An Analysis of the Hemoglobin Glycation Rate in the A1C-Derived Average Glucose Study Population Applying a Monte Carlo Method

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Abstract— **Diabetes is a huge and growing problem, and the costs to society are consistently increasing. The glycated hemoglobin A1c (HbA1c) concentration is a frequently used marker of the metabolic control. In 2008 the results of the A1c-Derived Average Glucose (ADAG) Study were reported establishing a linear relationship between HbA1c and the preceding glycemia. The aim of this study was to analyze the overall hemoglobin glycation rate constant (*k*) in the ADAG population applying a Monte Carlo method. We reproduced the distribution of HbA1c and plasma glucose concentrations in the ADAG population and we obtained an estimate of *k* equal to $1.239 \pm 0.175 \times 10^{-9}$ L/(mmol s) assuming that red blood cells' (RBCs) life span was equal to 120 days. The *k* value estimated in this study is in a good agreement with values of this parameter reported earlier. It was also demonstrated that a normal distribution of RBCs' life span with the standard deviation of 14 days is sufficient to explain the inter-subject variability of the hemoglobin glycation rate. The obtained distribution of RBCs' life spans is even less scattered around the mean than previously reported distributions for healthy individuals and for patients with diabetes. However, an assumption that *k* is the same for all the individuals implies a positive correlation of RBCs' life span and HbA1c concentration, which was inconsistent with earlier reports. This result suggests that the hemoglobin glycation rate might increase with a deterioration of the metabolic control.**

Keywords— **Hemoglobin A1c, Diabetes mellitus, Mathematical modeling, Monte Carlo method.**

I. Introduction

According to the International Diabetes Federation – diabetes is a huge and growing problem, and the costs to society are high and escalating [1]. Insufficient production of insulin by a pancreas or ineffective use of this hormone by the body are two causes of the three main types of diabetes – type 1 diabetes, type 2 diabetes and gestational diabetes affecting more than 380 million patients worldwide.

Regardless of the treatment method that is used, it is of a crucial importance to continuously monitor the metabolic control resulting from this treatment. The glycated hemoglobin A1c (HbA1c) concentration is the most frequently used marker of the metabolic control. HbA1c has a glucose particle linked to the N-terminus of the β-globin chain of a hemoglobin particle. For the last few decades it has been considered that HbA1c reflects a weighted average of blood glucose levels during the preceding 4 months (i.e. the average erythrocyte's life span) with recent glycemia levels contributing considerably more to the level of HbA1c. Not until 21st century it was possible to quantitatively determine a relationship of HbA1c and plasma glucose due to the lack of continuous glucose monitoring systems, the limited accuracy of HbA1c measurements and the slow pace of hemoglobin glycation. Hence, just in 2008 the results of the A1c-Derived Average Glucose (ADAG) Study were reported [2]. This study established a linear relationship between HbA1c and the Estimated Average Glucose (eAG) over the preceding 3 months that was based on the continuous glucose monitoring in 507 participants including 80 non-diabetic subjects, 268 patients with stable type 1 and 159 patients with stable type 2 diabetes.

We demonstrated previously that a non-linear chemical model assuming first-order reaction in respect to glucose and hemoglobin, which is characterized by a single overall glycation rate constant (*k*) is able to predict changes of HbA1c with a high accuracy in non-diabetic subjects [3, 4].

The aim of this study was to analyze the overall hemoglobin glycation rate constant in the ADAG population applying a Monte Carlo method.

II. Materials and Methods

HbA1c Calibration

During the ADAG study all the HbA1c values were measured using methods calibrated according to the National Glycohemoglobin Standardization Program (NGSP) [2]. This is the most commonly used HbA1c scale and it is also used throughout this manuscript. However, this scale reports biased HbA1c values. To avoid negative influence of this bias on results of calculations presented in this work the measured HbA1c values were corrected according to the master linear equation to obtain unbiased values, i.e. as if they were measured using methods calibrated according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [5]:

$$HbA1c_{NGSP} = 0.915 \times HbA1c_{IFCC} + 2.15 \qquad (1)$$

B. Model of Hemoglobin Glycation

According to the model that was previously tested in healthy subjects, HbA1c formation in each single red blood cell (RBC) can be expressed by a simple differential equation [6]:

