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Correlation of fasting blood glucose and haemoglobin A_{1c} measured with an automated analyser

Akira Furota,† Teikichi Miyagawa, Izumi Tsuda,‡ and Noriyuki Tatsumi‡

Midori Health Care Foundation, Tarumicho 3-22, Suita 565, and ‡ Department of Clinical and Laboratory Medicine, Osaka City University Medical School, Asahimachi 1-5-7, Abeno-ku, Osaka 545, Japan

A subtype of glycohaemoglobin, haemoglobin (Hb) A_{1c}, in specimens of whole blood was assayed on a new automated analyser that makes use of high-pressure liquid chromatography. The analyser provided precise and reproducible values. The mean of the HbA_{1c} values was lower than that with an older instrument. The mean tended to increase with the age of the subjects, who were undergoing routine health examinations. No sex difference was found. When measurement was made 1 h after the subjects drank 50 g of glucose, the value of HbA_{1c} was unaffected. Correlation was strong between the HbA_{1c} value and the fasting blood glucose value, which suggested that fasting blood glucose could be estimated from the HbA_{1c} value.

Introduction

There are several methods for the measurement of haemoglobin A_{1c} (HbA_{1c}) in red blood cells, involving electrophoresis, colorimetry, minicolumns and high-pressure liquid chromatography (HPLC). Each method has different advantages but a problem shared by all of these is the long testing time.

Here, we evaluate the performance of an automated apparatus for the assay of HbA_1 and HbA_{1c} (Hi-Auto A_{1c}, model 8121, Kyoto Daiichi Kagaku Co., Ltd) first marketed in 1987. In this model, HbA_{1a}, HbA_{1b}, HbA_{1c}, and HbF are separated more completely than in the earlier analyser (model 8120) tested here for comparison, because of changes made in the column filler and in the eluent. In the newer model, unstable HbA_{1c} is eluted first by use of tetrapolyphosphoric acid, which competes for the site on the haemoglobin molecule where glucose binds.

Table 1. Effects of anticoagulants on HbA_{1c} and A_1 values (%) found with models 8120 and 8121.

Anticoagulant	Model	HbA_{1c}, %	HbA_1, %
NaF	8120	5·41 ± 0·51	8·09 ± 0·76
(N = 204)	8121	4·92 ± 0·44	7·09 ± 0·76
EDTA	8120	5·17 ± 0·55	8·05 ± 0·81
(N = 246)	8121	4·85 ± 0·55	6·97 ± 0·84
Na-citrate	8120	5·08 ± 0·36	8·07 ± 0·65
(N = 85)	8121	4·78 ± 0·38	6·80 ± 0·57

Note: Values for each haemoglobin are the mean percentage ± 1 SD of total haemoglobin, found by HPLC.

† Correspondence to Akira Furota at: Department of Clinical and Laboratory Medicine, Osaka City University Medical School, Asahimachi 1-5-7, Abeno-ku, Osaka 545, Japan

Materials and methods

Instruments

The machine tested was a Hi-Auto A_{1c} model 8121, which assays HbA_{1c}, HbA_1, and HbF in 4 min with 3 µl specimens [1]. For comparison, an older model from the same manufacturer (Hi-Auto A_{1c} model 8120, which resembles the model 8110 described in [2]) was used.

Materials

Everyone who comes to our health care centre is a company employee with no known current health problems who is to undergo routine health examinations.

Table 2. Day-to-day reproducibility of HbA_{1c} and HbA_1 and retention time in assay of control material. The same column was used to measure about 90 specimens daily for 20 days. Each specimen was from a different subject. Control material was assayed once at the end of each work day.

	Item			
Day	HbA_{1c}, %	HbA_1, %	Retention time	Numbers of specimens
1	6·5	9·5	2′09	90
2	6·4	9·3	2′08	88
3	6·5	9·5	2′06	89
4	6·4	9·3	2′06	90
5	6·5	9·5	2′11	88
6	6·4	9·4	2′09	89
7	6·5	9·5	2′09	91
8	6·4	9·3	2′11	90
9	6·5	9·8	2′09	87
10	6·4	9·5	2′06	90
11	6·5	9·5	2′09	88
12	6·4	9·6	2′09	89
13	6·5	9·6	2′09	89
14	6·5	9·5	2′09	88
15	6·4	9·5	2′08	89
16	6·5	9·5	2′06	92
17	6·4	9·5	2′09	90
18	6·5	9·5	2′09	90
19	6·4	9·5	2′12	89
20	6·5	9·5	2′06	90
CV	0·79	1·18	1·37	Total, 1786

