Do Glycoalbumin Levels Preferentially Reflect Changes in Postprandial Glucose Excursions?

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Aims/objective: To evaluate whether plasma glycated albumin (GA), which provides an integrated measure of plasma glucose levels over the preceding 2-4 weeks, better reflects changes in postprandial glucose excursions than glycosylated haemoglobin (HbA_{1c}).

Methods: Patients with suboptimal glycaemic control on dual oral therapy were randomised in the Treating-to-Target-in-Type 2 diabetes trial (4-T) to the addition of once-daily basal insulin, twice-daily biphasic insulin or thrice-daily prandial insulin. GA levels were assayed enzymatically in baseline and one-year fasting plasma samples. Robust correlations of GA and HbA_{1c} with fasting and post-prandial glucose levels were evaluated at these two time points, and with the insulin-induced changes in the post-prandial excursion.

Results: Requisite data were available for 625 of the 4-T patients. They were mean (±SD) age 62±10 years, body weight 85.8±15.9 kg and median (IQR) diabetes duration 9 (6, 13) years. Partial correlations at baseline and one-year between postprandial glucose excursions and GA/HbA_{1c}, after adjusting for fasting glucose, were 0.27/0.15 and 0.22/0.18 respectively. GA, compared with HbA_{1c}, explained 66% more of the variation in postprandial glucose excursions at baseline. At one year postprandial glucose excursions on basal, biphasic and prandial and insulin therapy were reduced by 0.43, 0.78 and 1.88 mmol/l respectively. These reductions were associated with changes in both GA and HbA_{1c} ($p < 0.01$), with a relatively stronger association for GA.

Conclusions/interpretation Changes in GA and HbA_{1c} reflect changes in postprandial glucose excursions to a similar extent.

Postprandial hyperglycaemia is an independent cardiovascular disease (CVD) risk factor that may help explaining the residual CVD risk seen after adjustment for other classical risk factors (1, 2). As an emerging therapeutic target for reducing CVD risk that could play an important role in diabetes management (3-5) it was the focus of the nateglinide arm of the NAVIGATOR trial (6) and the ongoing ACE trial (7). Postprandial glucose levels, however, are difficult to measure in practice, with any differences in the post-meal measurement time introducing additional variation.

Glycated albumin (GA), which reflects prevailing blood glucose concentrations over the preceding 2-4 weeks, has been proposed as an alternative marker for glycaemic control in patients with diabetes that preferentially reflects postprandial glycaemia (8-13) . Unlike HbA1c it is not affected by changes in erythrocyte survival time (14), nor changes in albumin concentration (15), and is not subject to interference by endogenous glycated amino acids (15, 16) . GA may be useful as an alternative to HbA1c to provide information on glycaemic control on short-term (2-4 weeks), and for monitoring glycaemic control in patients with hemoglobinopathies and iron deficiency (renal diseases) where HbA1c measures are not considered as robust (17-19). GA has also been evaluated as a useful marker for assessing the risk of atherosclerosis and microvascular conditions (12, 20-22). Recently, the usefulness of GA as a predictor of diabetic complications compared with HbA1c was examined in two studies: the Atherosclerosis Risk in Communities (ARIC) Study and the Diabetes Control and Complications Trial (DCCT) (12, 20-22). The cross-sectional analysis of data from ARIC Study reported that, compared to HbA1c, GA could be more strongly associated with prevalent chronic kidney disease, albuminuria, and retinopathy (20). Using data from DCCT/EDIC study in patients with type 1 diabetes, Nathan and colleague have reported similar association of GA and HbA1c with retinopathy and nephropathy (12).

Only a few studies have reported high correlation between GA and HbA1c, and their individual association with preprandial and postprandial glucose concentrations (8-10, 12, 23, 24). A recent study based on three groups of patients with established diabetes, impaired glucose regulation and normal glucose tolerance, has reported significantly high correlation of GA with HbA1c (r=0.94), fasting plasma glucose (r=0.86) and 2-hr post load plasma glucose (r=0.81). In DCCT study, the associations of mean pre and postprandial glucose concentrations in multivariate additive model setup were found to be significantly correlated with HbA1c and GA (12). In patients with type 1 (n=93) and type 2 (n=75) diabetes, GA was reported to be more strongly correlated with maximum blood glucose levels compared to HbA1c (8). In 51 patients with type 2 diabetes, GA was reported to be more closely associated with postprandial glucose, compared with HbA1c (23). Most of these studies were based on small number of patients and the inferences on the correlation (degree of linear relationship) or association (regression) between GA or HbA1c with fasting and postprandial glucose levels were drawn without considering the inherent dynamic relationship between pre and postprandial glucose levels in patients with established diabetes.