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$$\frac{d[HbA(t)]}{dt} = -k[HbA(t)][G(t)] \quad (2)$$

where square brackets denote a measure of concentration and G stands for glucose, HbA for non-glycated hemoglobin, k for the overall glycation rate constant and t for time. The model is used under the following assumptions that had been validated earlier [3, 4]:

- RBCs' count *in vivo* is constant.
- HbA1c concentration in the newly generated reticulocytes is equal to zero.
- Concentration of all other types of hemoglobin except HbA and HbA1c is negligible.
- Influence of hemoglobin loss from RBCs on HbA1c is negligible.
- Short-term glucose variability does not influence HbA1c formation in individuals with stable long-term metabolic control (i.e. with constant mean glycemia).

Under these assumptions HbA1c concentration in percents of the total hemoglobin for a particular subject i, with a stable mean plasma glucose concentration, can be expressed in the following way:

$$HbA1c_i = 91.5 \times \left(1 - \frac{1 - e^{-kT_i[G_i]}}{kT_i[G_i]}\right) + 2.15 \quad (3)$$

where T_i is a life span of RBCs and $[G_i]$ is considered to be constant and equal to the mean plasma glucose concentration over T_i.

By approximating the exponential function in Eq. 3 with first four elements of its Taylor series we can obtain a relatively simple formula to calculate k:

$$k = \frac{3 - \sqrt{9 - \frac{24 \times (HbA1c_i - 2.15)}{91.5}}}{2T_i[G_i]} \quad (4)$$

C. Estimation of the Hemoglobin Glycation Rate Constant in the ADAG Population

Based on the data presented in the ADAG study report [2] we made a statistical model of the ADAG population. HbA1c values were sampled from three normal distributions representing the healthy subjects, patients with type 1 diabetes and patients with type 2 diabetes, respectively [2]:

$$\begin{aligned} HbA1c_{Healthy} &\sim N(5.2, 0.09) \\ HbA1c_{Type\,1} &\sim N(7.3, 1.21) \\ HbA1c_{Type\,2} &\sim N(6.8, 1.21) \end{aligned} \quad (5)$$

A percentage of samples from each of these three subpopulations was equal to the percentage of participants of the ADAG study in each subpopulation, i.e. 15.78%, 52.86% and 31.36%, respectively.

The plasma glucose concentration for a particular HbA1c concentration was modeled by a normal distribution, with the mean value $\mu_{[G]}$ being a linear and variance $\sigma^2_{[G]}$ being a power function of HbA1c concentration according to the relationships reported in the ADAG study:

$$\mu_{[G]} = 1.5944 \times HbA1c - 2.594 \quad (6)$$

$$\sigma^2_{[G]} = 0.0148 \times HbA1c^{2.03} \quad (7)$$

The distribution of hemoglobin glycation rate constant k in the ADAG population, sampled as described above, was estimated using a Markov Chain Monte Carlo (MCMC) method according to Eq. 4. We used OpenBUGS 3.2.1 to conduct the analysis (http://www.openbugs.info/w/) [7]. Initially, we assumed a constant T equal to 120 days to be able to compare the obtained k value with the previously reported value for the non-diabetic subjects [4]. Then, we demonstrated how changes of T influence the k value.

D. Analysis of the Hemoglobin Glycation Rate Constant

An analysis was performed to check whether RBCs' life span heterogeneity is sufficient to explain the inter-subject variability of k that occurs when a constant RBCs' life span is assumed.

Initially, we used distributions of $[G]$ and HbA1c of the ADAG population (formulas 5-7) to sample 1 000 pairs of $[G_i]$ and $HbA1c_i$ values (where $i = 1,..,1\,000$). We used these data as surrogates of the data from the "real" participants of the study.

Then, we defined a model, which assumed that each sampled in such a way $HbA1c_i$ value is an approximation of the real HbA1c concentration that is normally distributed:

$$HbA1c_i \sim N(\mu_{HbA1c_i}, \sigma^2_{HbA1c}) \quad (8)$$

where μ_{HbA1ci} is related to k, T_i and $[G_i]$ according to Eq. 3.

