

ENDOCRINE PRACTICE Rapid Electronic Article in Press

Rapid Electronic Articles in Press are preprinted manuscripts that have been reviewed and accepted for publication, but have yet to be edited, typeset and finalized. This version of the manuscript will be replaced with the final, published version after it has been published in the print edition of the journal. The final, published version may differ from this proof.

DOI:10.4158/EP161363.OR

© 2016 AACE.

Original Article

EP161363.OR

**HBA1C AND MEAN GLUCOSE DERIVED FROM SHORT TERM CONTINUOUS GLUCOSE
MONITORING (CGM) ASSESSMENT**

DO NOT CORRELATE IN PATIENTS WITH HBA1C >8%

*Eijiro Yamada, MD, PhD¹, Shuichi Okada, MD, PhD¹, Yasuyo Nakajima, MD, PhD¹,
Claire C Bastie, PhD^{2,3}, Manu Vatish, MBBSchir, MD, D.Phil.⁴, Yuko Tagaya, MD, PhD¹,
Aya Osaki, MD, PhD¹, Yoko Shimoda, MD, PhD¹, Ryo Shibusawa, MD¹, Tsugumichi
Saito, MD, PhD¹, Takashi Okamura, MD, PhD, Atsushi Ozawa, MD, PhD¹, and
Masanobu Yamada, MD, PhD¹*

Running title: Reassessment of HbA1c and CGM

From: ¹ Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan; ²Division of Biomedical Sciences, Warwick Medical School, Coventry, United Kingdom; ³Department of Medicine & Endocrinology, Albert Einstein College of Medicine, Bronx, New York, USA; ⁴Nuffield Department of Obstetrics & Gynaecology, University of Oxford, Oxford, UK

Correspondence address: Dr. Eijiro Yamada

Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan

Email: eijiro.yamada@gunma-u.ac.jp

DOI:10.4158/EP161363.OR

© 2016 AACE.

Disclosure Statement: The authors have nothing to disclose.

Abstract

Aims: Optimum therapy for patients with diabetes depends on both acute and long-term changes in plasma glucose, generally assessed by HbA1c levels. However, the correlation between HbA1c and circulating glucose has not been fully determined. Therefore, we carefully examined this correlation when glucose levels were assessed by continuous glucose monitoring (CGM).

Methods: 51 patients (70 % women, 30% male) were examined; among them were 28 type 1 patients with diabetes and 23 type 2 patients with diabetes. Clinically determined HbA1c levels were compared with blood glucose determined by CGM during a short time period.

Results: Changes of HbA1c levels up to 8.0% showed a clear and statistically strong correlation ($R=0.6713$, $P<0.0001$) with mean blood glucose levels measured by CGM, similarly to that observed in the A1c-derived Average Glucose (ADAG) study where patients were monitored for a longer period. However, we found no statistical correlation ($R=0.0498$, $P=0.8298$) between HbA1c and CGM-assessed glucose levels in our patient population when HbA1c was greater than 8.0 %

Conclusions: Short term CGM appears to be a good clinical indicator of long-term glucose control (HbA1c levels), however cautions should be taken while interpreting CGM data from patients with HbA1c levels above 8.0%. Over or under estimation of the actual mean glucose from CGM data could potentially increase the risks of inappropriate treatment. As such, our results indicate that a more accurate analysis of CGM data might be useful to adequately tailor clinical treatments.

Abbreviations:

CGM = continuous glucose monitoring; **ADAG study** = A1c-derived Average Glucose study; **SD** = standard deviation; **MAGE** = mean amplitude of glycemic excursion; **CV** = percentage coefficient of variation; **M100** = M-value from 100 mg/dL.

Keywords: HbA1c, average glucose, continuous glucose monitoring (CGM)

1. Introduction

Diabetes mellitus is a worldwide chronic disease due to a reduction of insulin secretion and/or impaired insulin action. Although short-term elevation of circulating glucose levels is typically asymptomatic, the long-term effects have serious complications including

both micro- and macro-vascular diseases (1). In Japan, a total of 7.2 million people (7.6 % of the population) were reported to have diabetes in 2013 (2), which mirrors the worldwide prevalence of diabetes estimated to be 8.3% among adults (20-79 years old) (2).

The diagnosis and clinical treatment of patients with diabetes is based on circulating fasting glucose and glycated hemoglobin (HbA1c) levels. HbA1c is a gold standard in routine practice, as HbA1c levels are considered more reliable than fasting glycaemia to determine the diabetic status of an individual. That is because the non-enzymatic glucose conjugation to hemoglobin represents the average glucose concentration over a 2-3 month period, the relative turnover time for red blood cells (3). Additionally, multiple clinical trials have demonstrated a direct relationship between HbA1c levels and diabetic complications, including microvascular complications (4-6) and as such, HbA1c levels are typically used in clinical trials to determine therapy effectiveness. The relationship between average glucose levels and HbA1c was first established in 1984 (7), however the average blood glucose levels were determined by daily 7-point measurements, which are insufficient to define the glucose profile of an individual.

