs. FPG)

		Classification based on FPG		
		Non-diabetes	Diabetes	Total
Classification based on HbA1c/GA	Non-diabetes	308	15	323
	Diabetes	42	97	139
	Total	350	112	462

FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; GA, glycated albumin. The number of subjects with the same diagnosis by both tests are underlined. Optimal cutoffs for diabetes: GA: 16.39%; 1,5-AG: 68.87μmol/L; FPG: 6.43mmol/L; HbA1c: 6.45%.

Supplementary Table 4. Baseline characteristics of subjects in the Obese Chinese Cohort sub-cohort (n=24).

	Baseline (N=24)	1 month (N=13)	3 months (N=14)	*P* value
Age (Years)	32.46 ± 9.58	30.46 ± 9.32	32.67 ± 10.64	0.983
Sex (Male %)	45.83%	46.15%	38.89%	0.885
BMI (kg/m^2)	42.44 ± 13.96	42.36 ± 14.08	33.47 ± 10.50	0.036
Waist circumference (cm)	121.72 ± 18.54	116.56 ± 17.58	97.88 ± 28.65	0.001
FPG (mmol/L)	8.27 ± 2.77	5.37 ± 0.83	5.23 ± 0.87	<0.001
HbA1c (%)	7.18 ± 1.56	6.24 ± 1.14	5.33 ± 0.52	<0.001
Insulin (mIU/L)	21.68 ± 12.00	13.66 ± 6.32	9.82 ± 6.10	<0.001
HDL (mmol/L)	1.06 ± 0.19	0.95 ± 0.10	1.09 ± 0.21	0.782
LDL (mmol/L)	2.96 ± 0.83	2.87 ± 0.65	3.10 ± 0.63	0.600
TG (mmol/L)	2.06 ± 1.18	1.31 ± 0.42	1.22 ± 0.42	0.002
GA (%)	19.01 ± 6.00	10.82 ± 3.07	11.69 ± 3.02	<0.001
1,5-AG (μmol/L)	61.08 ± 48.17	75.05 ± 53.76	80.28 ± 40.79	0.187

Data are presented as mean ± SD. BMI, body mass index, HDL; High-density lipoprotein; LDL, Low-density lipoprotein; TG, triglycerides; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; 1,5-AG, 1,5-anhydroglucitol.

Supplementary Table 5. Baseline characteristics of subjects in the CRISPS sub-cohort.

	Non-diabetes (n=158)	Incident diabetes (n=158)	*P* value
Age (Years)	50.47 ± 11.02	50.98 ± 11.10	0.684
Sex (Male %)	49.4	49.4	1.00
BMI (kg/m^2)	24.19 ± 3.65	26.01 ± 3.53	< 0.001
Waist circumference (cm)	77.39 ± 9.10	83.30 ± 9.70	< 0.001
FPG (mmol/L)	4.93 ± 0.45	5.20 ± 0.56	< 0.001
2hG (mmol/L)	5.75 ± 1.51	7.38 ± 1.95	< 0.001
HbA1c (%)	5.65 ± 0.47	5.85 ± 0.45	< 0.001
TC (mmol/L)	5.25 ± 0.90	5.49 ± 0.95	0.021
HDL (mmol/L)	1.39 ± 0.35	1.26 ± 0.32	0.001
LDL (mmol/L)	3.35 ± 0.80	3.55 ± 0.85	0.030
TG† (mmol/L)	0.90(0.70-1.30)	1.20(0.90-1.80)	< 0.001
Use of lipid-lowering drug (%)	7.6	13.9	0.448
SBP (mmHg)	126.23 ± 19.81	130.27 ± 19.95	0.072
DBP (mmHg)	77.36 ± 10.82	81.18 ± 10.78	0.002
HT (%)	40.5	55.7	0.007
Use of anti-hypertensive medication (%)	7.6	13.9	0.101
Exercise within the last 1 month	38.0	48.7	0.069
GA (%)	11.83 ± 2.35	13.74 ± 2.81	< 0.001
1,5-AG (μmol/L)	131.99 ± 50.50	102.80 ± 42.34	<0.001