The T_i was also assumed to be normally distributed:

$$T_i \sim N(\mu_{T_i}, \sigma^2_T) \quad (9)$$

Finally, we set: (1) a constant k equal to the mean value estimated for the ADAG population, (2) vague prior uniform distribution for σ_{HbA1c}, (3) prior normal distributions for μ_{Ti} with the mean value of 120 days and the standard deviation of 23 days [8] and we used MCMC method to

obtain posterior distributions of T_i and σ_{HbA1c} given the $HbA1c_i$ and $[G_i]$ data. Calculations were performed using four chains. First 10 000 iterations with thinning set to 10 were discarded as 'burn-in' and we used results of the consecutive 50 000 iterations with the same value of thinning for inference. Convergence of the chains was confirmed by the Gelman-Rubin statistics.

III. Results

The k estimated for the ADAG population under an assumption that RBCs' life span is equal to 120 days is equal to $1.239 \pm 0.175 \times 10^{-9}$ L/(mmol s). The coefficient of variation of k is equal to 14.1%. It is noteworthy that k value calculated directly from Eq. 3 based on the mean values of HbA1c and $[G]$ is equal to 1.213×10^{-9} L/(mmol s).

Fig. 1 illustrates how changes of T influence the k value assuming that T is constant for all the study participants.

The mean k for a particular T expressed in days can be calculated using the following formula:

$$k = 1.479 \times 10^{-7} \times T^{-0.999} \tag{10}$$

It was also confirmed that in the sampled HbA1c distribution was characterized by the mean value of 6.8% and the standard deviation of 1.3%, which were exactly the same as in case of the real ADAG data. The same was true for $[G]$, which was equal to 8.3 ± 2.2 mmol/L.

Fig. 2 shows the posterior distribution of T_i that was estimated given 1 000 pairs of $HbA1c_i$ and $[G_i]$ sampled from the ADAG population. The k value was set to $1.239 \pm 0.175 \times 10^{-9}$ L/(mmol s).

The posterior estimate of the standard deviation of T is equal to 14 days, which is smaller by 39% than the value of 23 days estimated using the end-alveolar carbon monoxide technique [8]. The posterior estimate of σ_{HbA1c} (median and 95% credible interval) is equal to 0.03% (0.005%, 0.09%) meaning that RBCs' life span heterogeneity is sufficient to explain the inter-subject variability of the hemoglobin glycation rate.

Fig. 3 demonstrates the estimated RBCs' life spans against HbA1c concentrations assuming that hemoglobin glycation follows the reaction described by Eq. 2 and given k equal to $1.239 \pm 0.175 \times 10^{-9}$ L/(mmol s).

It can be seen in Fig. 3 that T increases by approx. 4 days with an increase of HbA1c concentration by 1% to satisfy the model when the same value of k is assumed for all the individuals.

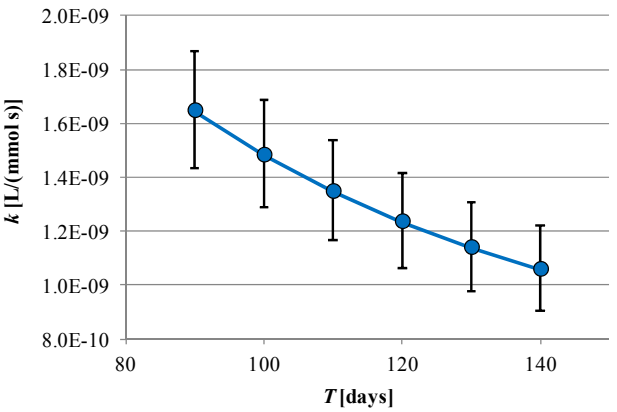

Fig. 1 The hemoglobin glycation rate constant k (mean ± SD) *vs.* RBCs' life span (T)

