Evaluation of the relationship between glycated hemoglobin A1c and mean glucose levels derived from the professional continuous flash glucose monitoring system

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Abstract. Previously, we reported that short-term continuous glucose monitoring (CGM) with the professional iPro2© CGM device is a good clinical indicator of glycated hemoglobin (HbA1c) levels. However, there was no significant correlation between CGM and HbA1c levels when HbA1c levels were >8.0%. To further investigate this issue, we performed a similar study using the FreeStyle Libre Pro©, a newer device that does not require glucose calibration and allows patients to be examined for up to 14 days. Fifty-nine patients (68% women, 32% men) were examined. Twenty-eight and 31 patients presented with type 1 and type 2 diabetes, respectively. Clinically assessed HbA1c levels were compared to blood glucose levels determined by the FreeStyle Libre Pro© for up to 14 days (10.7 ± 3.7 days). We found a significant correlation between HbA1c and CGM levels even when HbA1c levels were >8.0%. Additionally, the correlation between HbA1c and average glucose was identified with the modern CGM and was found to deviate substantially from the new suggested formula. More importantly, we found a more robust correlation between HbA1c and CGM levels in patients with type 2 diabetes. Overestimation or underestimation of blood glucose levels through CGM might increase the risks of inappropriate clinical treatment of diabetes patients. Our results indicate the need for proper CGM data interpretation individualized for each patient to better assist the determination of customized treatments for patients.

Key words: Average glucose, Continuous glucose monitoring (CGM), Diabetes, FreeStyle Libre Pro©, HbA1c

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TECHNOLOGY for diabetes treatment continues to advance, and the continuous glucose monitoring (CGM) system is considered one of the most advanced technologies of the last decades [1]. The use of CGM has enabled physicians to detect unexpected glucose fluctuations otherwise undetermined by simple glycated hemoglobin (HbA1c) measurement or self-monitoring of blood glucose. CGM systems are classified into two types: personal and professional [2]. Personal CGM is also referred to as real-time CGM, and with personal CGM, patients can monitor their blood glucose values continuously. Professional CGM, also referred to as masked CGM, is typically performed by physicians, and patients remain unaware of their monitoring results until glucose values are downloaded [2]. The benefit of the blinded CGM is that theoretically, results are not affected by the patient’s behavior, enabling physicians to make therapeutic decisions based on unbiased data.

The A1c-Derived Average Glucose (ADAG) study, published in 2008, showed a strong correlation between average glucose levels and HbA1c levels. The formula used to assess the relationship was as follows: average glucose [mg/dL] = 28.7 × HbA1c [%] – 46.7 [3]. Using this formula, the CGM could calculate estimated A1c (eA1c): eA1c = (average glucose [mg/dL] + 46.7)/28.7, which is one of the CGM metrics which was recently recommended for patient education by an international consensus group [4]. Moreover, with modern CGM, the formula for assessing the relationship has been updated...
to the following: average glucose [mg/dL] = 41.806 × HbA1c [%] – 138.38 [4]. Using this formula, CGM could be used to calculate the estimated A1c. This parameter is now called the glucose management indicator (GMI). The formula has been updated to the following one: GMI (%) = 3.31 + 0.02392x (average glucose [mg/dL]). It has recently been reported that 10 days of modern CGM data is usually sufficient for estimating the average glucose value [5].

Recently, to investigate this correlation over a shorter time period, we studied the correlation between average glucose levels assessed with the professional iPro2© CGM device (iPro2, Medtronic, Northridge, CA, USA) and HbA1c levels over a 2–7-day period (4.1 ± 1.4 days) in a Japanese cohort [6]. Despite the shorter time frame of our study, we found a correlation similar to that in the ADAG study between average glucose levels during CGM and HbA1c levels for individuals with HbA1c levels <8%. However, contrasting results were obtained in patients with HbA1c levels >8%, where no significant correlation between glucose levels and HbA1c levels was found [6].

In the present study, we re-evaluated the relationship between glycated hemoglobin A1c and mean glucose levels derived from the FreeStyle Libre Pro© system (Abbott Diabetes Care, Alameda, CA, USA), one of the modern CGMs recently introduced in Japan.

**Materials and Methods**

**Ethics statement**

This study was approved by the Gunma University Institutional Review Board (ID: 150008), and it conforms to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). Patients provided written informed consent before undergoing any study-related procedures.

**Patients**

All out-patients underwent CGM with the FreeStyle Libre Pro© device in the Department of Internal Medicine, Division of Endocrinology and Diabetes, Gunma University Hospital, between December 2016 and August 2017. We excluded participants who were included in our previous report [6]. Patients treated with steroids, patients with anemia (hematocrit level <39% in men and <36% in women), and patients with glucose levels >500 mg/dL during CGM tests were excluded, as this technology is only accurate for up to 500 mg/dL of subcutaneous glucose concentrations [7]. Additionally, the FreeStyle Libre Pro© was not approved for pregnant women in Japan before the study; therefore, pregnant women were excluded.