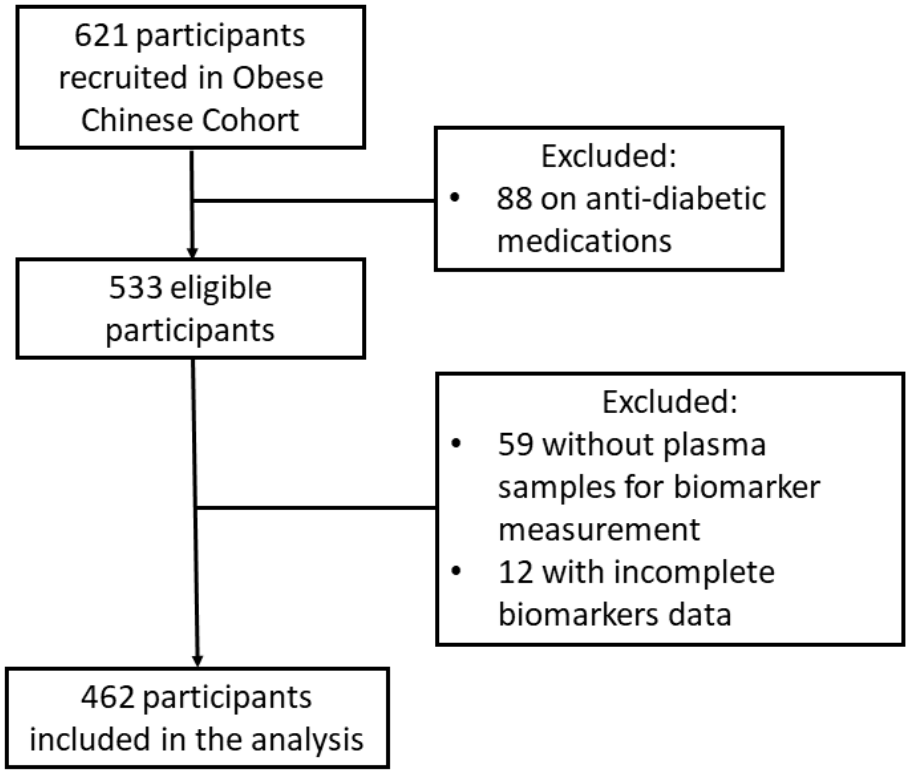
Data are presented as mean ± SD or median(interquatile range). BMI, Body mass index; FPG, fasting plasma glucose; 2hG, 2-hours glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL, High-density lipoprotein; LDL Low-density lipoprotein; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension; GA, glycated albumin; 1,5-AG 1,5-anhydroglucitol. †Natrual log-transformed before analysis.

Supplementary Table 6. Multivariable conditional logistic regression analyses.