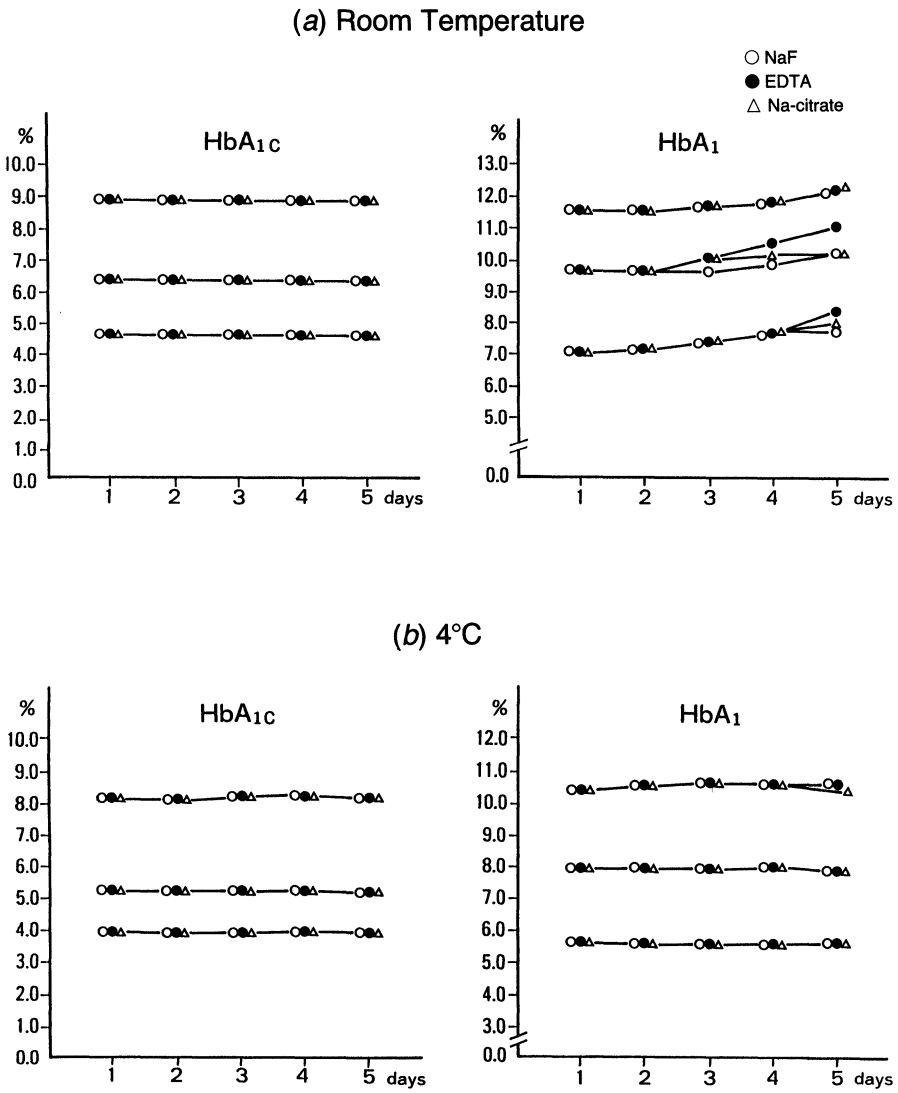

Figure 1. Effects of storage at two temperatures in three different anticoagulants on values found for HbA_{1c} and HbA_1 with the newer analyser. (a) Room temperature; (b) 4 °C. ○, NaF; ●, EDTA; △, Na-citrate.