Recently, continuous glucose monitoring (CGM) has become available and using this strategy a continuous glycemic profile over a few to several days can be assessed. It is

believed that this technique is more reliable to determine the association between average glucose levels and HbA1c in an individual. Supporting this, the A1c-Derived Average Glucose (ADAG) study published in 2008 took advantage of both CGM and frequent capillary glucose testing and provided the tightest correlation between average glucose levels and HbA1c ($\text{Average Glucose (mg/dl)} = 28.7 \times \text{HbA1C (\%)} - 46.7$) (8). Importantly, this linear regression equation did not significantly differ across subgroups based on age, sex, and diabetes type. However, this large study did not exclude patients with higher glycemic levels that are not accurately determined by CGM. Indeed, there was not significant correlation between average glucose and HbA1c in patients with the highest HbA1c levels and this had been already reported in several other studies (9-12).

In order to investigate this paradigm in more details, we studied the correlation between CGM and HbA1c levels in patients with HbA1c > 8% compared to patients with lower HbA1c levels. Participants were of Japanese background and regrouped patients with type 1 or type 2 diabetes in similar proportion. Although patients were monitored for a 7-day period, a shorter time than the ADAG study, we found a similar correlation between glucose levels monitored by CGM and HbA1c for individuals with HbA1c levels lower than 8%.

However, contrasting results were obtained in patients with HbA1c levels greater than 8%,

where no significant correlation between glucose levels and HbA1c was found. Our results might support the need for clinicians to more carefully interpret CGM data to offer more adequate treatments to their patients.

2. Materials and Methods

2.1. Ethics Statement

This study was approved by the Gunma University Institutional Review Board.

2.2. Subjects

All patients have undergone CGM in the Department of Internal Medicine, Division of Endocrinology and Diabetes, Gunma University Hospital during the period 2013-2015.

Individuals treated with steroids and anemic patients (hematocrit < 39 % in men and < 36 % in women) were excluded. Importantly patients with glucose levels above 400 mg/dl during CGM tests were also excluded, as this technology is only accurate up to 400 mg/dl of subcutaneous glucose concentration (13, 14). Additional conditions with possible effects of glucose levels (such as pregnancy) or interfering with HbA1c measurement (such as hemoglobinopathies) were also excluded.

2.3. Glycemia assessment

CGM was performed with iPro2 Professional CGM (Medtronic, Northridge, CA, USA) that measures glucose levels every 5 min for at least 2 days and up to 7 days. For calibration purposes and as an independent measure of glycemia, subjects were asked to self-monitor their glucose levels at least 4 times a day (before their meal and before their bed time) using OneTouch Ultra (Lifescan, Milipitas, CA, USA) for at least 2 days while on-going CGM. Patients were previously advised to continue with their normal routine and asked not to attempt unusual behavior, especially in terms of dietary intake and exercise. The results from CGM and fingerstick monitoring were downloaded to Carelink iPro, a web-based software (Medtronic, Northridge, CA, USA). To be acceptable for analysis, the CGM data had to include at least one successful 24-h profile out of the 2–7 days of monitoring with no gaps over any 120 min continuous interval. The CGM data sets were further analyzed using the Web-based application GlyCulator to check the confidence level and data whose confidence levels were less than 0.9 were excluded (15). Also mean glucose, median glucose, standard deviation (SD), mean amplitude of glycemic excursion (MAGE), percentage coefficient of variation (%CV), the M100 value that measures the stability of the glucose excursions in comparison with an “ideal” glucose value of 100 mg/dl were investigated (15). Blood samples

were collected in EDTA anticoagulant tubes and HbA1c measurement was performed using a HPLC ion exchange chromatography on an ADAMS A1c, HA-8180 (ARKLEY inc. Kyoto, Japan).

2.4. Statistics

Data are presented as mean \pm standard deviation of the mean (SD) and as n (%) for frequency data. All results are expressed as the mean \pm SD for continuous variables and as absolute numbers and relative percentages for categorical variables. Associations between continuous variables were examined using Spearman's coefficients. All tests for significance and resulting P values were two-sided, with a level of significance of 5%. Statistical analyses were performed using JMP 9.0.2 (SAS Institute, Cary, NC, USA).

