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Performance characteristics and clinical utility of an enzymatic method for the measurement of glycated albumin in plasma

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Abstract

Objective: The measurement of plasma glycated albumin is particularly useful in the short-middle term monitoring of glycometabolic control in diabetics. The aim of this work is to evaluate a new enzymatic method for the measurement of glycated albumin in plasma, with particular attention to some selected cases and comparison with other relevant tests (fasting plasma glucose, after glucose load, fructosamine, glycated hemoglobin).

Design and methods: We have performed a multicenter study by which sample collection was performed in three different centers (Milano, Padova and Cagliari) and serum samples, frozen at −80 °C, were then delivered under dry ice to the centralized laboratory in Milano. Glycated plasma albumin was measured with reagents from Asahi Kasei Pharma (Lucica GA-L enzymatic assay; AKP, Tokyo, Japan) on a Modular P Roche system. Fructosamine was assessed by a Roche method and HbA_{1c} (measured separately in the three centers on fresh EDTA blood) by DCCT-aligned HPLC systems. We have investigated 50 type 2 diabetics, 26 subjects with gestational diabetes, 35 subjects with thalassemia major, 10 subjects with cirrhosis, 23 patients with end-stage renal disease subjected to dialysis treatment and 32 healthy adult control subjects.

Results: The main analytical performance characteristics of the new GA test were the following: (a) the within-assay reproducibility was between 3.0 and 3.9% (in terms of GA% CV, measured on 2 serum pools and 2 control materials at normal and pathological glycated albumin levels); (b) the between-assays reproducibility was from 2.8 to 4.1%; (c) the linearity was tested in the interval between 13 and 36% and found acceptable ($r^2=0.9932$). Concerning the clinical utility of the new test, we have evaluated the relationships between GA, HbA_{1c}, fructosamine and fasting and post-prandial glucose in several patients, as well as the changes in the abovementioned parameters in a sub-group of type 2 diabetic patients for 18 weeks as they progressed from severe hyperglycemia ($HbA_{1c} \geq 10.0\%$) toward a better glycemic control. The correlations between glycated albumin and HbA_{1c} were as follows: (a) type 2 diabetics: $r^2=0.483$ (good glycemic control), $r^2=0.577$ (poor control); (b) diabetic patients under dialysis: $r^2=0.480$; (c) liver disease: $r^2=0.186$; (d) transfused non-diabetics with thalassemia: $r^2=0.004$. Glycated albumin, as well as HbA_{1c} and fructosamine, was of little value in the study of women with gestational diabetes, mainly because of the very limited glucose fluctuations in this particular category of subjects. In 11 type 2 diabetic patients under poor metabolic control, GA was better correlated with fasting plasma glucose then HbA_{1c} ($r^2=0.555$ vs. 0.291, respectively), and decreased more rapidly than HbA_{1c} during intensive insulin therapy.

Abbreviation: GA, glycated albumin

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Conclusions: The experience we have acquired with the new enzymatic test demonstrates its reproducibility and robustness. We confirm that plasma glycated albumin is better related to fasting plasma glucose with respect to HbA_{1c}. Moreover, glycated albumin is more sensitive than HbA_{1c} with regard to short-term variations of glycemic control during treatment of diabetic patients. This test is also very appropriate when the interpretation of HbA_{1c} is critical.

Keywords: Glycated albumin; Diabetes; Enzymatic assay; Glycated hemoglobin

Introduction

Glucose in humans is able to bind to several proteins, such as hemoglobin, collagen, immunoglobulins and others, thus producing non-enzymatic adducts in the form of ketoamine and other compounds [1,2]. In diabetic patients, because of chronic hyperglycemia, such compounds may have a direct link to the development of glucose-related complications, as clearly proven by some important clinical trials, such as the DCCT and UKPDS [3,4]. In the case of hemoglobin, the measurement of glycated hemoglobin in blood is very useful to estimate the average mean blood glucose over the previous 2 months [5], and is now also indicated for estimating the risk of developing macrovascular complications in non-diabetic subjects [6].

The measurement of glycated albumin (GA) in serum has not gained the same popularity as glycated hemoglobin, partly because of the lack of specificity or poor standardization of the proposed methods, and partly because the main clinical trials dedicated to defining the relationship between hyperglycemia and diabetic complications have been designed with attention focussed to glycated hemoglobin. Indeed, the concentration of GA in serum can be measured, as in the case of glycated hemoglobin, on the basis of different principles, such as affinity chromatography [7,8], ion exchange liquid chromatography [9,10], colorimetry [11,12] and immunochemistry [13,14]. However, all these methods suffer from a number of disadvantages, namely, poor precision and long processing times.

