Glycated albumin is correlated to insulin resistance and β-cell secretory function in subjects at risk of developing diabetes

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ABSTRACT

Insulin resistance and β-cell secretory function represent two main issues in the pathogenesis of type 2 diabetes mellitus (T2DM). Conflicting results have been obtained about the association between glycated albumin (GA) and body mass index (BMI), insulin resistance and β-cell function in diabetic patients. Actually, the relationship (if any) between GA and the markers of glucose homeostasis and insulin resistance in subjects at risk of developing diabetes, has not been completely elucidated yet. Two hundred and one patients undergoing to oral glucose tolerance test (OGTT) were enrolled in the study. Routine laboratory tests, including fasting insulin, were performed at enrollment. GA was measured on plasma-EDTA by quantILab® Glycated Albumin (Instrumentation Laboratory, A Werfen Company) on ILab Taurus analyzer. According to the plasma glucose concentration measured after 2 hours of glucose intake (2h-PG), 13 subjects (6.4%) were classified as impaired glucose tolerance (IGT). GA weakly correlated with fasting plasma glucose (FPG) ($r=0.21$; $P=0.002$), with HbA1c ($r=0.16$; $P=0.024$) but not with 2h-PG ($P=0.7$). GA, but not HbA1c, was negatively correlated to HOmeostasis Model Assessment for β cell fuction (HOMA-β) ($r^2=0.23$; $P<0.001$), to HOMA for insulin resistence (HOMA-IR) ($r^2=0.15$; $P<0.0001$) and to BMI ($r^2=0.05$; $P=0.001$). In a stepwise multivariate regression analysis including HbA1c, HOMA-β, plasma albumin, BMI, eGFR, age, FPG, and HOMA-IR as predictors of GA, only HbA1c (β-coefficient: 0.04; $P=0.038$) and HOMA-β (β-coefficient: -0.01; $P<0.0001$) were able to predict GA levels ($r^2=0.26$; $P<0.001$ for the model). Our results demonstrated that GA was associated to HOMA-β and, to a lesser extent, to HOMA-IR and BMI. The increase of GA values can be explained by the reduction of β-cell secretory function in subjects with no significant increase of FPG and 2h-PG.

INTRODUCTION

Insulin resistance and β-cell secretory function represent two main issues in the pathogenesis of type 2 diabetes mellitus (T2DM) (1). In the natural history of the disease, postprandial hyperglycemia is the first abnormality that arises from the increased endogenous production of glucose, impaired post-prandial glucose control and reduced peripheral glucose uptake by skeletal muscle cells. All these can be considered features of peripheral insulin resistance and are accompanied by progressive hyperinsulinemia (2). Gradually, β-cells became incapable of secreting adequate amount of insulin to overcome the metabolic dysfunction. In clinical practice, the evaluation of β-cells secretory function and insulin resistance are not feasible, so several indexes have been proposed, including the HOmeostasis Model Assessment (HOMA) based on fasting glucose and insulin (3). Several biomarkers have been evaluated in relation to the onset of diabetes and its related micro- and cardiovascular complications (4-6), although it is clear that the main issue in the reduction of diabetes incidence, its metabolic features and the cardiovascular outcomes is the control of the main risk factors (7-10).

Glycated albumin (GA) has been proposed as a short-term marker of glucose homeostasis. Glycation of plasmatic proteins is a non-enzymatic process and can

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occur at different rates based on the substrate. It has been demonstrated that albumin is glycated in the bloodstream at a higher rate than other plasmatic proteins, including hemoglobin (11). The GA distribution has been described in both healthy subjects and diabetics, showing a significant increase in the latter (12, 13), supporting thus the GA determination in the management of diabetes. Several clinical studies demonstrated that GA decreased faster than HbA1c when the hypoglycemic therapy was initiated in patients with diabetes (14). Moreover, GA has been evaluated for the screening of diabetes in the general population and in subjects at risk, showing high diagnostic accuracy in diabetes diagnosis (15-17).