3. Results

We screened a total of 145 patients for CGM analyses. 94 were excluded for the criteria indicated in materials and method. The excluded patients included 13 patients on steroid treatments, 15 pregnant women, 31 patients presenting anemia, 14 diagnosed with chronic kidney disease and an additional 21 patients were removed for insufficient data

consistency. Parameters of the 51 included patients are displayed in table 1. Table 1 also shows hypoglycemic episodes affecting HbA1c levels. Those episodes were observed in patients with type 1 diabetes as well as others.

We first considered patients with glucose levels $<400\text{mg/dl}$ and excluded patients with higher glucose levels. As such, patients with HbA1c $>10\%$ were not included in this analysis. Under these conditions, we found that HbA1c showed a clear and statistically strong relationship ($R=0.6713$, $P<0.0001$) with mean blood glucose levels measured by CGM (Fig.1A), however the slope was significantly different from the ADAG study (8). Our criteria of exclusion were not taken into consideration in previous studies and it is possible that this could account for these differences. To address this issue we then included all patient data. Although we found a correlation with mean blood glucose levels closer to this of the ADAG study ($R=0.7054$, $P<0.0001$), it remained significantly different (Fig. 1B).

In addition to overall glycemic control, glucose variability has been shown to be closely associated with cardiovascular diseases (16-18). We next evaluated the relationships between HbA1c and the glycemic variability indexes: standard deviation of glucose measurements (SD), mean amplitude of glycemic excursion (MAGE), coefficient of variation (%CV) and M-value from 100 mg/dl (M100). To note, most of insulin treated subjects used

long-acting insulin (100% and 89% of Type 1 diabetes and Type 2 patients, respectively, did not use pumps). We did not observe any significant differences in these parameters between the different groups (not shown). As shown in Figure 2, the relationships between HbA1c and all four indices of glycemic variability were still statistically significant but were not as robust as that between HbA1c and mean blood glucose (Fig. 1). In particular, the correlation between %CV and HbA1c was low ($R=0.2537$, $P=0.0315$).

Since the correlation between HbA1c and mean blood glucose was distinct from the ADAG study, we examined the association of mean glucose levels over various ranges of HbA1c. As shown in Fig. 3, the relationship between HbA1c and mean glucose was statistically significant when HbA1c ranged less than 8.0 % (HbA1c: 5.0-7.0%, $R=0.7141$, $P=0.0002$. HbA1c: 7.0-8.0%, $R=0.7797$, $P<0.0001$). However, when HbA1c ranged between 8.0 and 9.0 % ($n=18$), there was no significant correlation ($R=-0.0498$, $P=0.8298$). Since the correlation was strong when HbA1c ranged less than 8.0 %, we re-examined the correlation between HbA1c (lower than 8.0 %) and mean blood glucose. As shown in Fig. 4, this relationship provided the strongest correlation ($R=0.7797$, $P<0.001$) and was nearly identical to that reported in the ADAG study.

4. Discussion

In this study we carefully analyzed the relationship between HbA1c and mean blood glucose assessed by CGM. In order to improve the accuracy of these data, we established a defined set of exclusion criteria that could be easily evaluated in our hospital setting. Importantly, we excluded patients with glucose levels greater than 400 mg/dl as CGM is not accurate at these elevated glucose concentrations. This is in contrast with the ADAG study, in which there was no description this criterion of exclusion. Likely, the ADAG included patients with glucose levels > 400mg/dl, since patients with HbA1c greater than 10.0 % were included in the analyses (21 out of 507 patients (4%) had HbA1c > 9.0% and 77 out of 507 patients (15%) had HbA1c >8.0% while their mean HbA1c was $6.8\% \pm 1.3$ and mean glucose was $149.4 \text{ mg/dl} \pm 39.6$).

Our data demonstrates a strong statistically significant correlation between HbA1c and mean blood glucose, however it was different from the ADAG study. Nonetheless when patients with pronounced hyperglycemia (> 400 mg/dl) were included in the analysis, this resulted in increased convergence of the two studies. Recent studies have shown that not only HbA1c but also glycemic variability is a likely causative inducer of diabetic complications. However, in our analysis, we found that the correlation between HbA1c and glycemic

variability was less than that observed between HbA1c and mean glucose, indicating that HbA1c might reflect mean blood glucose more accurately than glycemic variability.

Clinical trials have shown that the rate of complications caused by diabetes continue to increase when HbA1c levels are greater than 8.0 % (4-6). Therefore, it is formerly possible that specific Hb glycation rather than glycemia itself might promote diabetic complications.

Several limitations need to be considered when interpreting the present findings.