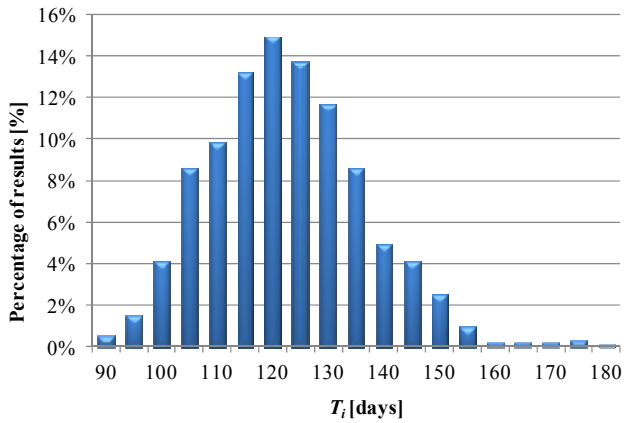

Fig. 2 Estimated distribution of RBCs' life span for 1 000 samples drawn from the distribution of the ADAG population given k equal to 1.239×10^{-9} L/(mmol s)

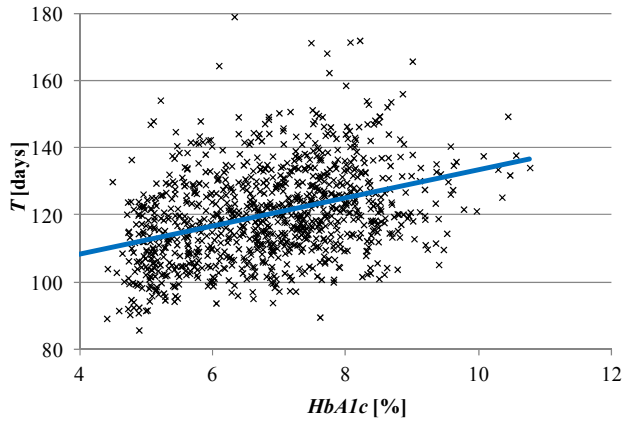

Fig. 3 Estimated RBCs' life spans *vs.* HbA1c concentrations for 1 000 samples drawn from the distribution of the ADAG population given k equal to 1.239×10^{-9} L/(mmol s)

IV. Discussion

The mean hemoglobin glycation rate constant obtained in this study based on the distribution of HbA1c and plasma glucose concentrations in the ADAG study is equal to $1.239 \pm 0.175 \times 10^{-9}$ L/(mmol s) under an assumption of a constant RBC's lifespan of 120 days. This value is close to the mean value of k that we reported earlier for a group of healthy subjects, i.e. $1.257 \pm 0.114 \times 10^{-9}$ L/(mmol s) that was obtained based on *in vivo* continuous glucose monitoring [4]. The mean k estimated during the current study is also in a good agreement with mean values reported earlier based on *in vivo* or *in vitro* experiments [e.g. 6, 9, 10].

The coefficient of variation of k estimated for the ADAG population is equal to 14.1%. This value is higher than the one obtained earlier for the healthy subjects (i.e. 9.2%) [4]. However, it seems understandable, taking into consideration much higher diversity of the ADAG population in terms of the ethnicity and the metabolic state.

We also demonstrated that a normal distribution of RBCs' life span with the standard deviation of 14 days is sufficient to explain the inter-subject variability of the hemoglobin glycation rate. The obtained distribution of RBCs' life spans is even less scattered around the mean than previously reported distributions for healthy individuals and for patients with diabetes [8, 10]. However, a positive correlation of RBCs' life span and HbA1c concentration was noted, which was inconsistent with earlier reports based on the end-alveolar carbon monoxide monitoring [11]. This result supports a hypothesis that the hemoglobin glycation rate increases with an increase of HbA1c, e.g. due to a susceptibility of patients with diabetes to oxidative stress and an association of hyperglycemia with free radical-mediated lipid peroxidation.

V. Conclusions

The mean hemoglobin glycation rate constant estimated in this study, based on the distribution of HbA1c and plasma glucose concentrations obtained in the ADAG study, is in a good agreement with values of this parameter reported earlier. Red blood cells' life span heterogeneity is sufficient to explain the inter-subject variability of the hemoglobin glycation. However, an assumption that k is the same for all the individuals implies a positive correlation of RBCs' life span and HbA1c concentration, which is inconsistent with previously reported data. This result suggests that the hemoglobin glycation rate constant might be positively correlated with a level of the metabolic control.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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