In the last years, a novel three-step enzymatic procedure to determine GA has been developed. The new procedure comprises: (a) the assay of GA concentration using an albumin-specific proteinase and ketoamine oxidase; (b) the measure of total albumin concentration by bromocresol green and (c) the calculation of GA percentage in total albumin [15]. Later on, such a method was improved by adding a step to remove the glycated amino acids and substituting bromocresol green with bromocresol purple in order to increase specificity in the measurement of total albumin [16]. Moreover, recently, a dry chemistry system (GA monitor) via this same enzymatic method was proposed in order to provide a GA value measurement for point of care testing (POCT) [17].

In this investigation, we report our experience with this new assay for measuring GA in serum, which has been evaluated for its analytical performance and clinical utility. To this regard, we have studied several diabetic patients under different conditions, including those suffering from nephropathy and thalassemia major, whose interpretation of glycated hemoglobin is particularly difficult because of repeated red cell transfusions. A comparison with fasting and non-fasting plasma glucose, glycated hemoglobin and serum fructosamine has also been performed to highlight the potential advantages and limitations of the proposed new test.

Materials and methods

Patients

Patients were enrolled in three different centers: University of Padova, University of Cagliari and S. Raffaele Hospital in Milano. Eight groups of patients were enrolled, and selected as follows. Group 1: type 2 diabetic patients in good metabolic control ($n=40$; 23 men, 17 women; mean age [±SD] 62±7 years; mean disease duration 7±8 years; all treated with diet and hypoglycemic drugs); group 2: type 2 diabetic patients in poor metabolic control ($n=11$; 5 men, 6 women; age 59±9 years; mean disease duration 8±8 years; all treated with insulin); group 3: patients with end-stage renal disease subjected to dialysis treatment without diabetes ($n=16$; 12 men and 4 women, age 71±9 years) and with diabetes ($n=7$; 4 men and 3 women, age 68±11 years); group 4: women with gestational diabetes mellitus (GDM) ($n=26$; age 35±4 years; 21 treated with diet, 5 with diet and insulin); group 5: thalassemic (Cooley's disease) non-diabetic patients ($n=19$; 11 men and 8 women, age 25±6 years); group 6: thalassemic (Cooley's disease) patients with diabetes ($n=15$; 8 men and 7 women, age 28±3 years); group 7: patients with cirrhosis ($n=10$; 4 men and 6 women, age 52±8 years); group 8: normal control subjects (blood donors; $n=32$; 12 men and 20 women, age 41± 10 years). Exclusion criteria for the patients with liver disease were the presence of diabetes mellitus. Group 2 patients were monitored at baseline and every 2 weeks for about 2 months, while group 4 patients were monitored every 2 weeks until delivery.

All patients gave informed consent to the study that was approved by the local Ethical Commission.

Laboratory measurements

EDTA-plasma samples were collected for measuring fasting glucose, GA and fructosamine. In group 1, 2 and 4, samples for post-prandial (1 hr after lunch) plasma glucose were also collected. Glucose measurements were performed directly in the three centers by enzymatic methods [18]. The remaining plasma aliquots were immediately frozen, stored at −80 °C and sent within 3 months to the centralized laboratory in Milano for GA and fructosamine quantification. A Roche Modular–P system automatic clinical analyzer (Roche, Milano) was used for

measuring GA by the Lucica® GA-L enzymatic assay (Asahi Kasei Pharma Corp., Tokyo, Japan), and fructosamine by Roche reagents [19]. A separate set of EDTA-blood samples was collected, stored and shipped in the same condition for measuring the HbA_{1c} concentrations to the same centralized laboratory in Milano. HbA_{1c} was measured by HPLC using an automated system (Bio-Rad Variant II dual kit, Bio-Rad Laboratories, Segrate, Milano) aligned to the DCCT method, as previously described [18].

Analytical evaluation of Lucica® GA-L enzymatic assay

Analytical imprecision was evaluated using different specimens. Within-run reproducibility was estimated using two control materials with normal (Level 1) or raised (Level 2) values of GA, and two plasma pool samples with normal (Level 1) and abnormal (Level 2) concentrations of GA with 9–11 replicates. The control materials with normal and raised GA levels were liophylized human sera obtained from the Manufacturer (BML Inc., Tokyo, Japan). In 1 day, we performed two runs. The same specimens were used for evaluating the between-run imprecision with 6 (controls) and 10–11 (pools) independent runs over 7 months. The controls analyzed for between-run imprecision were only 6 because we had to change a lot of controls during the period of test evaluation.

Linearity was assessed by assaying 11 samples prepared by mixing different volumes of a low-level serum pool (approximately 13% GA) and a high level serum pool (approximately 35% GA) to obtain various GA values between these limits. Each specimen was measured in duplicate and the means of the GA percentage were plotted on the on *y*-axis vs. dilution on the *x*-axis.