In this study, we evaluated whether GA is more closely related to postprandial glycemia than HbA1c, and whether it better reflects the insulin-induced changes in postprandial glucose excursions seen in the Treating-to-Target-in-Type 2 Diabetes (4-T) trial (25, 26).

RESEARCH DESIGN AND METHODS

The design of the 4-T trial and the follow-up results at 1- and 3-years have been published (25, 27). Briefly, this was an open-label, randomised controlled, multicenter trial, which assigned 708 patients who were receiving maximally tolerated doses of metformin and sulfonylurea but had suboptimal HbA1c levels (7.0 to 10.0%) to the addition of twice-daily biphasic insulin aspart, thrice-daily prandial insulin aspart, or once-daily basal insulin detemir

(twice if required). The primary outcome at one year was the HbA1c level achieved, with a target of ≤ 6.5%.

Biochemical Analysis

HbA1c levels, 8-point self-monitored capillary glucose (SMCG) profiles and fasting plasma samples for biomarker measurements were collected at baseline and at one year. Samples were surface-mailed overnight at ambient temperatures to the central laboratory. Diabetes Control and Complications Trial aligned HbA1c (normal range 4.5-6.2%) was measured by high performance liquid chromatography (Biorad Variant II, Biorad, Hemel Hempstead, UK). GA levels were assayed enzymatically in duplicate at baseline and one year (Lucica GA-L kit, Asahi Kasei Pharma Corporation).

Statistical Methods

Demographic and clinical characteristics were presented as number (%), mean±SD or median (IQR), as appropriate. The reproducibility of the duplicate baseline and one-year GA measurements were confirmed using Bland-Altman plots and the coefficient of variation (CV).

The arithmetic mean of duplicate GA measurements was used for all analyses. To avoid the influence of outliers in the distributions of fasting / postprandial glucose measures, HbA1c and GA, and to account for possible non-linearity in the relationships, 'robust correlation coefficients' were calculated to explore the degree of (linear) relationships between them (28). Linear regression models were used to compare the changes in the levels of the parameters for glycaemic control between three insulin treatment regimens, and the robust standard errors were estimated with 'study centre' as clusters.

The exploration as to whether GA is more closely related to postprandial glycemia than HbA1c is not straightforward, given the complex dynamics inherent in the 8-point SMCG profile and the fact that GA and HbA1c both reflect the overall glycaemic level. We used a statistically intuitive approach on a regression platform to address this issue: The association of fasting/preprandial SMCG (F/PSMCG) values, defined as the mean of the three pre-prandial and pre-bed values, with concomitant GA and HbA1c values at baseline and one-year were assessed using linear regression models, with adjustment for multiple treatments. The residuals from these regressions provided the GA or HbA1c components which remained 'unexplained' by F/PSMCG. These unexplained residual components were then regressed on the mean post-prandial SMCG rise, defined as the mean of the three post-prandial values minus the F/PSMCG. Given the approximate normality of the post-prandial SMCG rise, simple regression models were fitted without adjustment for treatment effects and 'study centre' effects. The formulation of this approach follows:

$$
\begin{aligned}
&GA_{ij} = \mu + FSMCG_{ij}^{/}\beta_1 + u_i + \varepsilon_{ij}, \\
&i(patient) = 1,2,....n;\ j(centre) = 1,2,...r \\
&\mathrm{Re}\, sidualGA = u_i + \varepsilon_{ij} = UGA_{ij} \\
&PPR_{ij} = \alpha + UGA_{ij}^{/}\beta_2 + e_{ij}
\end{aligned}
$$

The goodness-of-fit measures included adjusted R^2, Root Mean Squares of Errors (RMSE), and Bayesian Information Criteria (BIC). R^2 estimates provide the proportion of variation in the post-prandial glucose excursion explained by 'residual' GA or 'residual' HbA1c. A smaller estimate of BIC would indicate stronger model fit in terms of information content, while compared between different regression models. Partial correlation coefficients were also used to explore the association of post-prandial SMCG rise with the GA and HbA1c, having adjusted for the fasting glucose levels.