Some authors demonstrated that a negative correlation exists between GA and body mass index (BMI) in T2DM patients (18), but this finding was not replicated in patients with acute-onset type 1 diabetes (19) suggesting that this association is influenced by the different role of β-cell function in the pathogenesis of the different types of diabetes. Actually, it is not clear the relationship (if any) existing between GA, the other markers of glucose homeostasis and insulin resistance in subjects at risk of developing diabetes.

The aim of this observational study is to evaluate the relationship between GA, fasting plasma glucose (FPG), plasma glucose concentration measured after 2 hours from glucose intake (2h-PG), HbA1c and insulin resistance in a group of subjects at risk of diabetes who underwent to an oral glucose tolerance test (OGTT).

MATERIALS AND METHODS

Subjects

Recruitment was conducted at two major referral centers in Italy (University Hospital in Palermo; University Hospital in Padua) from September 2016 to July 2017. All patients presenting with one or more of the following risk factors for diabetes (20) were enrolled: overweight (BMI ≥25 kg/m^2 and <29.9 kg/m^2) or obesity (BMI ≥30 kg/m^2); previous HbA1c from 39 to 47 mmol/mol or impaired fasting glucose (IFG) (FPG ≥5.6 mmol/L and <7.0 mmol/L) or impaired glucose tolerance (IGT) (2h-PG ≥7.8 mmol/L and <11 mmol/L); family history of diabetes; women with previous gestational diabetes; history of cardiovascular disease; hypertension (≥140/90 mmHg or on therapy for hypertension); HDL-cholesterol <0.9 mmol/L or triglycerides >6.46 mmol/L; women with polycystic ovary syndrome; physical inactivity. Exclusion criteria were: anemia, hemoglobinopathies, recent transfusions, pregnancy, major surgery in the last six months, hypo- or hyperthyroidism, chronic liver disease, malignant diseases, any acute critical condition. Baseline demographic, biochemical and clinical characteristics of the participants undergoing to OGTT are shown in Table 1.

Blood samples, complete medical history and written informed consent were collected at enrollment. The study protocol was approved by the local Ethics Committees in accordance with the Declaration of Helsinki, and all study participants gave informed consent.

Laboratory analysis

HbA1c was measured by the D100 instrument and reagents (BioRad) at the University Hospital of Palermo and by the HA8180V instrument and reagents (Menarini Diagnostics) at the other site. Both assays are based on an ion exchange high performance liquid chromatography (HPLC) method. It has been demonstrated that the two assays are highly correlated (21). Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (22). GA was measured on plasma-EDTA by quantILab® Glycated Albumin (Instrumentation Laboratory, A Werfen Company) on ILab Taurus analyzer. This enzymatic colorimetric method includes the measurement of GA and albumin. GA concentration is reported as percentage of total albumin, and corrected with the inter-method arithmetic expression designed to adhere to the

Table 1
Baseline demographic, biochemical and clinical characteristics of the participants

N	201
Age, years	36 (25-54)
Males, n (%)	48 (23.9)
Caucasians, n (%)	187 (93.0)
Family history of diabetes, n (%)	74 (37.8)
BMI, kg/m^2	23 (21-26)
Obesity, n (%)	20 (10)
Waist circumference, cm	85.5±17.5
Albumin, g/L	42.2±3.6
Total Cholesterol, mmol/L	4.7±0.9
HDL Cholesterol, mmol/L	1.5±0.5
Triglycerides, mmol/L	0.8 (0.6-1.2)
Insulin, μU/mL	7 (4.5-11)
HOMA-β	93 (65-130)
HOMA-IR	0.9 (0.7-1.5)
Glycated Albumin, %	13±1.4
FPG, mmol/L	4.9 (4.6-5.5)
HbA1c, mmol/mol	36 (33-38)

Data are mean±SD or median (interquartile range).
BMI, body mass index; HOMA-β, Homeostasis Model Assessment for β cell function; HOMA-IR, Homeostasis Model Assessment for insulin resistance; FGP, fasting plasma glucose, HbA1c, glycated hemoglobin; 2h-PG, plasma glucose after two hours from glucose intake.