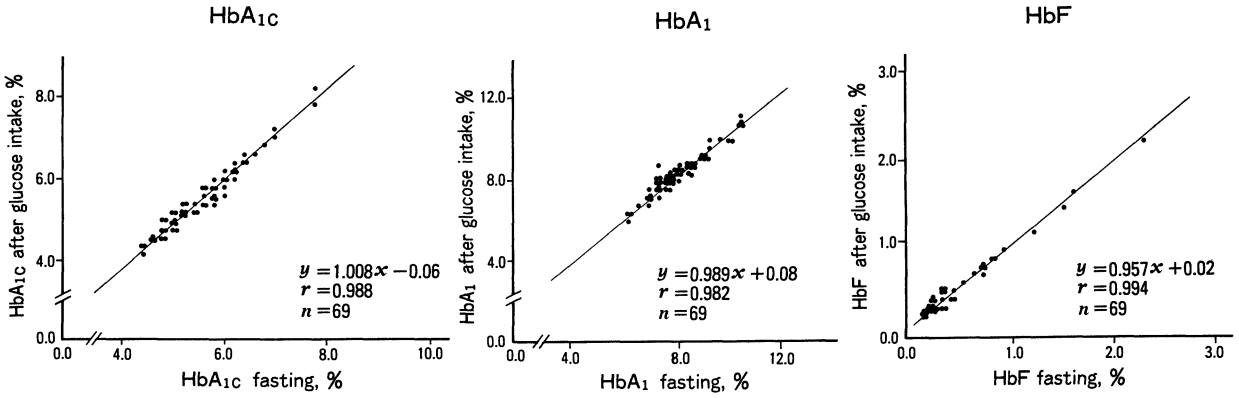

Figure 2. Comparison of HbA_{1c}, HbA_1, and HbF values measured with the newer analyser in blood of fasting subjects and in blood of the same subjects one hour after glucose intake.

Tests of urine, stools, blood chemistry and pulmonary function are carried out, and a complete blood cell count, X-ray films of the chest and the upper gastrointestinal tract, and electrocardiographs are taken. Glucose metabolism is examined by tests of urinary glucose and fasting blood glucose (FBG), and by a 50 g oral glucose tolerance test (OGTT), from which the blood glucose concentration 1 h after the intake of glucose (postprandial blood glucose, or PBG) was used here. In the OGTT, blood was collected into bottles containing NaF when the subject was fasting and again at 1 h.

Venous blood was withdrawn from the examinees with a Venoject syringe (Terumo Co., Osaka) containing NaF (1·25 mg/ml blood), sodium citrate (0·5 ml of a 3·85% solution per 4·5 ml of blood), or a mixture of EDTA-2Na (3·7 mg/ml blood) and heparin (12·5 U/ml blood). The glycohaemoglobins in the blood were assayed within 1 h of sampling. Blood glucose was assayed by the neocuproine method [3].

Control material was purchased from International Reagents Corporation (Kobe).

Statistics

Results were evaluated by Student's *t*-test and analysis of variance.

Results

HbA_{1c} measurements by the new model

The effects of the anticoagulant used on the value of HbA_{1c} are shown in table 1. The mean values obtained for HbA_{1c} were highest with NaF, intermediate with EDTA plus heparin, and lowest with sodium citrate. Mean values for HbA_1 were almost the same with all three anticoagulants. The newer model gave lower values than the older model in assays of both of these haemoglobins. With NaF, the mean values for HbA_{1c} for the two glycohaemoglobins were significantly different. The differences probably arose from differences in the column fillers and in the elution method, which cause differences in the efficiency of the removal of unstable glycohaemoglobin. With NaF, the blood glucose value of the specimens was not lowered as much as with the other anticoagulants, so that nearly all of the unstable form remained unbound in the specimens. For these reasons, the HbA_{1c} values obtained by the older model, in which removal of the unstable glycohaemoglobin was less efficient, were higher.

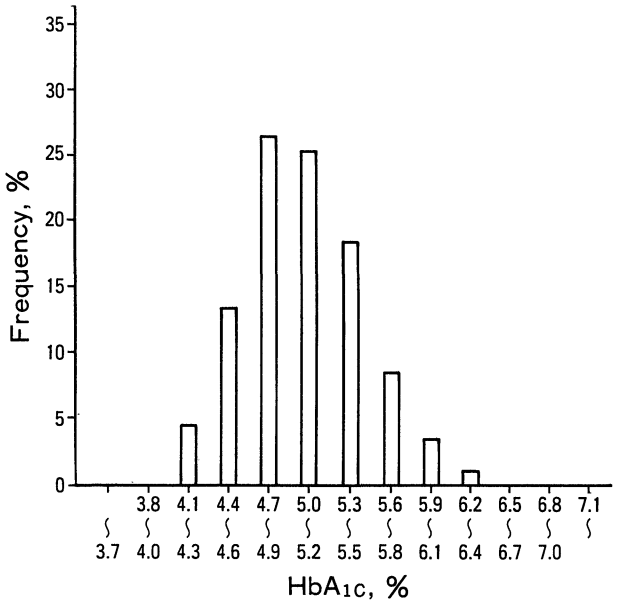

Figure 3. Distribution of HbA_{1c} values of 7260 specimens measured with the newer analyser.