Results

Of the 708 4-T patients, 625 had the requisite data available for these analyses. They were mean±SD 62±10 years old with 65% male, 92% Caucasian, and median (IQR) duration of diabetes of 9 (6, 13) years (Table 1). The distributions of anthropometric, clinical and biochemical study parameters, including GA and HbA1c, did not differ significantly at baseline between the three allocated insulin regimens. The GA assay was highly reproducible with duplicate measurement CVs of 2.9% and 2.6% at baseline and at one-year respectively. The numbers (%) of samples falling outside the 95% confidence lines of Bland-Altman plot were 18 (3.04%) and 22 (3.52%) respectively at baseline and one-year.

At one-year, 4-T achieved similar mean±SD HbA1c values on biphasic (7.3±1.0%) and prandial (7.2±0.9%) insulin, but values on basal insulin were higher (7.6±1.0%, $p<0.001$ for both comparisons). Similarly, achieved one-year GA values did not differ between the biphasic (15.8±3.2%) and prandial (15.4±3.9%) arms, and were higher in the basal arm (16.7±3.4%, Table 2).

Robust correlation coefficients at baseline and at one-year between HbA1c and GA were 0.66 and 0.64 respectively ($p<0.01$ in both cases). The relationships between GA and HbA1c changes from baseline to one-year in each treatment arm were linear independent of insulin treatment effect, with only few outliers. The scatter plots between GA and HbA1c with fitted Lowess regression lines are presented in Figure 1 (A-C).

The correlations between fasting SMCG and HbA1c/GA at baseline and one year were 0.55/0.53 and 0.29/0.28 respectively (Table 3). However, the degree of relationship of postprandial SMCG with GA were relatively higher compared to that with HbA1c: 0.62 vs 0.53 at baseline, 0.42 vs 0.35 at one-year. The mean±SD post-prandial glucose excursion at

baseline and one year were 2.67±2.08 mmol/L and 1.77±1.80 mmol/L respectively. The partial correlation coefficients between GA and the post-prandial rise in glucose controlling for the fasting mean glucose, were marginally higher compared to those with HbA1c (0.27 vs 0.15 at baseline; 0.22 vs 0.18 at one-year, Table 3). The patterns of association of fasting glucose, post-prandial glucose and the post-prandial glucose excursion, separately with HbA1c and GA at baseline are presented in Figure 2 (A-F).

The changes in the levels of average SMCG, HbA1c and GA were not independent of the treatment effects (Table 2). The fall in post-prandial glucose excursion at one year was highest among patients treated with prandial insulin (1.88 mmol/L). From baseline to one year postprandial glucose excursions fell by 29% with biphasic, 70% with prandial and 16% with basal insulin. Corresponding reductions in GA/HbA1c of 24/16%, 24/16% and 17/10% respectively were associated significantly (both $p<0.001$).

We further revealed the patterns of associations of post-prandial glucose rise with HbA1c and GA at one year, separately by insulin regimens, using polynomial smoothing regression technique (Figures 2 (C and F)). Clearly for individual insulin regimen, the patterns of association are very similar, reassuring the similarity of these two biomarkers in relation to their association with post-prandial glucose level in patients with type 2 diabetes.

The effect sizes of 'residual' GA and HbA_{1c} (after eliminating the possible contribution of fasting glucose, and adjusting for treatment effects) on the post-prandial glucose rise at baseline and one-year are presented in Table 4. These regression coefficients are statistically significant, suggesting that both HbA1c and GA can differentially explain post-prandial rise in glucose level. Compared to the regression estimates associated with the variant of HbA1c (residual HbA1c), the higher estimates of standardised regression coefficients associated with residual GA (0.26 and 0.15 at baseline line and year one respectively, Table 4) coupled with

marginally smaller root mean square errors and BIC estimates suggest that GA could explain the post-prandial rise in glucose level better only marginally compared to HbA1c. Also, compared to HbA1c, the correlation coefficients between post-prandial excursions and 'residual' GA were 44% higher at baseline (0.27 vs 0.15) and 18% at one year (0.22 vs 0.18).