It is worth noting that this study had a cross-sectional retrospective design and was conducted by recruiting patients within a single center. Therefore, variability in patient characteristics and/or treatment might vary according to the center. As such, a multicenter study might be necessary to palliate these issues and harmonize the data in the future. More importantly, we have to acknowledge the remaining discrepancy between the ADAG study and our analyses could have resulted from a racial effect, since our study population was exclusively of Japanese origin, a population that was along with other Asian populations, minimally represented in the ADAG study (8). The differences may have resulted from the smaller sample size in our study versus that of the ADAG study. In this regard, the sample size estimated for the study to be significant was calculated with a two-sided type 1 error of

less than 5% and a power of 80%. The sample size for the patients with HbA1c>8% only had a power of 70%, yet our statistical analyses revealed that this was sufficient to reach significance.

Lastly, the protocol for data inclusion used in our investigation was similar to this of the ADAG study, and despite the use of the iPro Professional CGM, Medtronic (11% MARD for adults (19)), only 2.8% of patients were excluded for inadequate monitoring (data not shown). However, in our study CGM data were collected for a few days only, rather than 3 times over the course of 12 weeks (ADAG ref); therefore assessing how this could reflect long-term complications might only extrapolation. Nonetheless, by comparing the changes in mean glucose levels within ranges of HbA1c levels (5-8%, 8-9% and 9-10%) we found a concordance of our data in the HbA1c range of 5-8% with the ADAG study where CGM was performed for at least 2 days at baseline and then every 4 weeks during the 12 following weeks.

In summary, we reassessed the relationship between HbA1c and mean blood glucose determined by short term CGM. We found a strong positive relationship between glucose measured by short term CGM and HbA1c levels ($R=0.7797$, $P<0.0001$) consistently

with the previously reported ADAG study where HbA1c levels were lower than 8%. However, for HbA1c levels greater than 8.0% there was no statistically significant correlation.

As such, our data strongly support that cautions in the use of short-term CGM to assess long term changes in HbA1c levels as an indicator of future diabetes complications should be taken when the HbA1c levels are above 8.0%. This is of particular relevance since CGM data and HbA1c are now used as critical indicators in clinical practice. Although CGM is becoming more and more popular in Japan, it is still not routinely used. Therefore, it is very important that in patients with HbA1c over 8% the interpretation of the short-term CGM data is done with caution. Overestimation or underestimation of the mean glucose from CGM could potentially lead to misguided changes in treatment. If not, and over estimation of HbA1c occurs, patients might be placed on insulin and oral therapy, which could potentially end up in life-threatening hypoglycemia. Additionally, unnecessary treatments in addition of being dangerous for the patient, would add costs to the health care system. Similarly, insufficient treatment in patients whom HbA1c levels have been underestimated, would also lead to the deterioration of the patients' health with possible chronic or acute complications, also costly for the society.

In summary, over- or down- estimations of CGM data might increase the risks of inappropriate clinical treatment of patients with diabetes and our results–could indicate the need for caution in interpreting CGM data to offer adequate treatments to the patients. Future studies are now needed in larger cohorts and within other Asian racial groups to determine if this conclusion remains valid and determine clinical therapeutic strategies.

FIGURE LEGENDS

Figure 1. Mean or median glucose determined by CGM in Japanese patients is highly correlated with HbA1c but differs from the ADAG study. (A) Correlation between mean blood glucose levels and HbA1c (patients with glucose levels <400mg/dl). (B) Correlation between mean blood glucose levels and HbA1c (All patients).

Figure 2. HbA1c is better correlated with mean glucose than with glucose variability. Correlation between HbA1c and SD (A), MAGE (B), %CV (C) or M value (from 100mg/dl) (D)

Figure 3. Correlations between HbA1c and mean glucose (A)(B)(C)(D) on various HbA1c levels. Correlations between HbA1c and mean glucose at HbA1c <7.0 (A), between 7 and 8 (B), between 8 and 9 (C) and between 9 and 10 (D).

Figure 4. HbA1c levels correlate with mean glucose when HbA1c are lower than 8%.

References

References:

1. Standards of Medical Care in Diabetes-2016: Summary of Revisions. *Diabetes care*. 2016;39 Suppl 1:S4-5.
2. **Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE.** Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice*. 2014;103:137-49.
3. **Koenig RJ, Peterson CM, Kilo C, Cerami A, Williamson JR.** Hemoglobin A1c as an indicator of the degree of glucose intolerance in diabetes. *Diabetes*. 1976;25:230-2.

4. **Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al.**

Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ*. 2000;321::405-12.

5. **Ohkubo Y, Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, et al.**

Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes research and clinical practice*. 1995;28:103-17.

6. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *The New England journal of medicine*. 1993;329:977-86.

7. **Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A.**

Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *The New England journal of medicine*. 1976;295:417-20.

8. **Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ.**

Translating the A1C assay into estimated average glucose values. *Diabetes care*.

2008;31:1473-8.

9. **Cohen RM.** A1C: does one size fit all? *Diabetes care*. 2007;30:2756-8.