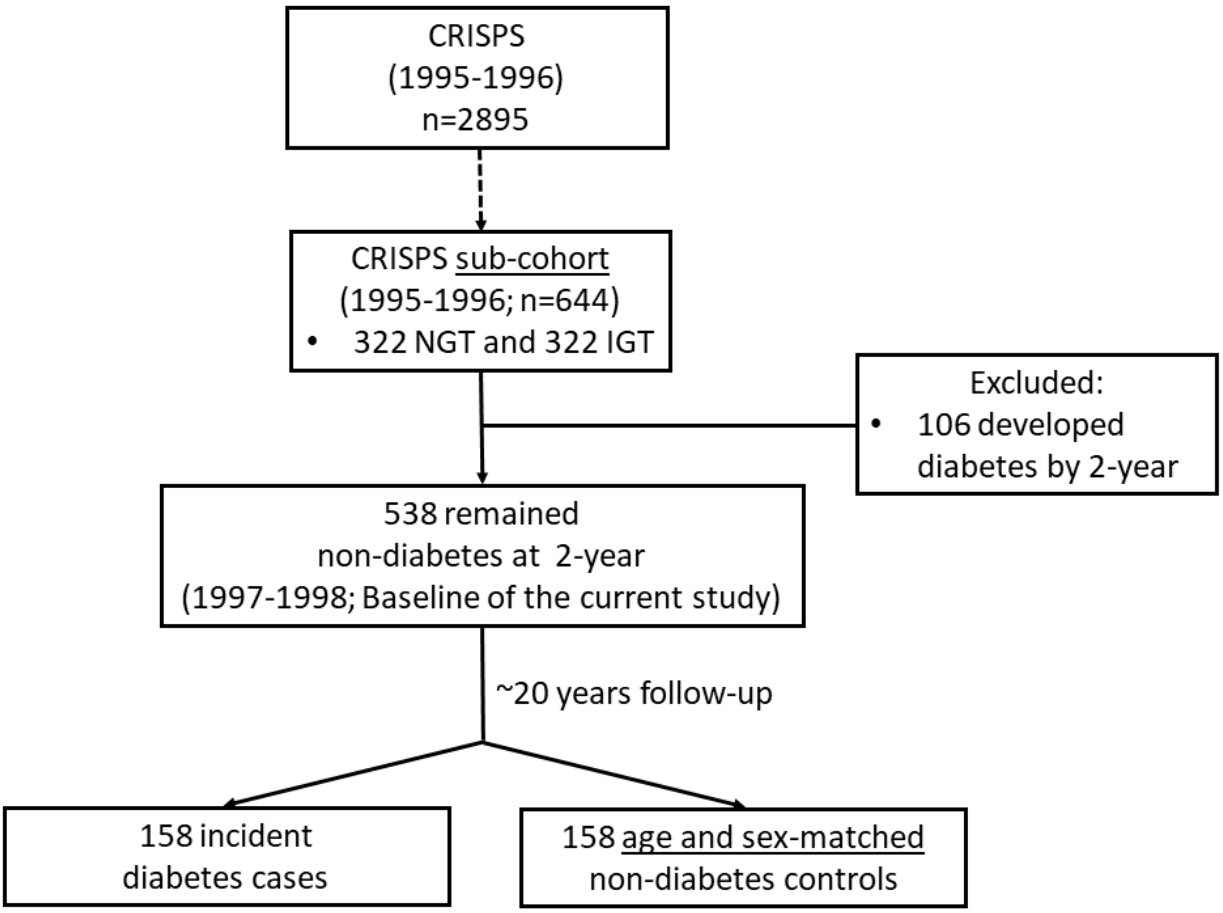
	OR(95%CI)	*P* value	OR(95%CI)	*P* value
BMI (kg/m^2)	1.08(1.00-1.17)	0.049	1.07(0.99-1.16)	0.077
HbA1c (%)	1.79(1.00-3.19)	0.049	2.18(1.22-3.92)	0.009
FPG (mmol/L)	1.62(0.92-2.84)	0.09	1.47(0.82-2.64)	0.192
2hG (mmol/L)	1.42(1.21-1.67)	<0.001	1.54(1.31-1.81)	<0.001
TG† (mmol/L)	1.82(1.00-3.32)	0.052	1.57(0.87-2.83)	0.133
GA (%)	1.29(1.16-1.45)	<0.001	-	-
1,5-AG (μmol/L)	-	-	0.99(0.98-0.99)	<0.001

BMI Body mass index, HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; 2hG, 2-hours glucose; GA, glycated albumin; 1,5-AG 1,5-anhydroglucitol. †Natrual log-transformed before analysis.

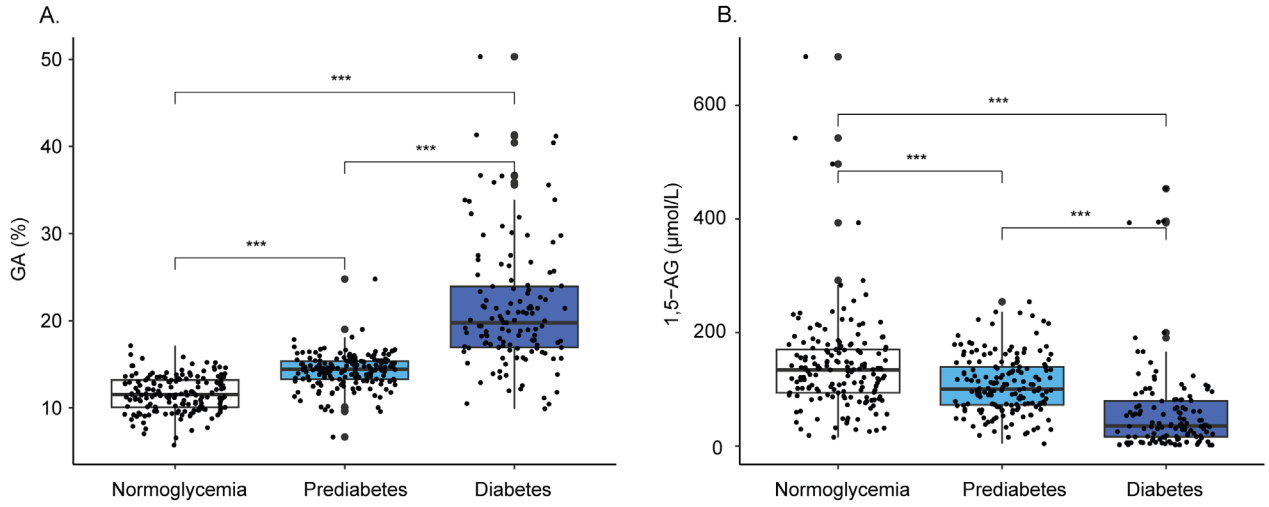
Supplementary Figure 1. Flow of study in the Obese Chinese Cohort.