Interference due to lipids, bilirubin and hemoglobin was evaluated by analyzing aliquots from a normal sera pool to which known amounts of a lipid emulsion (Intralipid, Pharmacia, Milan, Italy; 1.13–13.5 mmol/L final concentration), bilirubin (Sigma, Milan, Italy; 7.7–1290 μmol/L final concentration) and hemoglobin (0.25–2.5 g/L) were added separately. Interference was assumed to be significant when bias resulted to exceed ±10% with respect to the basal (i.e. without interference) level.

Table 1
Within-run imprecision

Parameter	Control		Pool	
	Level 1 (n=9)	Level 2 (n=9)	Level 1 (n=11)	Level 2 (n=10)
Albumin (g/dL)				
Mean (SD)	4.49 (0.17)	4.49 (0.10)	4.33 (0.07)	3.65 (0.10)
CV (%)	3.8	2.2	1.6	2.7
Glycated albumin (g/dL)				
Mean (SD)	0.58 (0.02)	1.77 (0.07)	0.57 (0.02)	0.86 (0.04)
CV (%)	3.9	4.2	4.2	4.2
Glycated albumin (%)				
Mean (SD)	14.3 (0.42)	37.4 (1.2)	14.6 (0.54)	23.5 (0.92)
CV (%)	3.0	3.2	3.7	3.9

Table 2
Between-run imprecision

Parameter	Control		Pool	
	Level 1 (n=6)	Level 2 (n=6)	Level 1 (n=11)	Level 2 (n=10)
Albumin (g/dL)				
Mean (SD)	4.57 (0.15)	4.53 (0.11)	4.33 (0.07)	3.64 (0.10)
CV (%)	3.3	2.8	1.7	2.8
Glycated albumin (g/dL)				
Mean (SD)	0.59 (0.02)	1.79 (0.06)	0.57 (0.02)	0.85 (0.04)
CV (%)	2.7	3.6	4.4	4.5
Glycated albumin (%)				
Mean (SD)	14.3 (0.42)	37.5 (1.1)	14.6 (0.56)	23.5 (0.97)
CV (%)	2.8	3.0	3.9	4.1

Statistical analysis

Values were expressed as mean ± SD, CVs or median (2.5th-97.5th percentiles) when not normally distributed. In order to assess the significance of the differences the Mann–Whitney Rank Sum Test was used. Linear regression analysis was performed by the least squares method. All statistical analyses were performed using SigmaStat software (Jandel Scientific, version 3.0).

Results

Analytical imprecision

The results of the reproducibility study for GA measurements are shown in Tables 1 and 2. Within-run and between-run CVs for GA% in the 4 tested materials were below 3.9% and 4.1%, respectively, the higher values being both found for the pathological pool. It is worth noting that the expression of GA as a ratio to total albumin (i.e. GA%) has generally a greater precision than that of GA expressed in terms of mass concentration.

Linearity and interferences

The Lucica® GA-L enzymatic assay showed an excellent linearity in all of the concentration ranges tested. In Fig. 1A, the mean values of 2 GA determinations are plotted against dilution. Bilirubin was not found to interfere in the analyses up to 342 μmol/L with a negative interference (−40%) at 1290 μmol/L (Fig. 1B). Turbidity interference was tested up to 13.5 mmol/L triglycerides, evidencing at this level a positive bias up to +80%. At 3.9 mmol/L, a bias of +26.2% was found (Fig. 1C). Hemolysis was tested up to 2.5 g/L, showing a significant negative bias above 1.75 g/L and about 30% underestimation at 2.5 g/L (Fig. 1D).

Clinical utility of the GA test

Levels of GA, HbA_{1c}, fructosamine and fasting glucose in all the categories of subjects studied are summarized in Table 3. In

