Reproducibility of HbA_{1c} in a Healthy Adult Population

The Telecom Study

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The Expert Committees of the American Diabetes Association and the World Health Organization recently suggested that glycated hemoglobin could be used for diagnosing diabetes in the future, when standardization of the glycated hemoglobin measurement will be improved (1,2). Indeed, a critical point—the reproducibility of glycated hemoglobin in healthy adults—needs to be evaluated before glycated hemoglobin A_{1c} (HbA_{1c}) could be proposed as a test for diabetes diagnosis. At the present time, no consistent information about the reproducibility of HbA_{1c} in healthy adult populations is available, leading the authors of an encouraging meta-analysis on the use of HbA_{1c} in diabetes diagnosis to deplore that "no clinical data are available on the reproducibility of HbA_{1c} levels in this setting" (3). More recently, an assessment of biological variation of HbA_{1c} in 12 nondiabetic subjects showed a low index of individuality, and the authors concluded that "HbA_{1c} measurements will always be of limited value when screening for type 2 diabetes" (4), but this paper raised strong controversies (5,6). We report here data that assess the reproducibility of HbA_{1c} in a large sample of healthy adult subjects.

RESEARCH DESIGN AND METHODS — The subjects were from the Telecom Study (7). The background population was 3,240 consecutive subjects, all employees of France Telecom attending a center for preventive medicine. They underwent a check-up that included a 0- to 2-h oral glucose tolerance test and an HbA_{1c} measurement. From this large and healthy sample, 202 nondiabetic subjects who were at risk for diabetes (family history of diabetes in first-degree relatives, past delivery of a newborn weighing >4 kg, or overweight), were selected for a second examination within 1 year, and fasting plasma glucose and HbA_{1c} were again determined. No instructions for changing lifestyle were provided before the second examination had been completed, and no significant change in body weight was observed. Patients with hemoglobinopathies, known diabetic patients, and pregnant women were excluded.

At both analyses, HbA_{1c} was measured by the same technician, who was blinded from the first result for the second measurement, and the same chromatographic technique was used. In the background population, HbA_{1c} was normally distributed with a mean value of 5.05, an SD of 0.56, and a median of 5.00 (7) (to avoid confusion, HbA_{1c} values are expressed in absolute units, e.g., 5.00, rather than 5.00% [of total hemoglobin]). A pilot study of 32 diabetic patients with samples measured in triplicate showed for HbA_{1c} a residual standard error (assessing analytical variability) of 0.22 with a range from 0.06 to 0.40 and an intra-assay coefficient of variation of 2.8%. Fasting plasma glucose was measured using the glucose oxidase method, with the same conditions throughout the study.

Statistical analyses used SAS software. An Altman plot was drawn which showed no correlation between the subject HbA_{1c} difference and the subject mean ($r = -0.07$) (8). The reproducibility of HbA_{1c} and of fasting plasma glucose was assessed by the intraclass correlation coefficient ρ, which is the ratio of the interindividual variance (σ_a^2) to the sum of σ_a^2 plus the intraindividual variance (σ_e^2):

$$\rho = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

When there is only one repetition, ρ is equal to the Pearson correlation coefficient (9). These analyses were applied to the whole sample and to the two subsamples obtained when the population was divided according to a delay of 120 days between the two examinations, corresponding to the erythrocyte lifespan. Data are shown as means ± SEM.

RESULTS — The mean values of HbA_{1c} and fasting plasma glucose did not differ between the two examinations and were more similar for HbA_{1c} than for fasting plasma glucose. Fasting plasma glucose had a smaller intraindividual than interindividual variance, while, strikingly, the reverse was true for HbA_{1c}. Therefore, the intraclass correlation coefficient was low for HbA_{1c}, nearly half that of fasting plasma glucose: 0.35 versus 0.67 (Table 1).

The better reproducibility of fasting plasma glucose compared with HbA_{1c} is confirmed by Figs. 1 and 2. Figure 1 shows a typical regression to the mean that indicates the high and random variability of HbA_{1c}. In the individuals ($n = 57$) reexamined within the erythrocyte life span (delay = 101 ± 2 days), HbA_{1c} had a better reproducibility compared with the group ($n = 145$) reexamined with a delay of >120 days (195 ± 4 days): intraclass correlation coefficients of 0.61 versus 0.28, intraindividual variances of 0.090 versus 0.197, and interindividual variances of 0.132 versus 0.075, respectively. In contrast, the intraclass correlation coefficient of fasting plasma glucose was not influenced by the delay between the two examinations: 0.64 versus 0.68 for each subgroup, respectively.

CONCLUSIONS — This study demonstrates that HbA_{1c} is poorly reproducible in healthy subjects. This was not to be ex-

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Reproducibility of HbA_{1c} and fasting plasma glucose: the Telecom Study*

	First exam	Second exam	Intraindividual variance	Interindividual variance	Intraclass correlation coefficient
HbA_{1c} (% of total Hb)	5.02 ± 0.04	5.00 ± 0.03	0.167	0.088	0.35
Fasting plasma glucose (mg/dl)	91.0 ± 0.6	89.2 ± 0.6	26.6	50.0	0.67

Data are means ± SEM. n = 202.