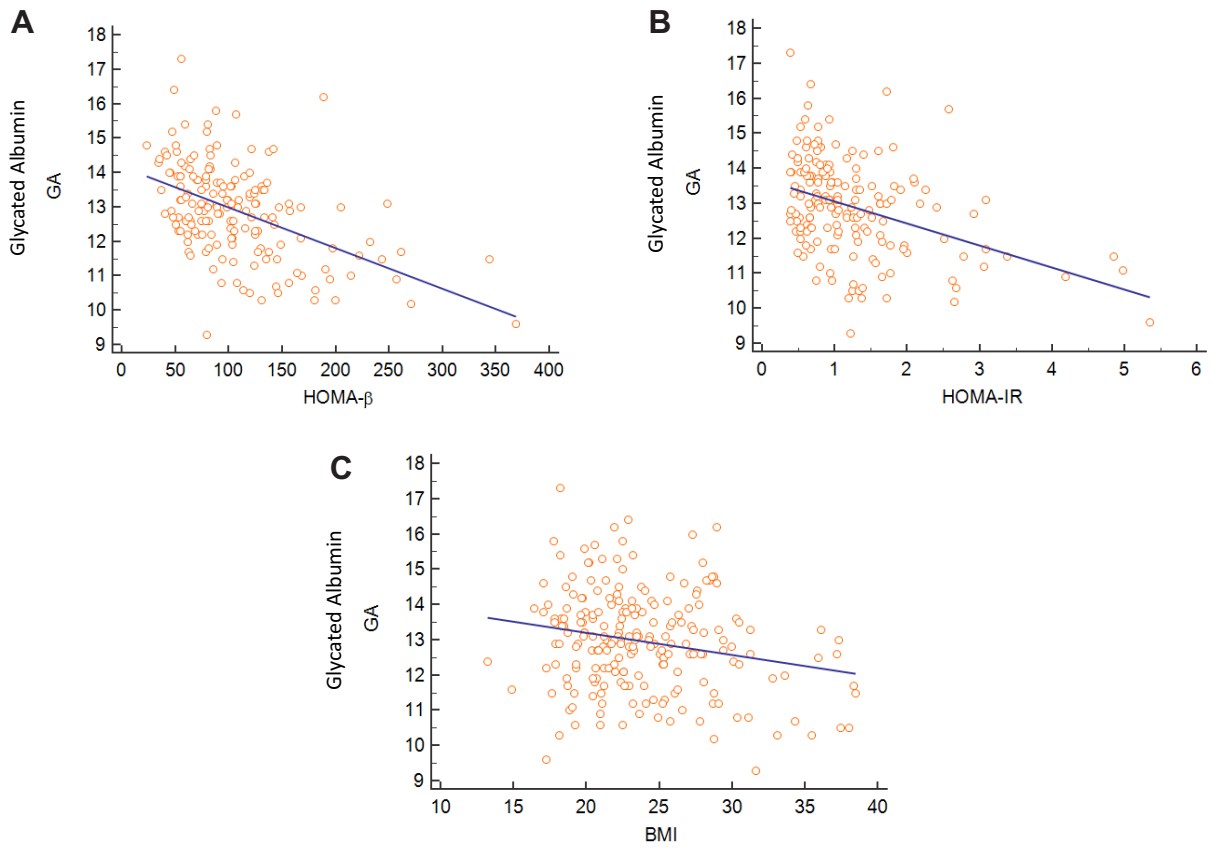

Figure 1
Univariate regression analysis between glycated albumin and HOmeostasis Model Assessment for β cell function (HOMA-β) (r^2=0.23; intercept=14.1 [95%CI: 13.8-14.5]; slope=-0.011 [95%CI: 0.015-0.008]); P <0.001) (panel A).
HOmeostasis Model Assessment for insulin resistance (HOMA-IR) (r^2=0.15; intercept=13.7 [95%CI: 13.3-14]; slope=-0.62 [95%CI: 0.85-0.40]; P=0.0009) (panel B).
Body Mass Index (BMI) (r^2=0.05; intercept=14.5 [95%CI: 13.5-15.4]; slope=-0.063 [95%CI: 0.10-0.024]; P=0.001) (panel C).

HPLC method (23). Imprecision of the assay was evaluated analyzing two control samples provided by the manufacturer. At low concentration (GA 15.2%), between-run and within-run CV were 3.1% and 1.3%, respectively. At high concentration (32.1%), between-run and within-run CV were 2.5% and 0.9%, respectively. HOMA of insulin resistance (HOMA-IR) and HOMA of β-cell function (HOMA-β) indexes was calculated by the HOMA2 Calculator v2.2.3 (©Diabetes Trials Unit, University of Oxford).

Statistical analysis

Variables were presented as mean ± standard deviation (SD) for continuous variables, as medians with interquartile ranges (IQR) for not-normally distributed continuous variables, and as a percentage for the categorical variables.

The correlation between values was assessed with Spearman's (Pearson) test and expressed as r.

To analyze the effect of explanatory variables on GA, univariate regression analysis was performed and results expressed as r^2. GA was considered as the dependent variable. In stepwise multivariate regression analysis GA was the dependent variable, and HbA1c, HOMA-β, albumin, BMI, eGFR, age, FPG, HOMA-IR were considered as explanatory variables. The statistical significance of P <0.05 was accepted for all the tests. Statistical analysis was performed using MedCalc software Version 17.9.2.

RESULTS