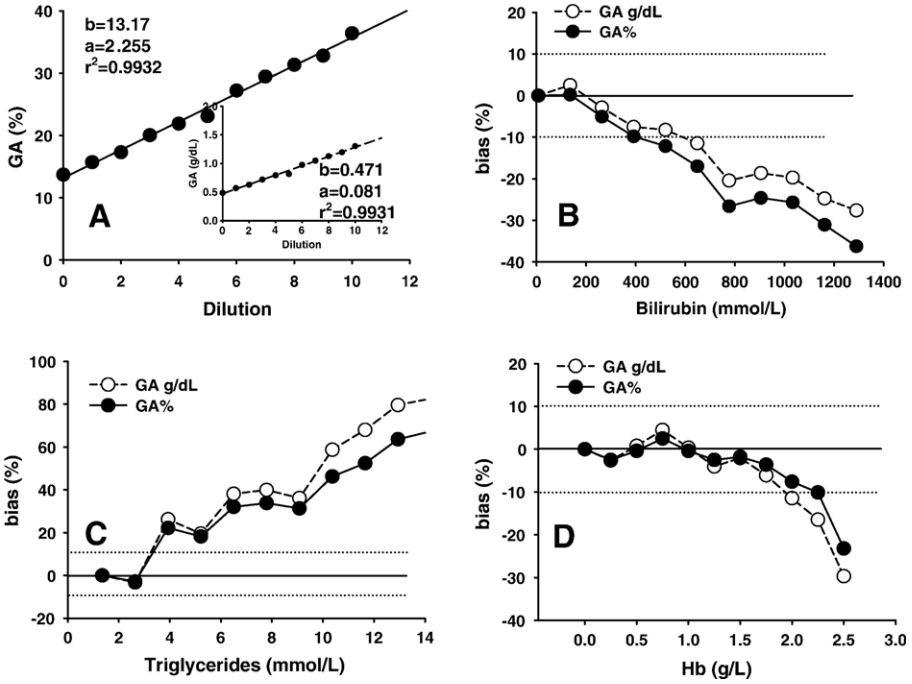

Fig. 1. Performance characteristics of the glycated albumin enzymatic assay. (A) Linearity in the range of 10–40%. In the insert, the linearity of glycated albumin expressed in terms of g/dL is reported. (B) Bilirubin interference. (C) Triglycerides interference. (D) Hemoglobin interference. The dashed lines in panels B, C and D highlight the bias limits of ±10%.

almost all the subject categories, the four parameters tested were significantly higher when compared to control subjects, and this was particularly evident in type 2 diabetic patients in poor metabolic control, renal patients and in the thalassemic diabetic patients.

Comparisons of GA with HbA_{1c} and fructosamine, and their relationships of each other with plasma glucose were evaluated. The most significant findings of these analyses are reported in Figs. 2 and 3.

In Fig. 2, the results obtained in healthy blood donors and type 2 diabetic patients in good metabolic control are reported. These two categories of subjects were pooled in order to cover most of the physiopathological range of the measured parameters. In these subjects, positive significant correlations were found between GA, HbA_{1c}, fructosamine and fasting plasma glucose (Figs. 2A–C), with the strongest correlation obtained for HbA_{1c} ($r^2=0.746$) (Fig. 2B) which appeared to be better correlated to fasting glucose with respect to GA ($r^2=0.504$) (Fig. 2A). Furthermore, positive correlations, even if weaker, were found between GA, HbA_{1c}, fructosamine and post-prandial plasma glucose (Figs. 2A–C). GA and fructosamine also resulted well correlated ($r^2=0.778$) (Fig. 2E). Finally, it is interesting to note that the correlation between GA and HbA_{1c} ($r^2=0.483$) (Fig. 2D) is weaker than that of both GA and

Table 3
Glycated albumin, HbA_{1c}, fructosamine and fasting glucose among the various groups of patients

Parameter	Type 2 diabetes		Renal disease	GDM	Cooley's		Cirrhosis	Controls
	Good control	Poor control			Non-diabetes	Diabetes		
GA (%)	17.4*	26.4*	15.6*	14.5	11.7*	18.3*	14.1	13.4
	14.2–270	22.6–49.9	12.8–35.8	12.3–16.5	10.3–13.6	11.0–41.2	11.7–15.9	11.7–16.9
	(n=39)	(n=10)	(n=23)	(n=22)	(n=19)	(n=14)	(n=10)	(n=32)
HbA_{1c} (%)	6.8*	11.1*	5.9*	5,4	6.6*	8.2*	5.4	5.3
	5.9–8.3	9.4–13.1	4.9–9.4	4.9–6.9	6.0–8.2	6.1–13.5	4.7–5.9	4.7–5.7
	(n=39)	(n=9)	(n=13)*	(n=22)	(n=19)	(n=15)	(n=10)	(n=32)
Fructosamine	254*	408*	315*	194	200	271*	228*	192
(μmol/L)	193–315	346–653	260–430	167–216	175–223	180–569	180–255	149–242
	(n=35)	(n=10)	(n=23)	(n=21)	(n=19)	(n=14)	(n=10)	(n=22)
Fasting glucose	7.72*	13.6*	13.8*	4.83	5.22*	8.55*	5.27*	4.66
(mmol/L)	6.33–11.9	8.6–24.0	5.05–15.5	4.22–6.72	4.72–5.88	4.77–18.26	4.33–6.61	4.05–5.44
	(n=39)	(n=9)	(n=23)	(n=22)	(n=19)	(n=15)	(n=10)	(n=32)

Values are reported as median (2.5th–97.5th percentile). *P<0.01 vs. controls by Mann–Withney Rank Sum Test.