Discussion

The 4-T study evaluated the efficacy of randomized addition of a basal, prandial or premixed insulin regimen to dual oral therapy in patients with type 2 diabetes. The differential imposed changes in postprandial glucose rises provided an ideal opportunity to examine possible differential relationships between HbA1c and GA measurements, compared with glucose measures. GA appears to reflect current glycemia as well or marginally better than HbA1c. Correlation coefficients between GA and post-prandial glucose were relatively higher both at baseline and one year (0.62 and 0.42), than those between HbA1c and post-prandial glucose (0.53 and 0.35). The partial correlation coefficient of post-prandial glucose excursion with GA was marginally higher compared to that with HbA1c, after adjusting for the effect of mean fasting glucose level.

Nathan and colleagues (2014) reported significant association of mean pre and post-prandial glucose concentrations with HbA1c and GA, with relatively stronger association for GA (in terms of model R^2) in patients with type 1 diabetes (12). Their analyses were based on various additive multivariate modelling, but did not report the standardised regression coefficients for comparisons with post-prandial glucose excursion, as the units of measurements for HbA1c and GA were different. We adopted a different approach – our regressor was a variant of GA or HbA1c measure, and post-prandial excursion was the dependent parameter. We have evaluated the association of post-prandial glucose excursion with GA or HbA1c, after eliminating the components of these glycaemic measures explained

by fasting or pre-prandial glucose values. Our study shows that GA is associated with but does not necessarily preferentially reflect postprandial glucose. This suggests that GA could provide similar results to postprandial glucose and act as an alternative early indicator of diabetes and the subsequent macro- and microvascular complications.

Being the product of chemical condensation of hemoglobin and glucose, HbA1c values are influenced by infection, hemoglobinopathies and renal failure (9, 29). Assessment of HbA1c in such patients might lead to underestimation as a result of either shortening the life span of erythrocytes or altering proportions of young to old erythrocytes by erythropoietin (30). There is some disagreement among researchers as to the degree that postprandial glucose as measured by HbA1c relates to overall glycaemic control and therefore having a reliable concomitant marker, such as GA, could provide more accurate results.

The assay methods used to analyze the samples have been previously tested, showing no interference by endogenous glycated amino acids or changes in albumin concentration (31). However, we also found that the GA assay gave reliable CVs. GA is an index of the prevailing blood glucose concentrations over the preceding 2-4 weeks and gives a more accurate short-term result than HbA1c. However, it does not capture long-term glycaemia measurements in the same way.

Although we found that GA and HbA1c, measures of glycation, do not preferentially reflect postprandial glucose rises, we have confirmed a reliable short-term measure of glycaemic exposure. Closely correlated in all analyses with HbA1c, GA showed a marginally stronger correlation with mean post-prandial glucose and post-prandial glucose excursion in a 24 hour profile. The ability of the assay to quantitate glycoalbumin, an index of the prevailing blood glucose concentrations over the preceding 2-4 weeks, makes it a favourable candidate for utilization in the clinical setting to monitor glycaemic control in diabetic subjects.

Simultaneous measurement of GA with HbA1c may be useful for the management of a postprandial hyperglycaemic state in diabetic patients and prevention of diabetic complications (32). Other markers that may be more accurate in measuring glucose control include 1.5-anhydroglucitol (33) and fructosamine (32), but these require further investigation using data from large clinical trials.

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RRH is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

RRH is a NIHR Senior Investigator.

Author Contribution

SKP conceived the statistical methodological ideas, conducted the statistical analyses and wrote the first draft of the manuscript. RRH designed the 4-T study, wrote the manuscript and researched the data. All authors contributed to the revision of the manuscript and approved the final version.