10. **Munshi MN, Segal AR, Slyne C, Samur AA, Brooks KM, Horton ES.**

Shortfalls of the use of HbA1C-derived eAG in older adults with diabetes. *Diabetes research and clinical practice*. 2015;110:60-5.

11. **Zhou J, Mo Y, Li H, Ran X, Yang W, Li Q, et al.** Relationship between

HbA1c and continuous glucose monitoring in Chinese population: a multicenter study.

PloS one. 2013;8:e83827.

12. **Ferenci T, Korner A, Kovacs L.** The interrelationship of HbA1c and

real-time continuous glucose monitoring in children with type 1 diabetes. *Diabetes*

research and clinical practice. 2015;108:38-44.

13. **Welsh JB, Kaufman FR, Lee SW.** Accuracy of the Sof-sensor glucose sensor

with the iPro calibration algorithm. *Journal of diabetes science and technology*.

2012;6:475-6.

14. **Calhoun P, Lum J, Beck RW, Kollman C.** Performance comparison of the medtronic sof-sensor and enlite glucose sensors in inpatient studies of individuals with type 1 diabetes. *Diabetes technology & therapeutics*. 2013;15:758-61.
15. **Czerwoniuk D, Fendler W, Walenciak L, Mlynarski W.** GlyCulator: a glycemic variability calculation tool for continuous glucose monitoring data. *Journal of diabetes science and technology*. 2011;5:447-51.
16. **Frontoni S, Di Bartolo P, Avogaro A, Bosi E, Paolisso G, Ceriello A.** Glucose variability: An emerging target for the treatment of diabetes mellitus. *Diabetes research and clinical practice*. 2013;102:86-95.
17. **DeVries JH.** Glucose variability: where it is important and how to measure it. *Diabetes*. 2013;62:1405-8.
18. **Service FJ.** Glucose variability. *Diabetes*. 2013;62:1398-404.
19. **Rodbard D.** Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities. *Diabetes technology & therapeutics*. 2016;18 Suppl 2:S23-213.

Baseline characteristics of analyzed subject			
	Type1	others	all
n	28	23	51
Age	39±12.0	53±17.2	44±15.2
Sex (% female)	20 (71)	17 (74)	43 (70)
A1C (%)	7.7±1.1	6.8±1.3	7.6±1.4
Treatment			
insulin pump	46%		
Three or more daily injections	54%		
Diet only		4%	
Oral agents only		18%	
Insulin only		39%	
Insulin + oral agents		39%	
hypoglycemia < 70.2mg/dl	86%	61%	75%
hypoglycemia < 54mg/dl	68%	17%	45%

Table.1

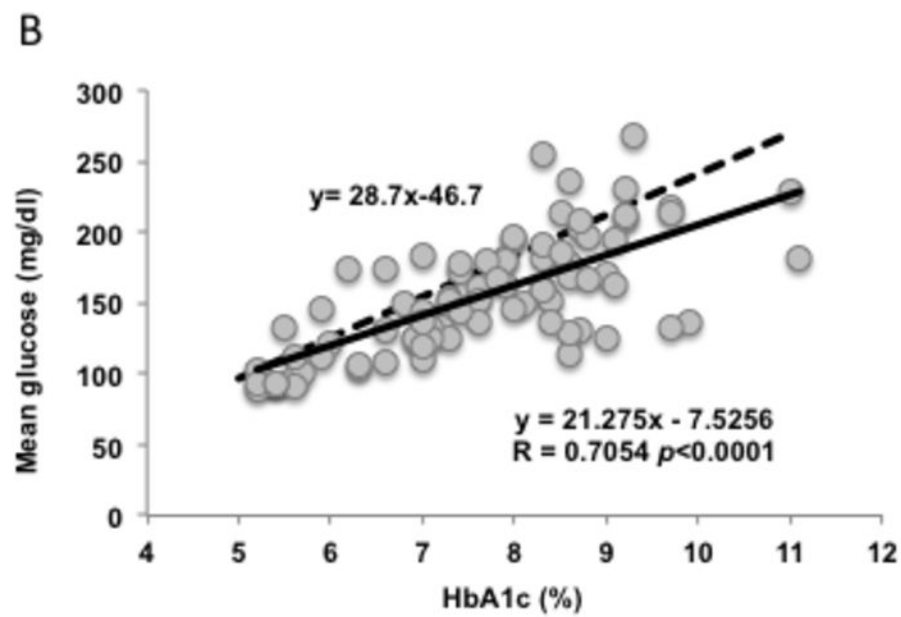
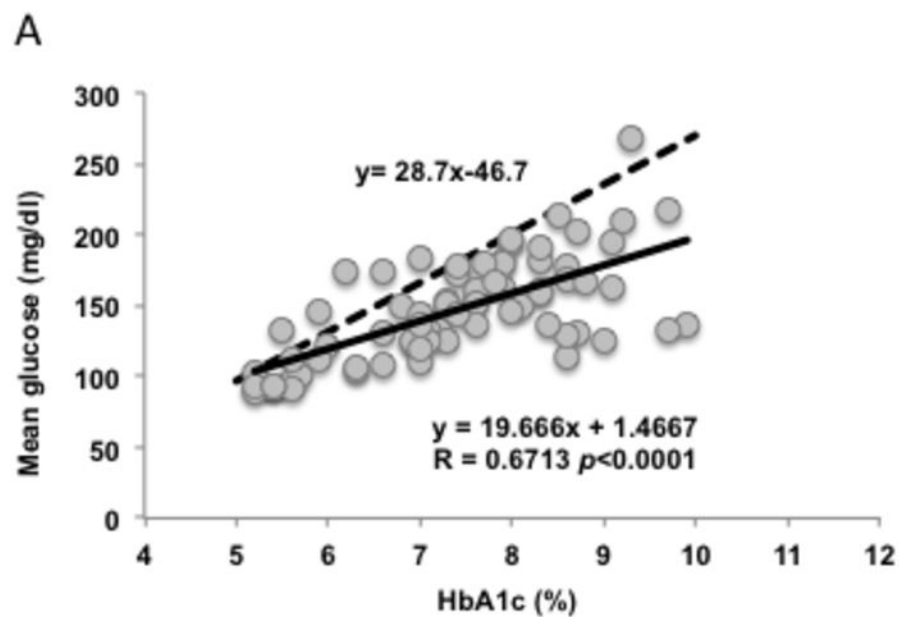