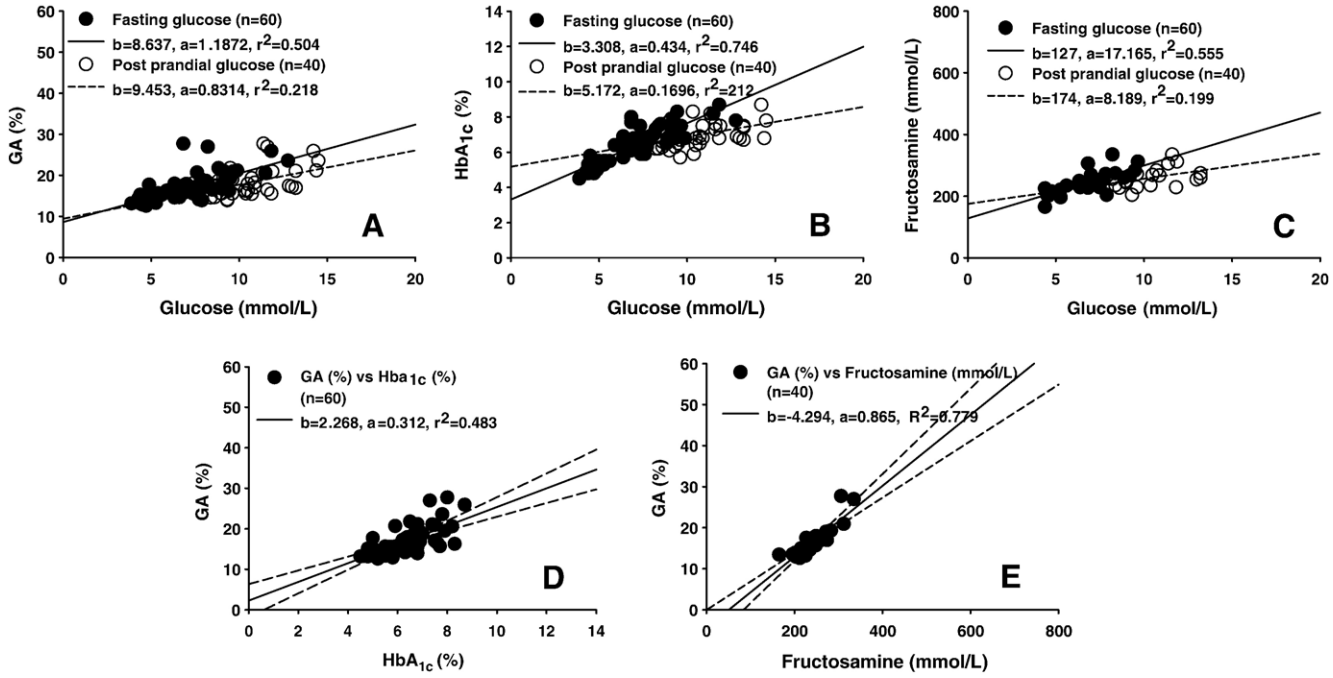

Fig. 2. Scatter plots of glycated albumin (A), HbA_{1c} (B) and fructosamine (C) vs. fasting plasma glucose in healthy blood donors and type 2 diabetic patients with good metabolic control. Relationships between glycated albumin and HbA_{1c} (D) and between glycated albumin and fructosamine (E) are reported in the lower part of the figure. Dashed lines in the regression plots represent 95% regression confidence intervals.

HbA_{1c} with fasting plasma glucose ($r^2=0.504$ and $r^2=0.746$, respectively) (Figs. 2A and B). It is also to be noted that apparently at very low glucose concentrations, a minimum of GA and HbA_{1c} could be still expected (as evident from the intercepts of the regression lines of Figs. 2A and B), but that no GA could be expected if HbA_{1c} would be absent and *vice versa*, as shown by Fig. 2D. The same kind of observations have been drawn when comparing fructosamine to plasma glucose (Figs. 2C and E).

The results obtained by analysing the same relationships in type 2 diabetic patients under poor metabolic control are reported in Fig. 3. In these patients indeed the relationships