**Assessment of glycemia**

CGM was performed with the FreeStyle Libre Pro©, which measures glucose levels every 15 minutes for at least 2 days and up to 14 days. The FreeStyle Libre Pro© does not need calibration, and patients were advised to continue their normal routine, especially in terms of dietary intake and exercise [8]. We typically started the examination after lunch, and the sensor was allowed to stabilize, as reported in the literature [7]. As such, sensor glucose values were downloaded after 9:00 am the following day and were analyzed with the FreeStyle Libre Pro© software. Downloaded data sets were further analyzed using EasyGV© (N. R. Hill, University of Oxford, Oxford, UK; available at www.easygv.co.uk) for the following parameters: mean glucose level, standard deviation (SD), mean amplitude of glycemic excursion (MAGE), and percentage coefficient of variation (%CV) [9]. Blood samples were collected, at the time CGM started and 1 month before or after this time, in ethylenediaminetetraacetic acid anticoagulant tubes, and HbA1c levels were measured using ion-exchange high-performance liquid chromatography on an ADAMS A1c, HA-8180 (ARKLEY, Inc., Kyoto, Japan).

**Statistical analysis**

Data are presented as a mean ± standard deviation (SD) and as n (%) for frequencies. All results are expressed as means ± SD for continuous variables and as absolute numbers and relative percentages for categorical variables. Associations between continuous variables were examined using Spearman 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).

**Results**

We screened 101 patients for CGM analyses. Forty-two patients were excluded based on the aforementioned exclusion criteria. Our average examination period was 10.7 ± 3.7 days. Characteristics of the 59 included patients are displayed in Table 1.

In the present study, we found a clear and statistically significant correlation between HbA1c levels and mean glucose levels using the FreeStyle Libre Pro© (r = 0.7248, p < 0.0001) (Fig. 1A). More importantly, the formula was more similar to the one used in the ADAG study (dotted line, Fig. 1A) than the one recently suggested for modern CGM (dashed line, Fig. 1A). Additionally, as HbA1c should also reflect the average previous glucose level for greater than a 1-month duration, we examined the concordance of data with HbA1c.
values obtained 1 month prior and after analysis (Fig. 1B–D).

Next, we evaluated the differences between HbA1c and eA1c by assessing the HbA1c levels (Fig. 2A); we also evaluated the differences between HbA1c and GMI by again assessing the HbA1c levels (data not shown) and found that there was no tendency. More importantly, we examined the relationships between HbA1c levels and glycemic variability indices: SD of glucose measurements, MAGE, and coefficient of variation (%CV), as

| Table 1 | Baseline characteristics of the included patients |
|---------|-----------------------------------------------|
|         | Type 1 diabetes (n = 28) | Type 2 diabetes (n = 31) | All (n = 59) |
| Age     | 44 ± 15.1                  | 54 ± 16.2                  | 49 ± 16.4    |
| Sex (% female) | 19 (68)                  | 21 (68)                    | 40 (68)      |
| HbA1c (%) | 7.5 ± 0.9                  | 7.5 ± 1.2                  | 7.5 ± 1.1    |
| Treatment |                              |                            |             |
| Insulin pump         | 43%                        |                            |             |
| Three or more daily injections | 57%                        |                            |             |
| Diet only            | 20%                        |                            |             |
| Oral agents only     | 19%                        |                            |             |
| Insulin only         | 13%                        |                            |             |
| Insulin + oral agents| 48%                        |                            |             |

Correlation between mean blood glucose levels and glycated hemoglobin A1c (HbA1c) levels of this study (solid line), ADAG study (broken line) and the new formula for GMI (dotted line) (A). HbA1c levels at the time CGM started and 1 month before or after CGM started (B). Correlation between mean blood glucose levels and HbA1c levels (this study (solid line), ADAG study (broken line) and the new formula for GMI (dotted line)) 1 month before (C) and after (D) CGM started.

Fig. 1  Mean glucose levels determined by continuous glucose monitoring (CGM) with the Freestyle Libre Pro© are highly correlated with HbA1c levels.
described in our previous report [6]. Interestingly, although the relationships between HbA1c levels and SDs or MAGEs were statistically significant (Fig. 2B–C), the relationship between HbA1c levels and %CV was not (Fig. 2D).