Fig.1

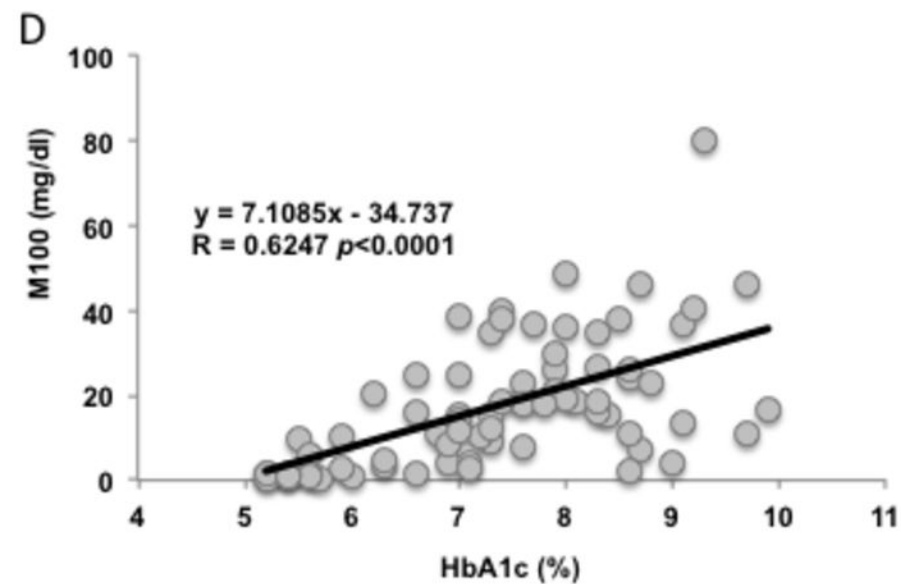
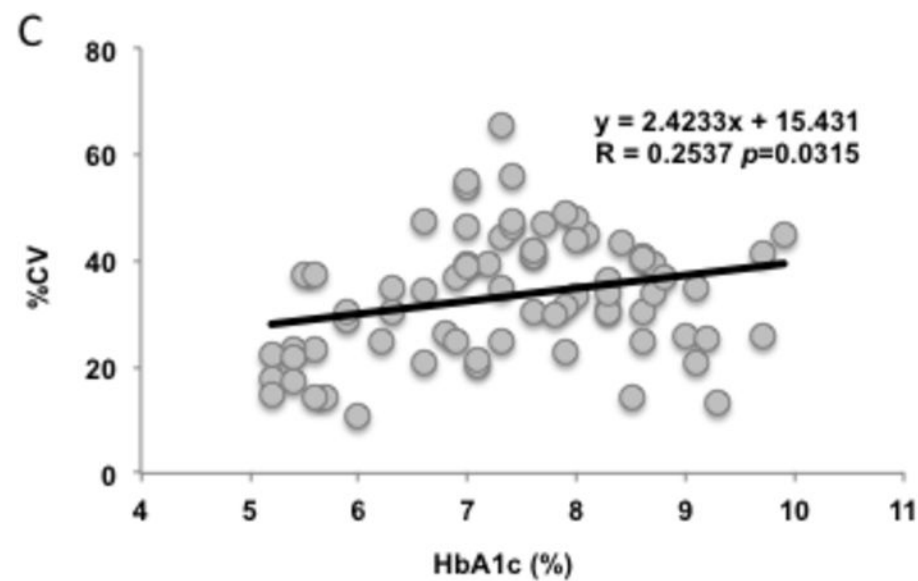
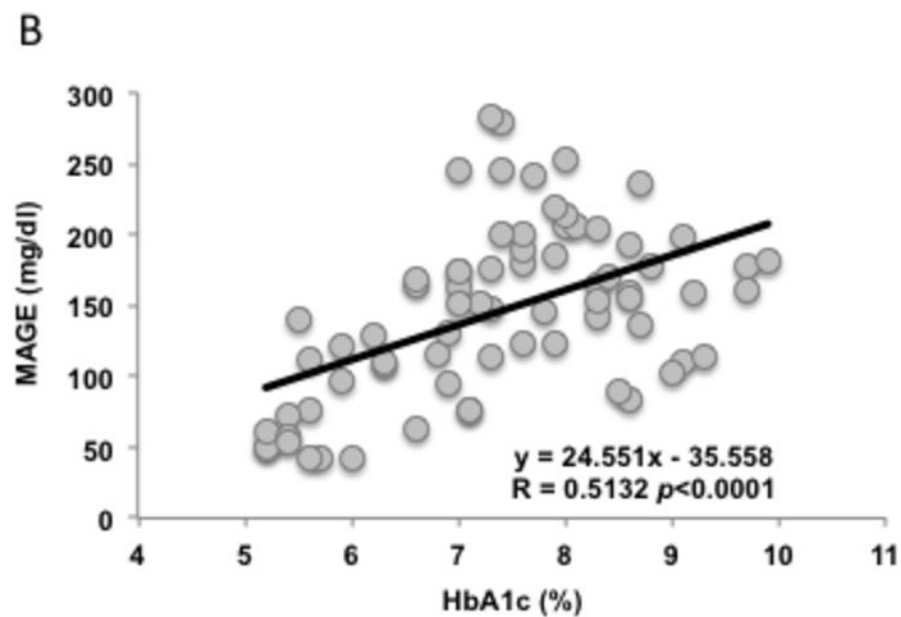