Disclosures

SKP has acted as a consultant and/or speaker for Novartis, GI Dynamics, Roche, AstraZeneca, Guangzhou Zhongyi Pharmaceutical and Amylin Pharmaceuticals LLC. He has received grants in support of investigator and investigator initiated clinical studies from Merck, Novo Nordisk, AstraZeneca, Hospira, Amylin Pharmaceuticals, Sanofi-Avensis and

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Figure Legends

Figure 1

Scatter plots with fitted Lowess regression lines showing relationship between (A) GA and HbA1c at baseline, (B) GA and HbA1c at one year, and (C) change in GA and HbA1c at one year from baseline. Scatters are separately coloured for the three insulin treatment regimens.

Figure 2

Scatter plots with fitted Lowess regression lines showing relationship between (A) mean fasting glucose and GA, (B) mean post-prandial glucose and GA, (C) post-prandial glucose excursion and GA, (D) mean fasting glucose and HbA1c, (E) mean post-prandial glucose and HbA1c, and post-prandial glucose excursion and HbA1c, at baseline. Scatters are separately coloured for the three insulin treatment regimens.

Table 1: Baseline demographic and clinical characteristics of participants.

Demographic	**(N=625)**
Male	403 (64.5%)
Age	61.7 (9.7)
Ethnicity	
White	577 (92.3%)
Mixed	7 (1.1%)
Asian	29 (4.6%)
Black	8 (1.3%)
Other	4 (0.6%)
Duration of diabetes	9 (6, 13)
Clinical & Biochemical	
BMI (kg/m^2)	29.8 (4.6)
Waist measurement (cm)	102 (12)
Systolic BP (mmHg)	138 (17)
Diastolic BP (mmHg)	79 (9)
Total cholesterol (mmol/L)	4.1 (0.9)
HDL cholesterol (mmol/L)	1.03 (0.25)
LDL cholesterol (mmol/L)	2.4 (0.7)
Triglycerides (mmol/L)	1.6 (1.2, 2.2)
Glycaemic Measures	
HbA_{1c} (%)	8.6 (0.8)
Glycated albumin (%)	20.11 (3.82)
Fasting SMCG (mmol/L)	9.8 (2.5)
Post-prandial SMCG (mmol/L)	12.5 (3.0)

Table 2: Changes in glycaemic measures and postprandial glucose excursion at one-year by insulin treatment regimens.

Biomarker	**Treatment**	**Marginal mean**	**95% C.I.**	**p *vs* biphasic**	**p *vs* prandial**
Glycated albumin (%)	Biphasic	-4.97	-5.59, -4.35		
	Prandial	-4.83	-5.43, -4.23	0.72	
	Basal	-3.25	-3.86, -2.64	< 0.0001	< 0.0001
HbA_{1c} (%)	Biphasic	-1.35	-1.50, -1.21		
	Prandial	-1.40	-1.59-1.26	0.63	
	Basal	-0.83	-0.69, -0.69	<0.001	<0.001
Fasting glucose (mmol/L)	Biphasic	-2.96	-3.34, -2.58		
	Prandial	-2.64	-3.02, -2.27	0.24	
	Basal	-2.06	-2.43, -1.70	0.001	0.031
Post-prandial glucose (mmol/L)	Biphasic	-3.78	-4.28, -3.29		
	Prandial	-4.59	-5.08, -4.09	0.024	
	Basal	-2.56	-3.04, -2.09	0.001	<0.001
Post-prandial glucose excursion (mmol/L)	Biphasic	-0.78	-1.17, -0.39		
	Prandial	-1.88	-2.27, -1.49	<0.001	<0.001
	Basal	-0.43	-0.81, -0.06	0.21	<0.001

Table 3: Robust correlation between Glycated albumin, HbA_{1c}, fasting glucose level and postprandial glucose level. Measures are presented for baseline and one year data.