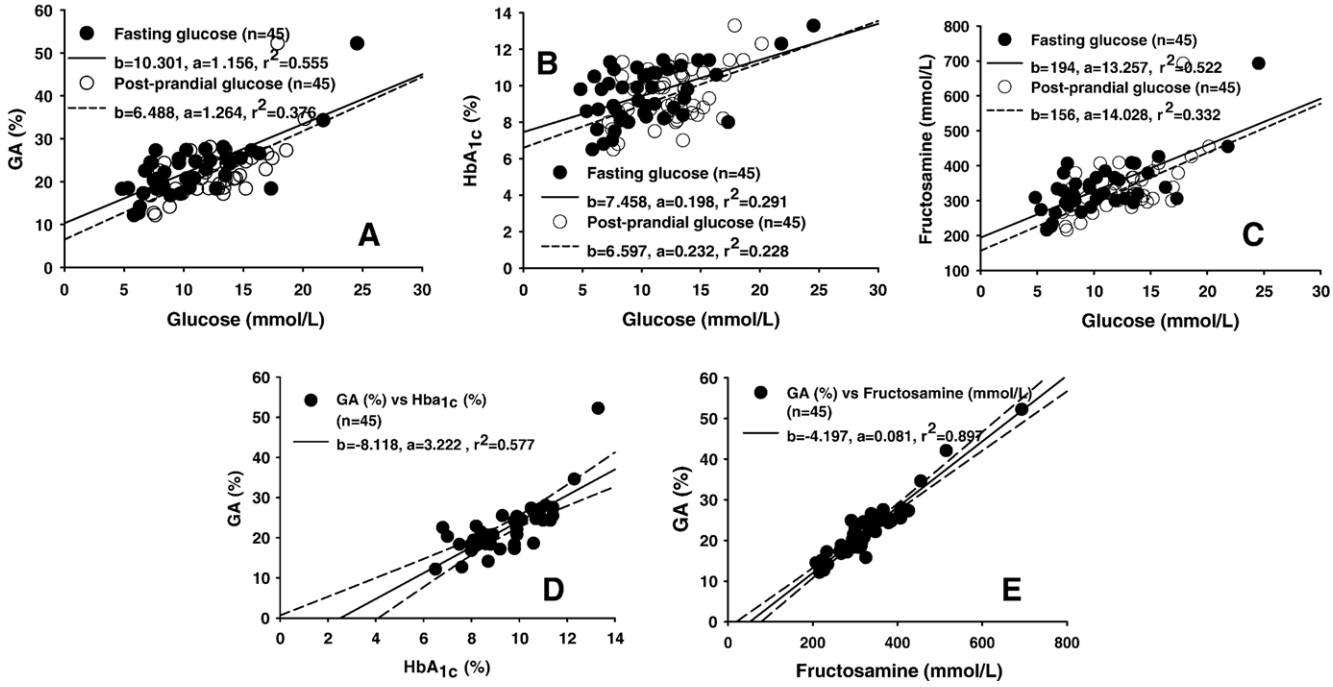

Fig. 3. Same parameters and analyses as in Fig. 2, related to patients with type 2 diabetes in poor metabolic control.

between GA and fructosamine with plasma glucose (fasting and post-prandial) or with each other (Figs. 3A, C and E) are essentially similar to those found in the subjects previously discussed as evidenced comparing the plots of Figs. 3A, C and E with those of Figs. 2A, C and E. However, the relationships between HbA_{1c} and fasting and post-prandial plasma glucose ($r^2=0.291$ and $r^2=0.228$, respectively) (Fig. 3B), and between HbA_{1c} and GA ($r^2=0.557$) (Fig. 3D), looked different, as clearly evident when comparing Figs. 3B and D with Figs. 2B and D, respectively. Indeed, the correlation between HbA_{1c} and fasting plasma glucose was much weaker in type 2 diabetic patients poorly controlled, with respect to healthy subjects and type 2 diabetic patients under stable glycometabolic control ($r^2=0.291$ vs. 0.746, respectively) (Figs. 3B and 2B).

The evaluation of glycometabolic control based on the measurement of glycated proteins in subjects having reduced red cell survival, such as in the case of patients subjected to regular blood transfusions, in patients with end-stage renal disease or in women with gestational diabetes, was also analyzed in our study. In patients with Cooley's disease, we found that GA is better related to fasting glucose with respect to HbA_{1c} ($r^2=0.693$ vs. 0.370) and that fructosamine and GA are strictly related to each other ($r^2=0.969$). A weaker relationship between GA and fructosamine was also found in patients with cirrhosis ($r^2=0.651$). On the contrary, in subjects under regular dialysis regimen for end-stage renal disease, the correlation between GA and fructosamine with fasting glucose was much weaker than in the other categories of subjects ($r^2=0.002$ and 0.010, with respect to values between 0.5 and 0.6), while the correlation between HbA_{1c} and fasting glucose was found to be stronger than in the case of type 2 diabetic patients under poor metabolic control ($r^2=0.420$ vs. 0.291). These were the results we obtained by pooling together the non-diabetic and the diabetic patients under constant regimen of hemodialysis. However, the analysis performed only in the diabetic patients under hemodialysis proved a stronger relationship between GA and fasting plasma glucose than between HbA_{1c} and fasting plasma glucose ($r^2=0.712$ vs. 0.125). In these patients, the relationship between GA and HbA_{1c} was weaker than in the diabetic non-hemodialyzed subjects ($r^2=0.480$ vs. 0.580). Finally, in the case of women with GDM, all the correlations between GA, HbA_{1c} and fructosamine with fasting or post-prandial glucose were found to be very weak (r^2 always <0.1).