When samples were stored at 4 °C or room temperature in one of the three anticoagulants for up to 5 days (figure 1), results for HbA_{1c} stayed about the same. However, at room temperature, values for HbA_1 increased during storage.

Table 2 shows the day-to-day reproducibility of HbA_{1c} and HbA_1 in control material assayed by the newer model with use of the same column for 20 days. The number of other specimens measured daily was about 90, each from a different subject; in all, 1786 specimens were assayed during these 20 days. Retention time with the HPLC column did not change. Each day, after all test specimens were assayed, control material was tested. Correlation was satisfactory for both glycohaemoglobins. Thus, repeated use such as is described here did not affect the results obtained with the column.

Relationship betweeen glycohaemoglobin values and blood glucose levels

In diabetic subjects, the unstable form of HbA_{1c} increases as the glucose level increases but the stable form is unchanged [4]; the same changes occur in healthy subjects, as in the OGTT. Thus, total HbA_{1c} (stable plus unstable forms) rises with increasing glucose. In the 69 subjects chosen at random during the test period to illustrate typical results here, the mean FBG level was 106 ± 17 mg/dl and the PBG level was 161 ± 45 mg/dl. Figure 2 shows the values for each of the three forms of haemoglobin assayed by the new model, HbA_1, stable HbA_{1c}, and HbF, when the subjects were fasting (*x*-axis) and at 1 h after glucose intake (*y*-axis). Correlation was satisfactory for all these three forms.

Distribution of HbA_{1c} values in specimens

Figure 3 shows the distribution of HbA_{1c} values in 7260 examinees screened over a 4 month period. The coefficient of variation (CV) was 5·22% and the standard deviation (SD) was 0·70%. The reference interval was calculated by the method of Hoffman [5]. The mean reference value and the SD were 5·13% and 0·43%, respectively.

Table 3 shows mean values of HbA_{1c} measured with the older model for different age groups and by sex for 5319 subjects. Table 4 shows mean levels of HbA_{1c} in the same way, measured with the newer model for 6807 other subjects. Results in table 4 are based on those in figure 4,

Table 3. HbA_{1c} values (%) obtained with the older model with subjects classified by age and sex.

Sex	Age							Total number
	−29	30−	40−	50−	60−	70−	Mean	
Male	5·04 (0·26)†	5·18 (0·35)	5·31 (0·39)	5·40 (0·43)	5·49 (0·46)	5·52 (0·41)	5·32 (0·42)	3660
Female	4·88 (0·22)	4·94 (0·26)	5·02 (0·28)	5·24 (0·35)	5·27 (0·32)	5·24 (0·17)	5·08 (0·34)	1659
Total	4·97 (0·26)	5·10 (0·35)	5·22 (0·39)	5·35 (0·42)	5·43 (0·44)	5·45 (0·39)	5·25 (0·42)	5319

† Numbers in parentheses represent 1 SD.

Table 4. HbA_{1c} values (%) obtained with the newer model with subjects classified by age and sex.

Sex	Age							Total number
	−29	30−	40−	50−	60−	70−	Mean	
Male	4·93 (0·36)†	5·07 (0·36)	5·15 (0·42)	5·24 (0·45)	5·30 (0·45)	5·33 (0·45)	5·18 (0·44)	4714
Female	4·78 (0·30)	4·91 (0·33)	4·95 (0·33)	5·15 (0·37)	5·25 (0·41)	5·34 (0·45)	5·03 (0·38)	2093
Total	4·87 (0·36)	5·02 (0·36)	5·09 (0·41)	5·21 (0·43)	5·29 (0·44)	5·33 (0·47)	5·13 (0·43)	6807

† Numbers in parentheses represent 1 SD.

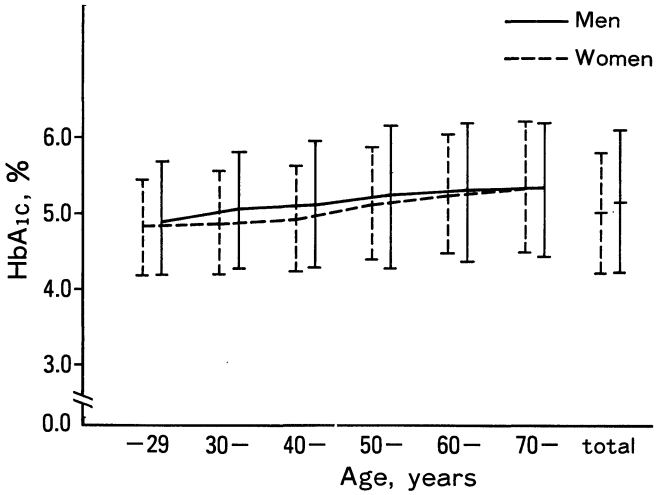

Figure 4. HbA_{1c} values by age and sex. The values are based on those in table 4, measured with the newer analyser. Curves connect the means and bars show 1 SD.