Supplementary Figure 2. Flow of study in CRISPS sub-cohort.

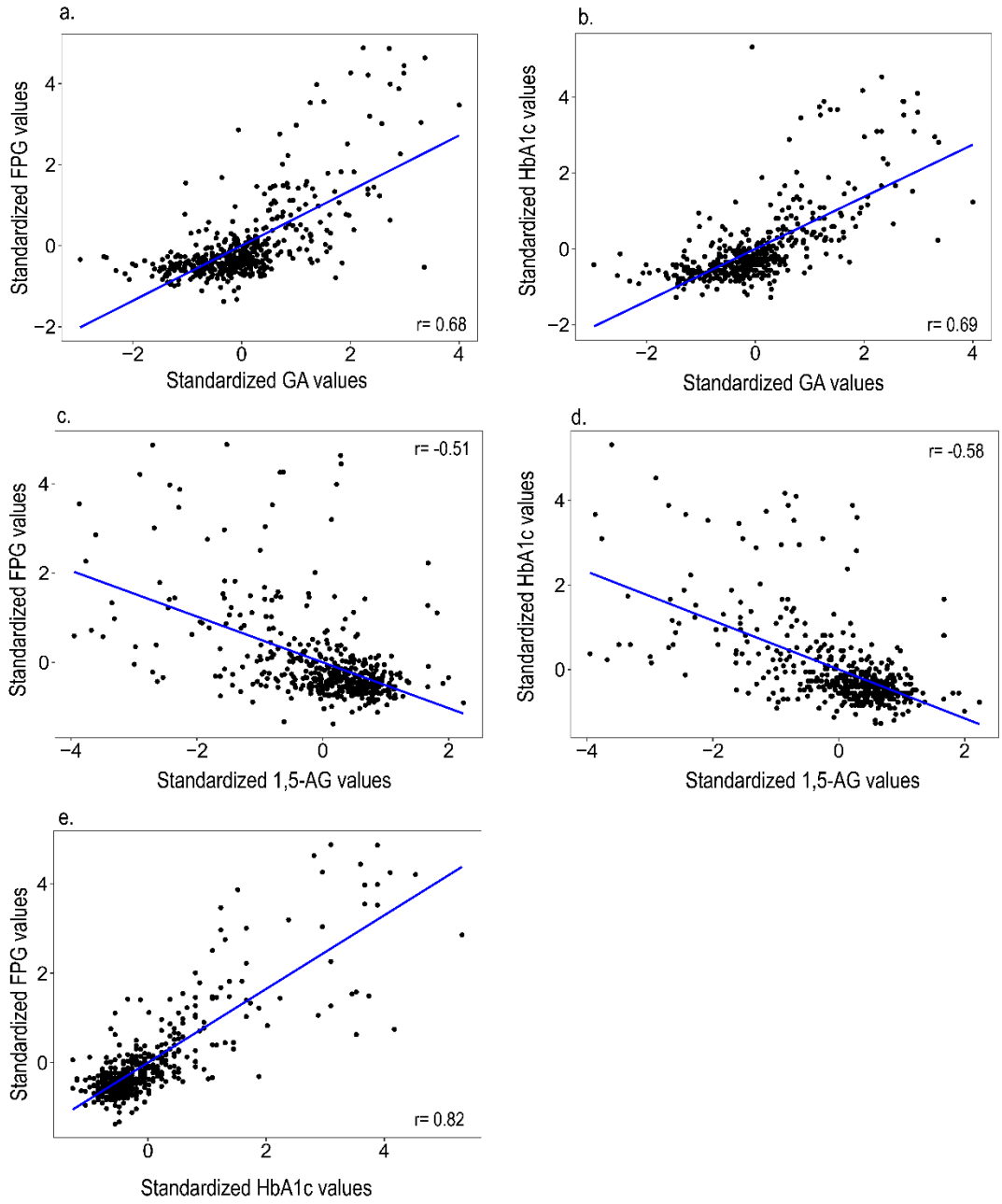


Supplementary Figure 3. Circulating levels of GA and 1,5-AG levels in different groups of subjects in the OCC.