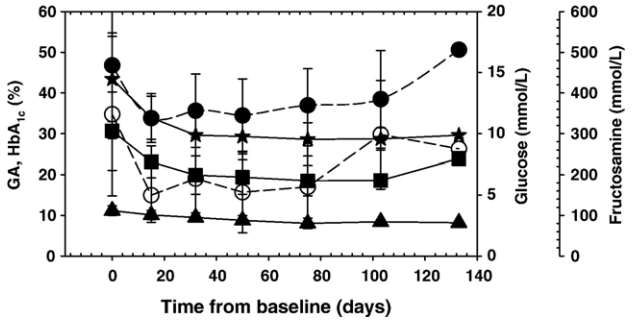

Fig. 4. Changes in fasting and post-prandial glucose (open and closed circles, respectively), glycated albumin, (-■-), HbA_{1c} (-▲-) and fructosamine (-★-) in 10 type 2 diabetic patients under poor metabolic control monitored at baseline (day 0) and every 2 weeks for about 2 months.

In order to compare the timeline of the changes in the investigated metabolic indices after the start of therapy, we compared the results obtained on 10 type 2 diabetic patients under poor metabolic control and plotted these data in Fig. 4. In this graph, the time-course of fasting and non-fasting glucose is plotted together with the time-course of the other metabolic indices monitored every 2 weeks in seven consecutive visits. Both GA and fructosamine were found to be strictly related to the fast metabolic changes that occurred in the first weeks of therapy.

Discussion

In this investigation, we have evaluated the performance characteristics of the improved version of Lucica® GA-L assay, an enzymatic method for the measurement of GA in serum, and we have tested its utility in a selected cohort of subjects by comparing its performance with two widely used indices, such as HbA_{1c} and fructosamine.

Our data indicate a more than acceptable analytical imprecision, with within- and between-run CVs almost in the same range and not exceeding the 4.5% limit. We were not able to understand why the imprecision for total albumin was better on pools than in controls, while that on glycated albumin (in terms of g/dL) was found to be more similar among the different samples. However, despite previously published data [16], we were able to obtain a better performance with this test. With an imprecision below 4.1% for GA%, our data are almost in the same range of the fructosamine test, which has been reported, with regard to inter-batch imprecision, to be in the 3.6–4.1% range [19]. Unfortunately, no analytical goals for imprecision of GA have been defined so far, so there is no target for comparison. However, with regard to total albumin, the actual analytical goal for imprecision (CV = 1.6% [20]) was found to be met in one of our specimen (i.e. on the Pool, Level 1 in the within-run experiment), and slightly higher CV_i values were found in the other specimen in the within- and between-run imprecision studies. These results can be compared to the analytical quality of the HbA_{1c} measurements in our laboratories, which is in the order of 2.0%, as CV, as previously reported [18].

With regard to the other analytical characteristics, the dilution study explored the physiological range up to 35% of GA, and gave satisfactory results by confirming the good linearity observed by Kouzuma T. et al. [15] in the 0–100% range. The limits chosen for testing method linearity (i.e. between approximately 10 to 35% GA) were decided by considering that it is quite unusual to find specimens with levels of glycated albumin >30%, according to the experience of Kozuma [15]. Indeed, in our experience we have found only 5 subjects over a total of 270 determinations with levels of glycated albumin slightly greater than 35%.

Our interference study evidenced a −2.8% bias at 264 μmol/L, a −7.5 % at 392 μmol/L and even higher negative bias for more elevated bilirubin concentrations. This result is

surprising if compared to that of Kouzuma et al. [16] who found that 279 μmol/L bilirubin did not affect the results of the assay. We also found that a 3.9 mmol/L triglyceride concentration (aquite common clinical situation) causes a +26% interference. These latest data are, however, difficult to compare with those of Kouzuma et al. [16] who expressed values as turbidity index of chyle. Concerning hemoglobin concentration, our observations are in agreement with those of Kouzuma et al. [16] and we found that hemoglobin affects less strongly the performance of the assay, and the bias exceeded the −10% limit only above 2.0 g/L. The concentration limits for testing lipids, bilirubin and hemoglobin interferences were chosen on the basis of previous experience in the evaluation of interference in clinical chemistry instrumentation [21].