	Baseline		**One Year**	
	HbA_{1c} (%)	Glycated albumin (%)	HbA_{1c} (%)	Glycated albumin (%)
Fasting Glucose (mmol/L)	0.55	0.53	0.29	0.28
Post-prandial glucose (mmol/L)	0.53	0.62	0.35	0.42
Post-prandial glucose excursion (mmol/L) [partial correlation, adjusting for fasting glucose]	0.15	0.27	0.18	0.22

Table 4: Regression results to compare the ability GA and HbA1c to explain post-prandial rise in capillary glucose levels (SMCG)

	Baseline		One Year	
	Standardised β	p	Standardised β	p
Residual GA	0.26	<0.01	0.15	0.008
Adjusted R^2	0.26		0.12	
Root MSE	3.34		2.62	
BIC	3018.32		2522.46	
Residual HbA1c	0.16	0.015	0.12	0.016
Adjusted R^2	0.10		0.10	
Root MSE	3.37		2.63	
BIC	3027.93		2524.31	

Figure 1

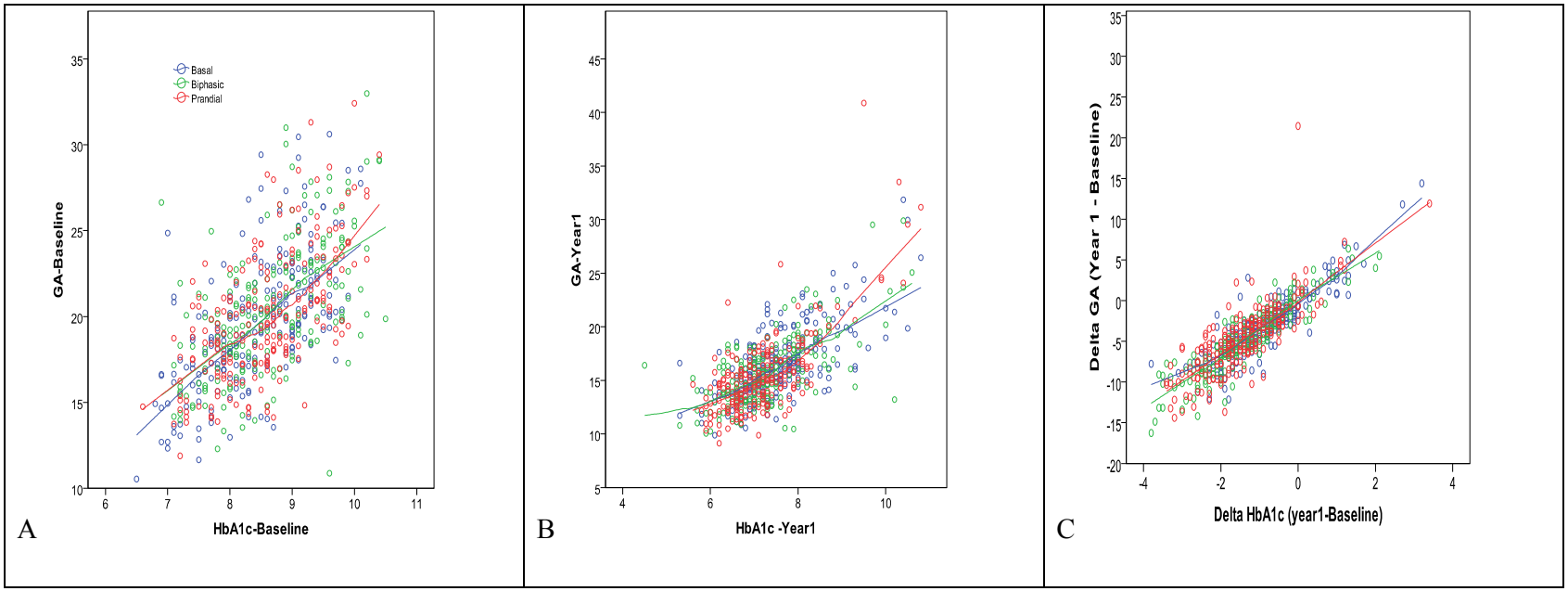
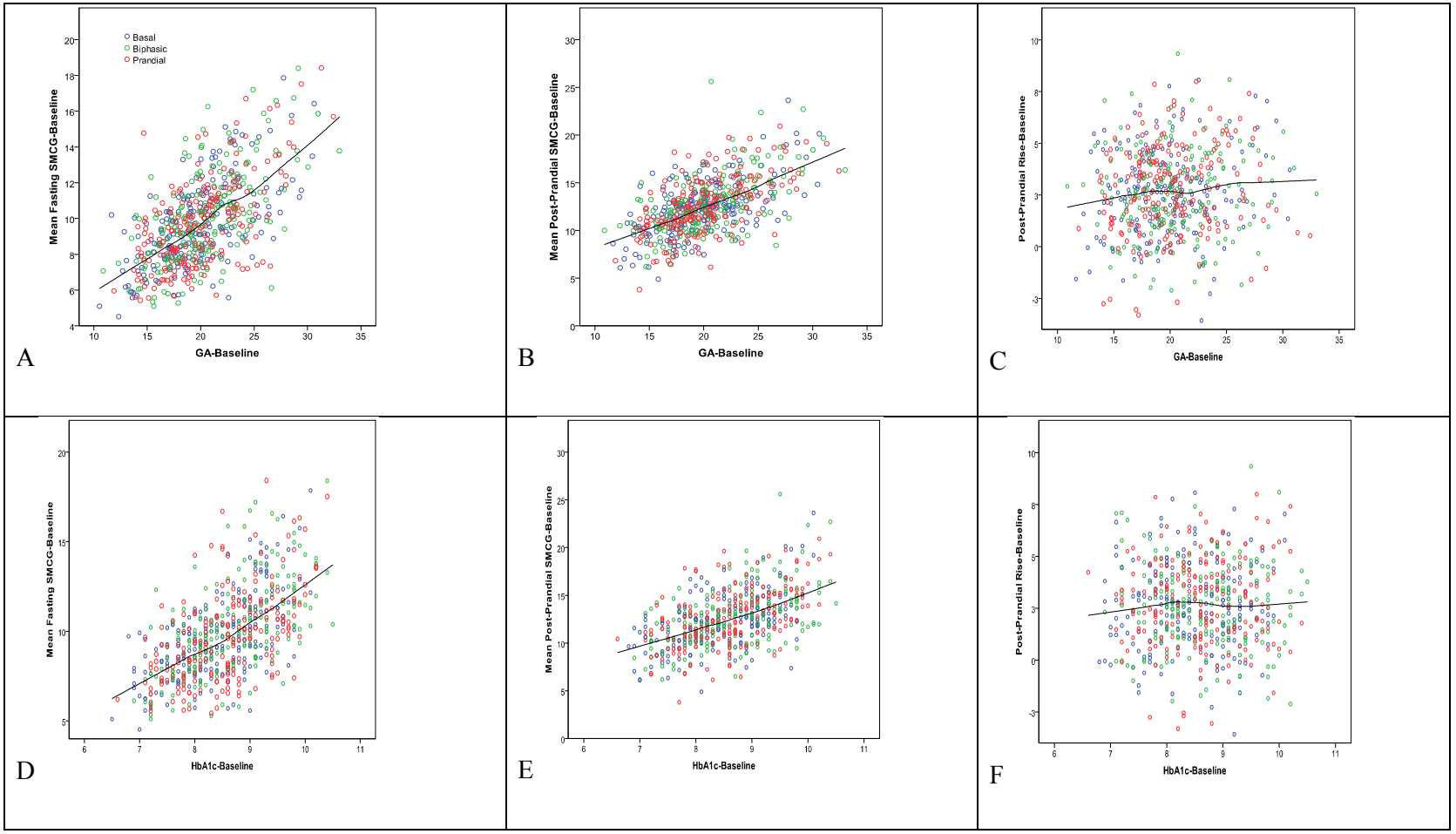

Basal
Biphasic
Prandial
GA-Baseline
HbA1c-Baseline
A
GA-Year1
HbA1c -Year1
B
Delta GA (Year 1 - Baseline)
Delta HbA1c (year1-Baseline)
C

Figure 2:


Basal
Biphasic
Prandial
Mean Fasting SMCG-Baseline
GA-Baseline
A
Mean Post-Prandial SMCG-Baseline
GA-Baseline
B
Post-Prandial Rise-Baseline
GA-Baseline
C
Mean Fasting SMCG-Baseline
HbA1c-Baseline
D
Mean Post-Prandial SMCG-Baseline
HbA1c-Baseline
E
Post-Prandial Rise-Baseline
HbA1c-Baseline
F