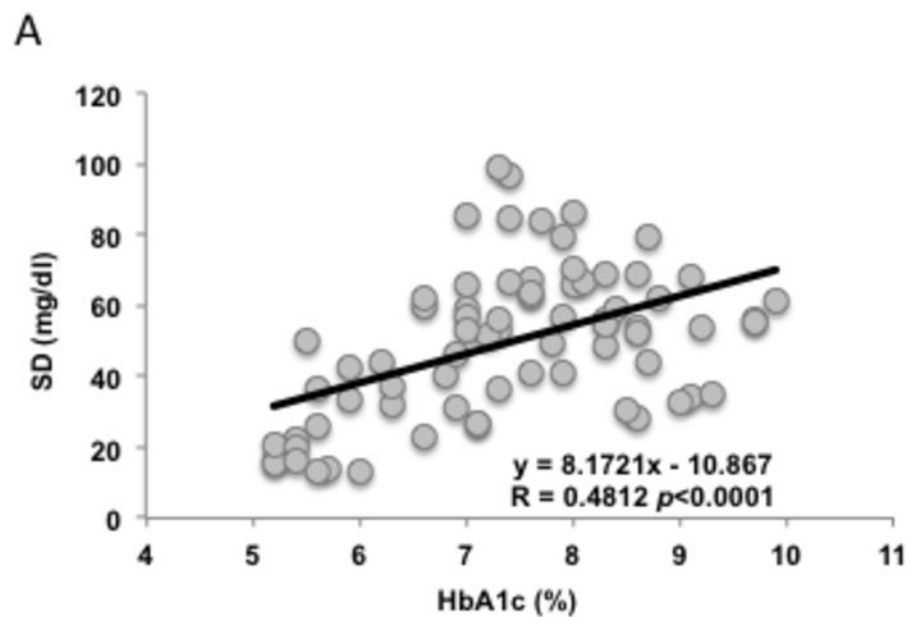
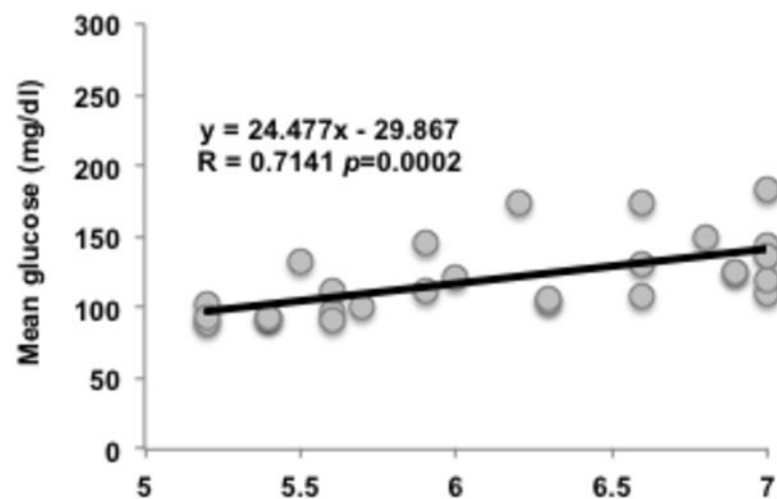
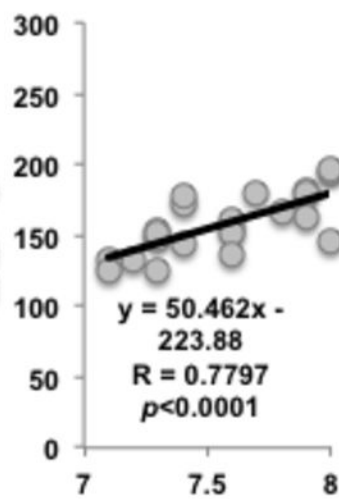