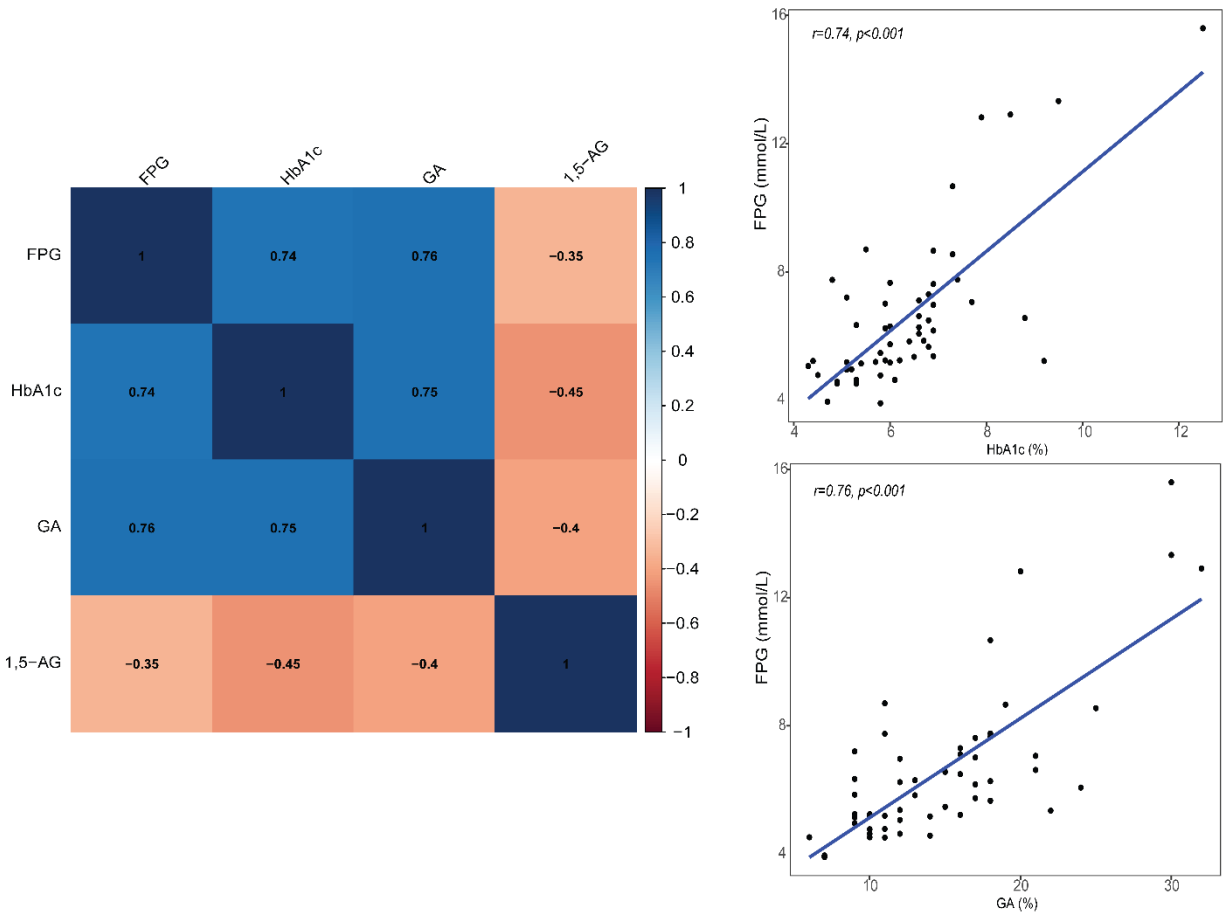
The circulating levels of (A) GA and (B) 1,5-AG in subjects with different glycemic statuses in Obese Chinese Cohort (*** *P* value< 0.001).

Supplementary Figure 4. Correlation analysis between biomarkers.



Pearson correlation coefficients between (a) GA and FPG; (b) HbA1c and FPG; (c) 1,5-AG and FPG; (d) 1,5-AG and HbA1c; and (e) FPG and HbA1c, all *P* value <0.001.

Supplementary Figure 5. The correlations between FPG, HbA1c, GA and 1,5-AG over the 3-month recovery period.



Data were collected at baseline, one and three months after the bariatric surgery.

Supplementary Figure 6. ROC analysis of the different biomarkers.