Considering that GA reflects the glycometabolic control over 1–2 weeks, in agreement with its half-life of approximately 17 days [22], we decided to evaluate the time changes of GA in diabetic patients under poor metabolic control, that were started on insulin therapy in order to achieve in a relatively short time an acceptable metabolic control. The results we report clearly show that GA is more useful than HbA_{1c} in detecting early response to treatment. Therefore, measurement of GA can be utilized in monitoring patients whose glycemic control is being improved. Furthermore, in type 2 diabetic patients under poor metabolic control, the better correlation obtained between GA and fasting plasma glucose, with respect to that obtained between HbA_{1c} and fasting plasma glucose, could mean that the worsening of glycemic control can be better recognized and monitored with GA rather than with HbA_{1c}.

GA, expressed as GA% and measured by the Lucica-GA test, was also found to be a robust parameter in patients with liver cirrhosis. This could be useful in monitoring diabetic patients with cirrhosis, whose metabolic control is often unstable and difficult to improve. Indeed, it has been recently reported that, in 40% of patients with chronic hepatitis, compensated cirrhosis and patients with chronic hepatitis treated with ribavirin, HbA_{1c} falls below the non-diabetic reference range [23].

In patients under constant dialysis regimen, the data on all the patients we analyzed do not seem to provide evidence that GA could be better than HbA_{1c} in monitoring glycometabolic control. However, unfortunately, not in all the patients we have studied it was possible to obtain HbA_{1c} values in the same number as for GA and fructosamine. Its is likely that the poorer relationship we have found between GA and fasting glucose, with respect to that between HbA_{1c} and fasting plasma glucose, could be simply an effect of the discrepancy in numbers of available measurement. Indeed, by analyzing separately the subgroup of diabetic patients under regular hemodialytic treatment, we have been able to demonstrate that GA was better correlated to fasting plasma glucose, with respect to HbA_{1c}. This finding is in agreement with some recent data collected from studies on a larger number of patients, where it was also proven that GA is a better indicator for glycometabolic control than HbA_{1c} on diabetic patients under hemodialysis [24]. The possible explanation is that in these patients the treatment with erythropoietin causes a significant reduction of red-cell life, and therefore produces lower HbA_{1c} value because of the increased proportion of young erythrocytes over older erythrocytes in peripheral blood of those patients.

Our data on gestational diabetes do not seem to indicate that the measurement of GA provides information particularly different from that of HbA_{1c} and fructosamine. Indeed in our experience these pregnant patients are usually under strict glycemic control, and we found no significative difference in the glucose levels among patients treated with insulin with respect to others not treated. Probably GA could be a better marker for glycometabolic control with respect to HbA_{1c} in case of pre-gestational diabetes (i.e. in pregnancy of type 1 or type 2 diabetic women) because of larger excursion of glycemic levels in these subjects, with respect to the GDM pregnancies.

It is interesting to observe that GA, HbA_{1c} and fructosamine still seem to be affected by a certain level of non-specificity, since, at very low glucose concentrations, a minimum of GA, HbA_{1c} and fructosamine could be expected in all our subjects, as evident from the intercepts on the y-axes of Figs. 2A–C and Figs. 3A–C. When comparing glycated proteins (Figs. 2D–E and 3D–E), the relationships were found to be better (i.e. with no y-intercept), probably because the degree of non-specificity is similar. We have already pointed out that if HbA_{1c} is measured by the recently developed IFCC reference method, the relationship between mean daily blood fasting glucose and HbA_{1c} is more consistent, since no HbA_{1c} should be expected if glucose is absent [25].

Concerning the relationship between GA and fructosamine, our findings indicate that these parameters were more strongly correlated to each other in 3 of the subjects groups studied (controls and type 2 diabetic patients under good metabolic control, type 2 diabetic patients poorly controlled and patients with Cooley's anemia). This is not surprising since GA and fructosamine are known to change both in relatively short term when glycemic control is changing rapidly, due to a shorter half-life of albumin with respect to hemoglobin. On the contrary, we found a weaker correlation between GA and fructosamine in patients with liver disease. It has been proven that fructosamine exhibited higher values with respect to the actual glycometabolic control [26] because of retardation of protein metabolism in the presence of chronic liver disease. Since GA is affected by albumin metabolism, we could expect that GA could also be elevated in presence of cirrhosis because of the prolongation of albumin half-life due to reduced albumin synthesis. Indeed mean fructosamine in cirrhosis cases was higher with respect to controls, while mean GA was not different between cirrhosis patients and controls (Table 3). Despite the small number of subjects with liver cirrhosis investigated ($n=10$), our data seem to indicate that in these cases GA and fructosamine behave differently, GA being more consistent with the degree of glycemic control respect to fructosamine.

In conclusion, we have provided some preliminary experiences with this enzymatic test for GA, and we can support that GA% as measured by the Lucica GA-L enzymatic assay is a useful test in the assessment of glycemic control in different subjects affected by diabetes mellitus or increased insulin resistance. The GA enzymatic test proved to be reproducible

and robust and was particularly useful in various clinical situations, especially when HbA_{1c} is difficult to interpret.

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