The study included 201 subjects with a median age of 36 years (IQR: 25-54), 23.9% were males. According to 2h-PG, 13 subjects (6.4%) were classified as IGT. GA correlated with FPG (r=0.21; P=0.002), with HbA1c (r=0.16; P=0.024) but not with 2h-PG (P=0.7). The GA% in the whole group was 13±1.4.

According to linear regression analysis, GA was negatively correlated to HOMA-β (r^2=0.23; P<0.001; Figure 1A) and to HOMA-IR (r^2=0.15; P<0.0001; Figure 1B). Interestingly, these correlations were not detected for HbA1c. GA was negatively correlated to BMI (r^2=0.05; P=0.001; Figure 1C) while plasma albumin was not.

Finally, in a stepwise multivariate regression analysis including HbA1c, HOMA-β, albumin, BMI, eGFR, age,

FPG, and HOMA-IR as predictors of GA, only HbA1c (β-coefficient: 0.04; P=0.038) and HOMA-β (β-coefficient: -0.01; P <0.0001) were able to predict GA levels (r^2=0.26; P <0.001 for the model).

DISCUSSION

The main finding of this study is that GA was associated to insulin resistance and, particularly, to β-cell secretory function in subjects with low-grade metabolic disturbances. This implies that GA could be considered a marker of β-cell dysfunction even in the initial stages of the disease. The cohort of patients included in this study was of particular interest because they represented the target for specific interventions to prevent the onset of diabetes, and, in turn, to control long-term complications associated to hyperglycemia exposure. In non-diabetic subjects from the ARIC study, GA showed similar associations with traditional risk factors, including older age, family history of diabetes, and black ethnicity, in comparison to common measures of hyperglycemia, supporting its value as a marker of the metabolic alterations resulting in the loss of glycemic control (24). Moreover, GA showed a good sensitivity for prediabetes in non-obese African immigrants living in the USA, considered a population at high cardio-metabolic risk (25).