Fig.2

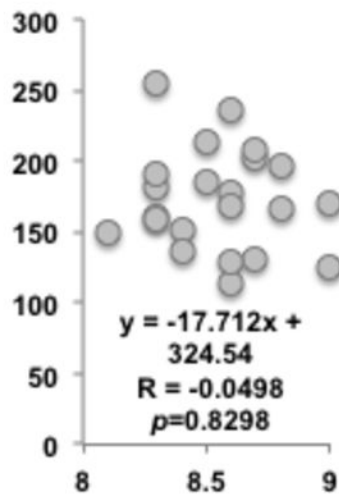
A



B



C



D

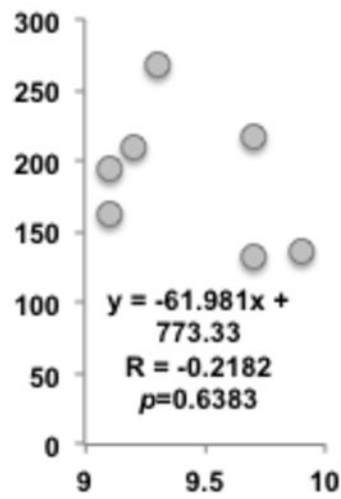


Fig.3

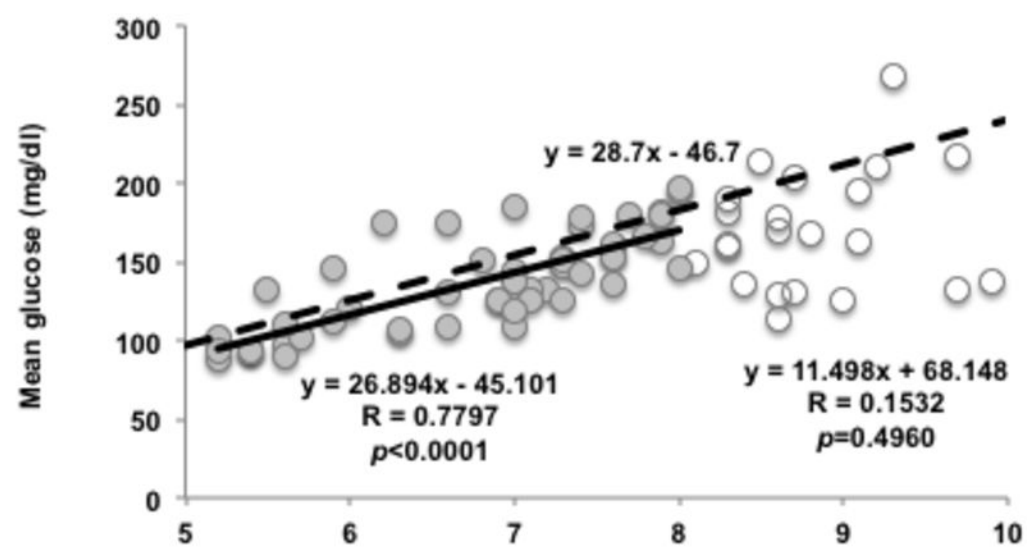


Fig.4