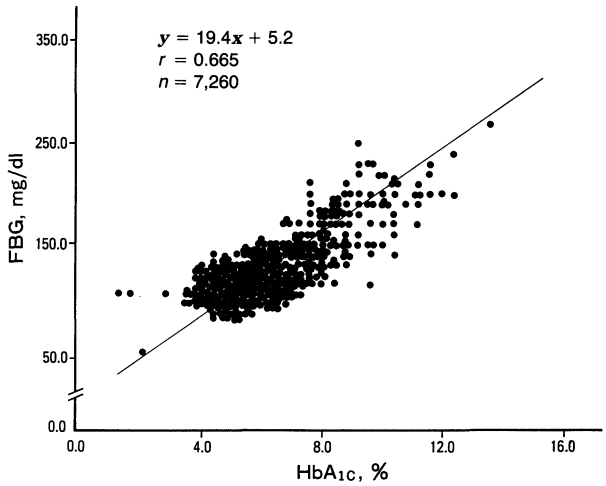

Figure 5. Correlation between HbA_{1c} and fasting blood glucose (FBG) measured with the newer analyser.

and one SD is shown. The mean values for this one large group measured with the older model were generally higher than those of the other large group measured with the newer model. HbA_{1c} tended to be higher in men than in women. It also tended to increase with age.

Figure 5 shows the correlation between HbA_{1c} and FBG in the 7260 specimens of figure 3. The correlation was high.

Discussion

The clinical significance of glycohaemoglobins has been established and their assay is becoming routine. However, the presence of HbF and the instability of HbA_{1c} [4] interfere with the accurate measurement of stable HbA_{1c}, which is of clinical interest. The number of samples sent for testing to clinical laboratories is on the increase and automation by a more reliable method is

desirable. The machine we tested separates subfractions of haemoglobins in an HPLC column at a controlled temperature, so that measurement is stable and data are both precise and accurate. The machine removed unstable HbA_{1c} and could assay 3 μl samples. The time taken for a single assay was 4 min; the retention time was short (table 2). The newer model gave lower values of HbA_{1c} than the older model, probably because of the removal of unstable HbA_{1c} by the use of tetrapolyphosphoric acid. After blood is sampled, the glucose level decreases and so the unstable HbA_{1c} in the blood changes gradually to the nonglycosylated HbA form [4]. If unstable HbA_{1c} is not removed before assay, then the extent of decomposition will influence the value obtained for total HbA_{1c}.

With the newer machine tested here, storage time did not affect the value obtained for HbA_{1c}, which value is closely correlated with the presence of diabetes. However, HbA_1, which has less correlation with diabetes, was affected by storage time and, if an accurate value for HbA_1 is needed, specimens should be assayed within 2 days of sampling. Because HbA_1 is unstable when blood is stored, and because correlation is good between HbA_1 and HbA_{1c} levels in blood assayed soon after being sampled, in general, assay of HbA_{1c} is more likely to give reliable results. Results for the assay of control material were highly reproducible (table 2).

The anticoagulants did not affect the level of HbA_1 and HbA_{1c}, so any of them can be used when HbA_{1c} is to be assayed. If the glucose level of the specimen is also to be measured, NaF can and should be used.

The mean difference in the glucose level when 69 of the subjects were fasting and 1 h after the intake of 50 g of glucose was about 50 mg/dl but the levels of HbA_1 and HbA_{1c} were unaffected by this difference.

The reference values for HbA_1 and HbA_{1c} obtained by groups using cation-exchange chromatography [6] are wider than ours, probably because of the removal of unstable HbA_{1c} by the machine we used. We found that HbA_{1c} tended to increase with the age of our subjects (aged 22 to 82 years). The same tendency has been reported for subjects divided into those aged 21 to their 45th birthday and those older. Because both the blood glucose level and glucose intolerance increase with age [8], the glycohaemoglobin level can be expected to increase, so our findings are reasonable. HbA_{1c} is of use in the monitoring of the glucose levels of a subject in the preceding few months. Because correlation between HbA_{1c} and FBG is high, the value of HbA_{1c} should also be of use for prediction of the likeliness that a subject will develop diabetes mellitus.

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