Finally, we re-assessed the correlation between HbA1c and mean blood glucose levels in patients with type 1 and type 2 diabetes. As shown in Fig. 3A, we still found a correlation in patients with type 1 diabetes; however, less significant than all the patients (Fig. 1A). Interestingly, the correlation of glycemic variability indices: SD of glucose measurements, MAGE was found to be completely diminished (Fig. 3C and E). However, we found the strongest correlation ($r = 0.8414$, $p < 0.0001$) in patients with type 2 diabetes (Fig. 3B) with the glycemic variability conserved (Fig. 3D and F).

**Discussion**

Our data demonstrated a strong statistically significant correlation between HbA1c and mean blood glucose levels measured by the FreeStyle Libre Pro®. We previously reported a strong correlation between HbA1c and mean glucose levels measured by the iPro2 in patients with a lower HbA1c level (<8%), reflecting the finding of the ADAG study. However, we did not find a statistically significant correlation between these parameters among patients with HbA1c levels >8% [6]. First of all, the differences obtained with the two devices might be due to their sensor accuracy. In this regard, the two devices have a similar mean absolute relative difference (MARD): the MARD for the FreeStyle Libre Pro® was 11.4 % and 11.0 % for the iPro2 [1]. Another possible explanation for the differences observed is that the FreeStyle Libre Pro® examination was longer than what was allowed with the iPro2 device and as such, patients likely did not modify their lifestyle in a consistent manner. Thus, data might actually reflect the patient’s actual values. Lastly, the FreeStyle Libre Pro® is very user-friendly as it is compact and does not need to self-monitor blood glucose for calibration. Therefore, it does not interfere with the patient’s every-day life. These emotional and practical factors may have affected patient behavior in previous studies [10-13]. We believe that these analyses assessing differences between iPro2 and FreeStyle Libre Pro® are important for patient education, since these...
could be factors for the interpretation of data during CGM. At the same time, direct comparison of the two devices might be limited since the patients assessed in this study were different than those in the previous study. Further studies in which patients are monitored using both the iPro2 and FreeStyle Libre Pro© at the same time are necessary for direct comparisons of glucose values obtained with each device.

The estimate A1c derived for the ADAG study was determined by both professional CGM (with use for a median of 13 days) and frequent capillary glucose testing for 39 days, which is one of the CGM metrics recently recommended for patient education by an international consensus group [4]. Importantly, with modern CGM, the formula for the relationship has been updated to the following: average glucose [mg/dL] = 41.806 × HbA1c [%] – 138.38 [4]. In the present study we used a FreeStyle Libre Pro©, which is considered a modern CGM; however, the formula used was closer to the ADAG study. One reason might be that these values were obtained from a Caucasian cohort, and based on genetic background differences, the values might not be completely applicable to our Japanese cohort.

It has been reported that not only the efficacy but also the accuracy of the FreeStyle Libre Pro© is ensured for patients with type 1 diabetes [7]; however, limited studies exist comparing differences between type 1 and type 2 diabetes. Our data showed a much stronger correlation.
between HbA1c and mean blood glucose levels in patients with type 2 diabetes than that for patients with type 1 diabetes (Fig. 3A and B). It has been shown that CGM may not be accurate if glucose viability is too high [14]. Since these clearly correlate with the glycemic variability indices, it might be due to the glucose fluctuation of patients with type 1 diabetes.

Several limitations need to be considered when interpreting the present findings. HbA1c supposedly reflects the average previous glucose levels that have occurred for at least 1 month. In the ADAG study, the predicted HbA1c was calculated using repeated average glucose levels for 3 months. In this study, we collected CGM data for only 14 days (10.7 ± 3.7 days); therefore, assessing how this could reflect long-term complications is only an estimated prediction. However, when using HbA1c data obtained 1-month prior and after our analysis, we also found a concordance of data (Fig. 1B–D). Lastly, it is worth noting that this study had a cross-sectional retrospective design and was conducted by recruiting patients within a single center. Therefore, patient characteristics and/or treatment could vary depending on the center, and a multicenter study might be necessary in the future to address this issue. More importantly, we have to consider the smaller sample size.

In this regard, the estimated sample size necessary for significance was calculated with a two-sided type 1 error of <5% and a power of 80% for this study.

In summary, we assessed the relationship between HbA1c and mean blood glucose levels determined by the FreeStyle Libre Pro®. We found a strong positive relationship between glucose levels measured by the FreeStyle Libre Pro® and HbA1c levels (r = 0.7248, p < 0.0001), which is more consistent with the findings of the ADAG study and more consistent than the updated formula with modern CGM. Moreover, we found a much stronger correlation between HbA1c and mean blood glucose levels in patients with type 2 diabetes than that for type 1 diabetes. Overall, our results indicate the need for proper CGM data interpretation individualized for each patient and choosing professional CGM to better assist determination of customized treatments for patients.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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