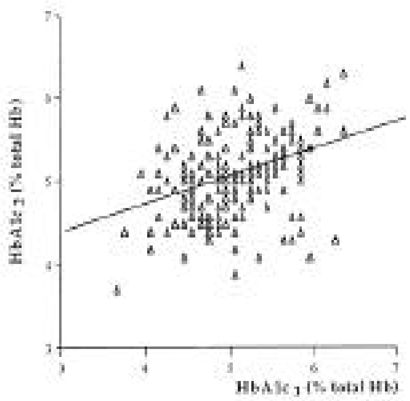

Figure 1—*HbA_{1c} at the first* (x-axis) *and second* (y-axis) *examinations: the Telecom Study* (n = 202).

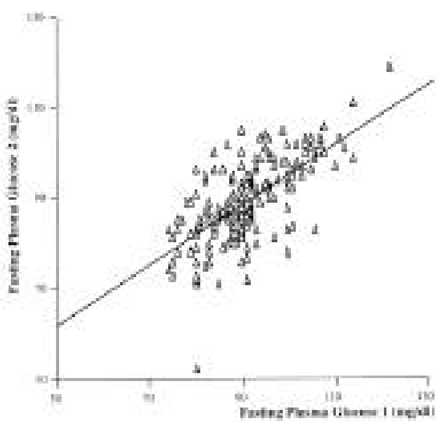

Figure 2—*Fasting plasma glucose at the first* (x-axis) *and second* (y-axis) *examinations: the Telecom Study* (n = 202).

plained by technical flaws in HbA_{1c} measurement. The same technician evaluated the analytical variability of HbA_{1c} measurement in a preliminary study, using the same technique, and showed that the method was reliable. Furthermore, the synthesis of 10 studies (including the background Telecom Study) selected for a meta-analysis showed a mean HbA_{1c} value of 5.30 and a standard deviation equal to 0.50 (3), similar to our reference values (7). Therefore, technical improvements in HbA_{1c} measurement could not considerably ameliorate the poor HbA_{1c} reproducibility in healthy subjects, since it appears to be essentially due to random variation.

It is noteworthy that HbA_{1c} reproducibility differed according to the delay between the two measurements: HbA_{1c} reproducibility was much better when the two blood samples were drawn within 4 months, the erythrocyte life span. Since no relationship was observed cross-sectionally in the background population between HbA_{1c} level and erythrocyte count, mean corpuscular volume, or the delay from the last period in women, and since no influence of seasons on HbA_{1c} level was shown (7), this lag-time variation in HbA_{1c} reproducibility is probably due to a wide variation in the process of hemoglobin glycation from one erythrocyte generation to another in healthy individuals. The role of an erythrocyte factor in the large biological variation of HbA_{1c} in nondiabetic subjects had been previously speculated (4,10) but never demonstrated, as in this study.

The poor reproducibility of HbA_{1c} in healthy adults is in contrast with the stability of HbA_{1c} in diabetic patients (11). This discrepancy could be due to an increase in the association between HbA_{1c} level and plasma glucose level when glucose tolerance deteriorates. Indeed, in the background population, in stepwise multiple regression analysis, fasting plasma glucose explained very little of the HbA_{1c} variability, with r^2 equal to 0.03 in men and 0.07 in women (7). Similar data were found in another community study, where only one-third of the variance of glycated hemoglobin levels was explained by the degree of glucose intolerance, and where the determinants of glycated hemoglobin variability could not be identified (10).

The limitations of our study mainly come from having examined a very healthy population with very few individuals having borderline HbA_{1c} values. If we had selected subjects to obtain a wider range of HbA_{1c} values, it would have inflated the correlation coefficient (12). Nevertheless, screening is usually performed in healthy populations, who are not always selected for diabetes risk factors, as in our study. Moreover, if less healthy subjects had been examined, an improvement in reproducibility would probably have been observed for fasting plasma glucose as well as for HbA_{1c}. Therefore, the striking contrast that we observed between HbA_{1c} and fasting plasma glucose reproducibility would have probably remained unaffected if less healthy subjects had been examined.

In conclusion, our data on 202 healthy subjects confirm the few small-scale studies that have previously shown the poor reproducibility of HbA_{1c} in healthy subjects. Our study demonstrates that erythrocyte life span is a major determinant of HbA_{1c} variability in nondiabetic subjects, and suggests that hematological factors could explain the poor reproducibility of HbA_{1c} in healthy populations. Even if further evaluation in less healthy populations would be useful to evaluate the impact of the poor reproducibility of HbA_{1c} on diabetes misclassification, these data provide strong arguments to suggest that a better standardization of HbA_{1c} measurement will not improve the ability of HbA_{1c} to be used as a tool for diabetes diagnosis and/or screening.

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