In our study, the association of GA with other markers of glucose homeostasis was lower than the ones reported by previous studies (26, 27). This result can be explained with the low prevalence of prediabetes (6.4%) observed in the sample population according to 2h-PG and the low prevalence of high FPG and HbA1c.

In the present study, GA was also associated to insulin resistance, β-cells dysfunction and BMI. Interestingly, HbA1c did not show similar associations. Both insulin resistance and β-cells dysfunction can be observed in prediabetes (2), defined as IFG or IGT, which is associated to a significant increase of the risk of diabetes (28). Nevertheless, it is reasonable that such risk might be present also when glucose concentration is within reference range, indicating that metabolic abnormalities might arise before the glycemic control is impaired. In the Whitehall II study, a prospective study conducted on a large middle-aged, metabolically healthy population, Tabak et al demonstrated that HOMA β-cell function decreased in a linear fashion, increasing between years 4 and 3 before the diagnosis of diabetes and then decreasing rapidly (29). This multistage model for the pathogenesis of the disease is in agreement with the hypothesis that a metabolic impairment characterized by β-cell dysfunction and transient impaired glycemic control occur before the onset of a stable postprandial or fasting hyperglycemia, both considered diagnostic criteria for the disease (20, 30). Moreover, it is known that metabolic alterations leading to diabetes can be considered as a continuum spectrum from normal glucose homeostasis to diabetes (29). In light of this view, a marker that can detect rapid fluctuation of plasma glucose before the onset of the disease, is appealing. Our results demonstrated that the reduction of HOMA-β is associated to an increase of GA, also in subjects with no significant alteration of FPG and 2h-PG. It can be hypothesized that in this prodromic state of the disease, the reduction of β-cell function might cause small fluctuations of plasma glucose allowing the glycation of some circulating proteins. Non-enzymatic glycation of albumin occurs 9- to 10-fold higher than other plasma proteins and, specifically, 4.5-fold higher than hemoglobin (31, 32) and it has been associated to a higher glycemic variability in diabetic patients (33, 34). Notably, HbA1c was not associated to β-cell dysfunction nor in the present study nor in a previous report (18). These results can be explained with the lower rate of glycation of hemoglobin in comparison to albumin and its reduced ability to reflect small glycemic excursions.

The negative association between GA and β-cell function has been documented in diabetic patients (18, 35, 36). It has been demonstrated that post-prandial hyperglycemia represents a different clinical phenotype from the fasting hyperglycemia (37), being the former the first sign of metabolic imbalance in the natural history of the disease. In light of this view, the confirmation of this finding in subjects at different stage of the metabolic impairment that prelude the disease could strengthen the associations between GA and markers of insulin resistance and β-cell function.

In our study, a negative association between GA and BMI was documented, confirming similar findings of previous studies. Indeed, GA was negatively correlated to BMI in both non-diabetic and diabetic patients (24, 25, 15, 38, 39), although the exact mechanism underlying this unexpected relationship remains unclear. It has been proposed that the chronic subclinical inflammation that characterizes overweight and obesity could also cause an increase of albumin turnover, thus reducing its glycation rate. Nevertheless, in our study BMI was not correlated to plasma albumin, suggesting that the correlation between GA and BMI is independent from the albumin plasma concentration. Further investigations are required to clarify the complex relationship between GA and BMI.

In conclusion, GA is negatively associated to peripheral insulin resistance and reduced β-cell secretory function in subjects with a low-grade metabolic imbalance. It has already been demonstrated that GA could help in the management of diabetic patients, especially in the short-term control of hypoglycemic therapy. Moreover, GA demonstrated good diagnostic accuracy in diabetes diagnosis, suggesting a role also in the diagnosis of the disease and, potentially, providing the opportunity to overcome the limitations of more complex diagnostic tests such as OGTT. Finally, GA can be considered a marker of reduced β-cell secretory function in the prodromic metabolic impairment considered at high risk of developing diabetes.

CONFLICTS OF INTERESTS

None.

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